

# The 2<sup>nd</sup> Berlin BedRest Study: protocol and implementation

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## Abstract

Longterm bedrest is used to simulate the effect of spaceflight on the human body and test different kinds of countermeasures. The 2<sup>nd</sup> Berlin BedRest Study (BBR22) tested the efficacy of wholebody vibration in addition to highload resistive exercise in preventing bone loss during bedrest. Here we present the protocol of the study and discuss its implementation. Twentyfour male subjects underwent 60days of sixdegree head down tilt bedrest and were randomised to an inactive control group (CTR), a highload resistive exercise group (RE) or a highload resistive exercise with wholebody vibration group (RVE). Subsequent to events in the course of the study (e.g. subject withdrawal), 9 subjects participated in the CTRgroup, 7 in the RVEgroup and 8 (7 beyond bedrest day30) in the REgroup. Fluid intake, urine output and axillary temperature increased during bedrest ( $p < .0001$ ), though similarly in all groups ( $p \geq .17$ ). Body weight changes differed between groups ( $p < .0001$ ) with decreases in the CTRgroup, marginal decreases in the REgroup and the RVE group displaying significant decreases in bodyweight beyond bedrest day51 only. In light of events and experiences of the current study, recommendations on various aspects of bedrest methodology are also discussed.

**Keywords:** Spaceflight, Microgravity, Countermeasures, Inactivity, Exercise

## Introduction

Spaceflight results in significant changes in a large number of systems in the human body<sup>1</sup>. Some of these changes, such as loss of muscular capacity, loss of bone, orthostatic intolerance

and impaired postural control could severely affect astronauts and cosmonauts upon return to Earth or performance of their mission tasks after a flight to Mars. In view of this background, a goal of space agencies worldwide is to develop countermeasures (be it exercise, pharmacological, nutritional or otherwise) against the effects of weightlessness. Rather than testing such countermeasures in space, it is far more cost effective to do so using an Earthbased methodology. Prolonged, strict bedrest, particularly in a head-down tilt (HDT) position, has become an established spaceflight analog methodology for testing countermeasures for spaceflight on Earth<sup>2,3</sup>.

Whilst using a groundbased methodology such as bedrest to test these countermeasures is more cost effective and easier than doing so in a weightless environment, the costs of bedrest studies typically run into millions of euros and the complex nature of these studies can be challenging. Consequently, the number

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of subjects participating in bedrest studies is typically kept to the minimum required for addressing the primary outcome measure. This limited number of subjects can impair our ability to examine the effect of bedrest on a particular body system. To alleviate this problem, one may attempt to pool data from a series of bedrest studies, but this can be hampered by differences in protocol between studies. These difference in protocols can also impair our ability to compare the effectiveness of different countermeasures across studies<sup>4</sup>. For these underlying reasons, the European Space Agency (ESA) has begun to standardize the conditions of the bedrest studies it sponsors across Europe.

One recent ESA sponsored bedrest study, the 1<sup>st</sup> Berlin BedRest Study<sup>7</sup>, focused on the effects of a highload resistive exercise with wholebody vibration (RVE) countermeasure, on bone and muscle adaptations in 56days of horizontal bedrest. The findings of this study showed that the countermeasure exercise programme was able to ameliorate bone loss<sup>8</sup> and changes in bone metabolism<sup>9</sup>; loss of muscle mass<sup>10</sup>; muscle fibre adaptations and molecular signalling<sup>11,12</sup> and impairment of muscle contractile capacity and motor control<sup>13</sup>. One important question that remained unanswered after this study was whether whole body vibration during highload resistive exercise provided an additional stimulus above that of resistive exercise (RE) alone, for the retention of muscle and bone during bedrest. For that reason, the 2<sup>nd</sup> Berlin BedRest Study was initially planned to compare RVE and RE during 56days of horizontal bedrest with 10 subjects in each group and with a view to using data from the 1<sup>st</sup> Berlin BedRest Study for the inactive control group. However, as part of standardisation of bedrest studies across Europe<sup>14</sup>, the study organisers were requested by ESA to implement 60days of sixdegree headdown tilt bedrest with lengthier prebedrest baseline data collection and postbedrest recovery periods (with the remaining aspects of standardisation, such as specific outcome measure protocols, more precise physical fitness assessment, amongst other procedures<sup>15</sup>, left for future projects). This necessitated changes of study methodology, such as the inclusion of a new control group, which was not previously planned for the current study, and more subjects. With this paper, we wish to: report the implementation of the 2<sup>nd</sup> Berlin BedRest Study including some specific aspects of the study protocol (e.g. open muscle biopsy, reambulation), discuss critical events that occurred during this study, provide data on changes in basic parameters (body weight, fluid intake, urine output, axillary temperature) during bed rest and in light of what we have learned during the operation of this study, discuss further plans for bedrest study standardisation and recommendations on other aspects of bedrest methodology. Detailed data on the main outcome measures (i.e. bone and muscle changes) from the 2<sup>nd</sup> Berlin BedRest Study are not presented here, but will be the subject of future publications.

## Materials and Methods

### *Study characteristics*

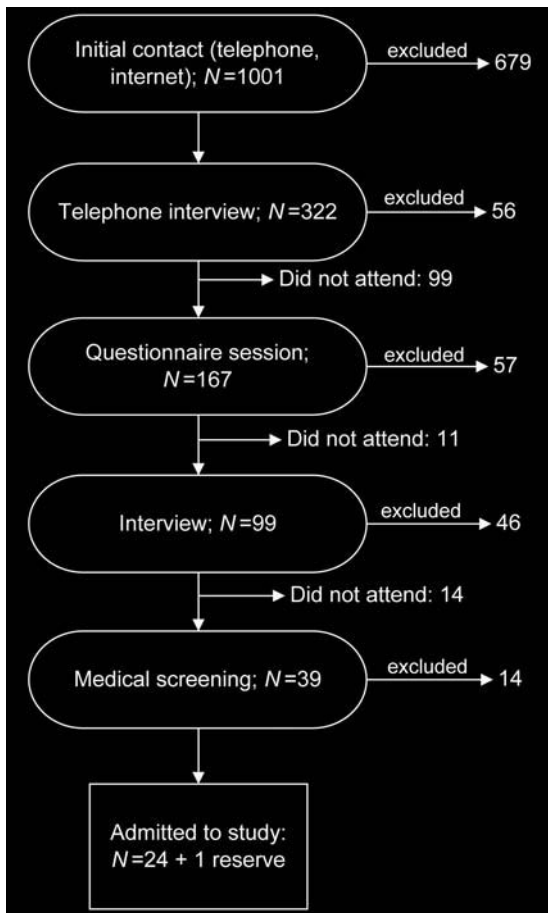
The 2<sup>nd</sup> Berlin BedRest Study was conducted at the Charité Campus Benjamin Franklin in Berlin, Germany, by the Centre for Muscle and Bone Research. Total bone mineral content of

the distal tibia was chosen as the primary outcome measure. Sample size estimates based upon data from an earlier bedrest study<sup>5</sup> suggested that for the comparison between RVE and control, six subjects were necessary per group and that for the comparison between RVE and RE, nine subjects per group were required.

Twentyfour male subjects were recruited. The bedrest phase of the study was conducted in four campaigns of six subjects each, in 2007 and 2008. For each campaign of six subjects, the subjects were paired as roommates according to psychological criteria and the pairs were then randomised to three different groups: one that performed resistive exercise only (RE) during bedrest, one that performed the same resistive exercises but with wholebody vibration (RVE), and one that performed no exercise and served as a control group (CTR). Thus, eight subjects were planned for each group.

Subjects were admitted to the facility nine days prior to the commencement of bed rest. Following this, they underwent nine days of baseline data collection (BDC9 to BDC1) and 60days of sixdegree headdown tilt bedrest (HDT1 to HDT60). Subjects remained in the facility for seven days after reambulation (R+1 to R+7) and returned to the facility 14, 30, 90, 180, 360 days after reambulation (R+14, R+30, R+90, R+180, R+360) with twoyear (R+720) followups still underway. For logistical reasons, R+14 was performed on days 13, 14, and 15 postbedrest (two subjects per day), R+30 on the 29<sup>th</sup> and 30<sup>th</sup> day postbedrest with R+90 and R+180 spread over two days, again for logistical reasons.

The inclusion criteria stipulated that subjects were to be: psychologically and medically healthy, male aged 20-45 years, of a height 155 to 195 cm, in possession of social insurance, available for more than 11 weeks (including 60 days of bed rest) and prepared to attend followup examinations up to 2 years after bedrest. The exclusion criteria were as follows: any addictions (alcohol, drug or medication), regular medical treatment or longterm hospital stays, smoking (>10 cigarettes per day) or not prepared to cease smoking for the duration of the study, regular intake of medication, chronic diseases, any kind of metabolic or hormonal disturbances, a need for dental therapy, history of psychological disease, history of any kind of vessel disease or surgery, cardiovascular disease, disturbances of blood clotting mechanism, any kind of muscle or bone disease, osteosynthesis or metal implants, any acute or chronic bacterial or nonbacterial inflammatory disease, vestibular disorders, migraine, donation of blood of more than 350 ml within 3 months of the commencement of participation in the study, simultaneous participation in other studies, orthostatic problems, any kind of allergy, active competitive sportsman, chronic low back pain or back pain in need of treatment, any history of spinal operation, severe scoliosis, sleep disorders (early riser or nightmares; requiring >10 hours or <5 hours sleep per day), epilepsy, any kind of cartilage disturbances of ankle, knee, hip or any kind of joint diseases (acute and/or chronic), prior knee surgery or ligamentous injury, low bone mass (dual energy Xray absorptiometry of the lumbar spine and hip <1.5 SD, or trabecular density of the lumbar spine via



**Figure 1.** Flow chart of the subject recruitment process. At initial contact, candidates gave age, weight and height and based upon this information a telephone interview was conducted to assess further inclusion/exclusion criteria. The “N” value within each box indicates the number of candidates attending each particular step of recruitment. *Did not attend*: number of candidates that did not attend the next step of the recruitment process despite being invited to do so.

quantitative computed tomography <120 mg/ml) and vegetarian/vegan dietary requirements. For successful completion of all aspects of the study, subjects received a royalty totalling eight thousand Euros. For completion of only part of the study, remuneration was reduced according to a fixed schedule.

The study was approved by the ethical committee of the Charité Universitätsmedizin Berlin. The radiological examinations were approved by the Bundesamt für Strahlenschutz. All subjects gave their informed written consent prior to participation in the study.

#### Subject recruitment and screening

Subjects were recruited via local, regional and national radio and television reports, advertisements in local and regional newspapers, advertisements on public transport, posters placed at the hospital and universities, internet in addition to 3000 direct mailouts to men between 25 and 35 years of age in Berlin.

Figure 1 describes the process of recruitment and screening: once contact was made, an initial telephone interview evaluated a number of inclusion and exclusion criteria. If the candidate was suitable, they were given one week to consider their participation in the study and then asked to attend a personality questionnaire session (Freiburger Persönlichkeitsinventar<sup>15</sup> and the Temperament Structure Scale [TSS], a standard personality testing instrument developed and used by the German Aerospace Center). If deemed suitable after the assessment of the questionnaire, the candidate was invited to attend a subsequent interview session (again after a oneweek consideration time) with a psychologist and study investigators. Candidates successful after this interview were invited to attend a medical screening session.

At medical screening, the following examinations were performed:

- lumbar and hip bone mass/density as measured via dual X ray absorptiometry (DXA). (Where necessary, trabecular density at the lumbar spine was measured via quantitative computed tomography).
- a stresscardiogram was performed by a sports physician and an additional echocardiogram where recommended by the sports physician.
- ultrasound of the pelvic and leg veins
- ultrasound of the kidneys
- blood and urine sampling (for biochemical and genetic testing to exclude genetic risk factors for blood coagulation (Factor V Leiden Mutation, Factor II Mutation [Mutation 20210], MTHFR Mutation [A223V]) and other biochemical disorders).

Five candidates were excluded on the genetic thrombosis risk criteria, three were excluded due to low bone density (one of which was also excluded due to thrombosis risk), two due to abnormal biochemical (blood/urine) results and five were found to be medically healthy but withdrew their interest in the study.

#### Routine Examinations and BedRest Conditions

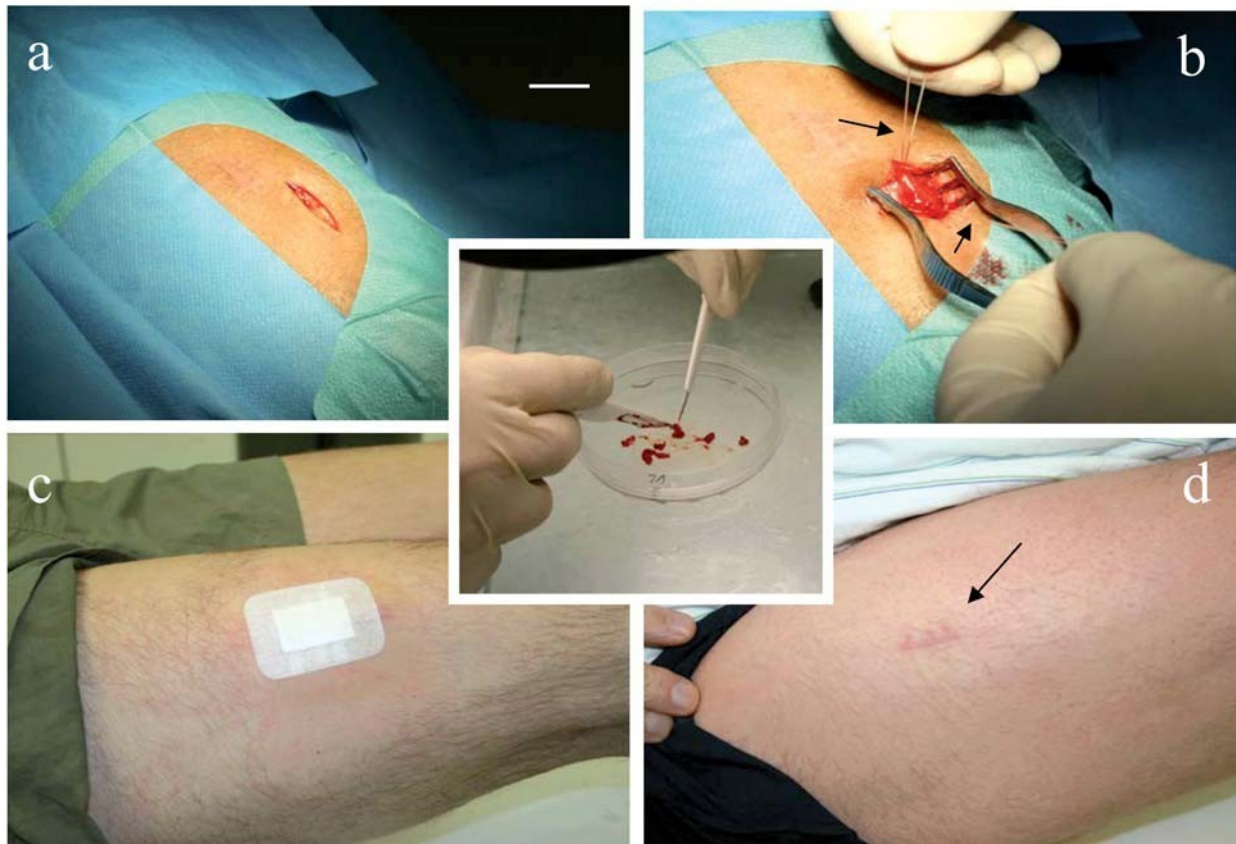
Whilst attending the bedrest facility, subjects received 24 hour nursing supervision.

*Medical consultations*: were conducted on a daily basis and a medical doctor was oncall 24 hours a day. A psychologist was available for consultation as deemed necessary. A consultant physician, uninvolved with the study or the research group, was available to provide independent medical examinations as needed.

*Blood drawings*: were performed two days prior to bedrest (BDC2) and then on days 5, 12, 19, 26, 33, 40, 47, 54, 60 of head down tilt bedrest as well as immediately prior to reambulation (R+1) and on post bedrest recovery days 3, 6, 30, 90 and 180.

*Bodyweight, urine output, fluid intake and body temperature*: Between 6.30 and 7.30 am every day, subjects were weighed<sup>a</sup> (Seca 985, Seca GmbH, Hamburg, Germany), urine output in the preceding 24 hours up until 7am (at which time the subject was requested to urinate) and axillary temperature

<sup>a</sup> on HDT7, HDT21, HDT35, HDT49, HDT54 and HDT55 a number of subjects could not be weighed due to other experiments being performed.



**Figure 2.** Openincision muscle biopsy, sampling procedure and wound care. (a): Incision of the skin for an open incision muscle biopsy following local anesthesia (m. vastus lateralis in this image) in a subject by a surgeon. (b): Open skin incision with exposed muscle fascia in the same subject, arrows indicate the position of ligatures. (Inset, middle panel): Preparatory steps in aliquot separation of a muscle biopsy by an operator on a sterile chilled glass plate. (c): Wound care (plaster) shortly after the biopsy. Compression bandages are then applied over the wound (not seen). (d): A small scar is seen on the skin (arrow) 8 weeks after an openincision biopsy of m. vastus lateralis. Bar in Figure section “a” = 1 cm

(with a standard mercury thermometer) were measured. Fluid intake from drinks was also recorded and fluid intake in 24 hour periods noted.

*Daily routine and bedrest conditions:* Subjects followed a day/night cycle of 7am wakeup and lightsout at 11pm. Showering was performed in a dedicated head-down tilt showerbed. Subjects were instructed that during bedrest they were allowed to change position in bed, but that physical activity was to be kept at a minimum level and all hygiene needed to be conducted in the head-down tilt position. Subjects were permitted to lie on their stomach, back or side, however, were instructed that, when in the supine position, the legs were to be kept straight and when in sidelying the trunk and head needed to remain in the head-down position (i.e. the head could not be held up with the hand/arm as one commonly does whilst reading). Video supervision on a 24-hour basis permitted further monitoring of subject adherence to the study protocol. Regular weekly meetings were conducted amongst study personnel to discuss the progress of each campaign.

*Diet:* Three meals per day were prepared by the hospital

kitchen under the guidance of a qualified nutritionist. The meal mixture was optimised according to German Nutrition Society (DGE) guidelines (50.55% carbohydrate, 30.35% fat, 15.20% protein). Subjects were advised that food could not be exchanged with other subjects and that meals were to be consumed completely. Subjects were not allowed any snacks between meals.

#### *Muscle Biopsy Protocol*

Prior to bedrest (BDC6) and shortly before the end of bedrest (HDT58) two open incision muscle biopsies (Figure 2) were taken under local anesthesia from the right leg of each subject. Biopsies were taken from the m. vastus lateralis, the lateral aspect of the thigh (with a mixed fast/slow fiber composition), and one from m. soleus, (more slow fiber composition). Before surgery, the cleaned skin surface area (about 5x5 cm) was anesthetized with 12% solution of Xylocain™ by a surgeon and a longitudinally short skin incision (approx. 1cm in length) was made taking special care to avoid small blood vessels or nerves (e.g., n. cutaneous femoris lateralis) in the



**Figure 3.** Countermeasure exercise training. Both the resistance exercise only (RE) and resistance exercise with wholebody vibration (RVE) groups performed their exercises on the specially designed Galileo Space exercise device. Subjects performed all exercises in the headdown tilt position and were positioned on a moveable platform with shoulder pads and hand grips preventing downward movement and permitting application of force via the platform. A pneumatic system generated the force, applied through the moveable platform, against which the subject needed to resist and move (via the shoulder pads and hand grips). The feet were positioned on either side of a platform which was set to vibrate during high load resistive exercises in the RVEgroup. Subjects were given visual feedback of their actual and target position in the exercise via a monitor placed in the subjects' field of view. As the force output was dictated by the exercise device, the feedback focused on ensuring the subjects performing the exercise in the desired range of motion and at the desired speed. During the heel raises the sport scientist monitored the range of movement and encouraged the subject to go to the end of range in each direction. Here the subject is performing squats with the knee and hip musculature.

subcutaneous connective tissue. A second short longitudinal incision was made to open the main fascia of the muscle and to expose the underlying muscle fascicles with their fibers clearly visible by eye. Two ligatures (separated by about 0.5–0.8 cm) were tied on an exposed small muscle fascicle and then the muscle was cut beside the ligatures to quickly remove a small sample. The samples were placed on a sterile plastic dish, and immediately separated into smaller samples on a chilled glass plate by a second operator using sterilized blades under an illuminated magnification lens in a separate small room attached to the surgery room. Separation of aliquot pieces took less than one minute, and samples were immediately frozen by another operator in liquid nitrogen (500ml Dewar), placed in plastic vials (cryotubes) coded for each of the subjects, and stored frozen in a liquid nitrogen container for further processing. Meanwhile, the fascia and skin incision were sutured by the medical doctor and a clip plaster placed over the wound. The upper leg was bandaged and the whole procedure was repeated for the second biopsy on the calf. In total, the entire procedure took about 15 min for each subject and leg using a team of one surgeon, one nurse, and two oper-

ators for biopsy sampling and freezing. After surgery, the subjects were transferred back to their rooms for recovery only. The next day, bandages were removed and subjects were able to continue their daily schedules with the exception being that exercise was not permitted on the day after biopsy.

#### *Preparation for countermeasure exercise: BDCphase*

A sport scientist (UG) supervised all exercise sessions with the subjects. During the BDCphase and prior to randomisation of subjects into the different groups, all subjects practised the exercises on three separate days (BDC4, BDC3 and BDC2) to ensure that those who were allocated to the RVE or RE groups were able to train optimally from the beginning of HDT. Familiarisation exercises were performed under low load (65% of body weight) and without wholebody vibration. On BDC2, the subjects' maximal force output during a squat exercise on the Galileo Space (Novotec Medical GmbH, Pforzheim, Germany) exercise device (Figure 3) was measured with a typical 1 repetition maximum test: 5 repetitions of warmup squat exercise was performed at low load (1000N), the operator then adjusted the force to a level just below what he

judged the maximum force output of the subject to be. The subject was required to perform a squat (starting from 10° bilateral knee flexion, increasing to 90° knee flexion and then returning to 10° knee flexion; as fast as possible without pause). When the subject successfully performed this, a 4 minute rest period was taken, force increased by 5% and the exercise repeated. This process was repeated until the subject could no longer perform the squat exercise. The force level at the final successful repetition was taken as the maximal force level to be used as a reference at the start of bedrest. In the evening after this maximal strength testing session, subjects were randomised into the three groups.

#### *Countermeasure exercise during bedrest*

Countermeasure exercises were performed on Mondays, Wednesdays and Fridays. On each exercise day, one exercise session was performed between 9am and 1pm. Training was scheduled such that subjects rotated their timeslots from one exercise session to the next, to avoid any “time of day” effects on training performance. The first session of exercise was performed on HDT1, which was always a Monday. The last exercise session was performed on HDT57. The RVEgroup performed the same exercises as the REgroup, however, with the addition of wholebody vibration (applied at the feet) simultaneously during highload resistance exercise. Subjects wore standard (i.e. no special air or gel “cushioning” in the sole) flat soled trainers on their feet during exercise.

In a given exercise session, the following exercises were performed:

- 1) *Warm up*: bilateral squat exercise (from 10° to 90° knee flexion and back to the starting position of 10° knee flexion) with 50% of maximal force for 64 seconds. Subjects performed both the concentric and eccentric phases of the exercise in four seconds each. Eight continuous (i.e. without pause) repetitions were performed. In the RVEgroup, the vibration was set to 24 Hz (amplitude 3.54mm). A two minute break was given after the warm up.
- 2) *Bilateral squats* (from 10° to 90° knee flexion and back with four seconds each for the concentric and eccentric phases) were performed on HDT1 at 75% and HDT3 at 80% of maximum and the subject performed the exercise continuously until exhaustion. From HDT5 and beyond, the force level was increased by 5% in each session until the subject could only perform eight repetitions. In sessions subsequent to this, if the subject improved such that they could perform more than ten repetitions in two adjoining sessions, then the force level was increased by 5% again. If a subject could not successfully complete six repetitions in two adjoining sessions, then the force was decreased by 5%. In the RVE group the vibration frequency was progressed from 20 to 24Hz (amplitude 3.54mm) from HDT1 to HDT5 and then maintained at this level for the rest of the study. A five minute break was given after this exercise.
- 3) *Single leg heel raises* were performed on the left and right legs from maximal plantarflexion to maximal dorsiflexion against a force equivalent to approximately 1.3 times their

HDT1 body weight. The forefoot was positioned on the bottom of the force plate (see Figure 3) with the heel hanging over the edge. Subjects were asked to perform the movement as quickly as possible whilst moving from full plantarflexion to full dorsiflexion. Typically, a movement frequency of 0.4 to 0.7Hz was achieved. The subject was instructed to hold the knee at full extension to avoid assistance via the knee musculature. The exercise was performed until exhaustion (i.e. when the subject could no longer perform the movement accurately). Vibration frequency was set to 26Hz (amplitude 3.54mm) in the RVEgroup. On completion of the exercise by the first leg, a 90 second break was given prior to commencement of the exercise by the other leg. At completion of the exercise by both legs, four minutes break was given. If the subject could not perform the exercise for more than 30 seconds then load was decreased by 5%. If the subject was able to perform the exercise for more than 50 seconds then the load was increased by 5%.

- 4) *Double leg heel raises* were performed in the same manner as for single leg heel raises except that the resistive force was set to approximately 1.8 times body weight. The exercise was performed until exhaustion. Vibration frequency was set to 26Hz (amplitude 3.54mm) in the RVEgroup. Two minutes break were given after the completion of this exercise. If the subject could not perform the exercise for more than 40 seconds then load was decreased by 5%. If the subject was able to perform the exercise for more than 55 seconds then the load was increased by 5%.
- 5) *Back and toe raises*: with their feet positioned on the platform, subjects extended their hips and lumbar spine, dorsiflexed their ankles, and maintained their knee at full extension. A planklike body lift was performed. Subjects were required to maintain this position for 60 seconds. During the exercise, a force 1.5 times body weight was applied at the shoulders. Vibration frequency was set to 16Hz (amplitude 3.54mm) in the RVEgroup. The exercise was not progressed.

A vibration amplitude of 3.54mm was chosen as it was the maximum possible on the device: higher vibration amplitude results in greater forces being imposed on the subject during exercise. Wholebody vibration frequencies for the squat exercise were chosen based on our prior experience that previously untrained people can more easily gain mastery of the manoeuvre with 20Hz vibration. Progression of vibration frequency during squats was done to increase exercise intensity and depended upon the ability of the subject. A maximum vibration frequency of 26Hz was chosen due to physiological time limitations of the muscle stretch reflex arc. At frequencies higher than 26 Hz, we assumed there to be inadequate time for muscle fibre contraction and relaxation. For the heelraise exercises, 26Hz vibration, as our experience shows that subjects can typically master this (simpler) exercise already with higher vibration frequencies. For the backraise exercise, a lower vibration frequency (16Hz) was chosen in order to better target type I (slow twitch) muscle fibres, which are typically activated with lower frequency motor unit potentials.



**Figure 4.** Experimental setup during reambulation.

### *Reambulation*

Two days prior to reambulation, subjects were informed of the procedures to be implemented. On the morning of reambulation, subjects were equipped with a cannula in the left antecubital vein and transferred from their beds to a tilt table, which was positioned horizontally. After cleaning the skin with alcohol pads, electrodes were placed on the chest and the neck for continuous recording of the electrocardiogram and beat-to-beat stroke volume by the thoracic impedance technique (CNSystems, Graz, Austria). For continuous plethysmographic measurement of blood pressure (CNSystems, Graz, Austria), finger cuffs were placed on the second and third finger of the right hand with the forearm resting in a sling on the chest. Finger plethysmography derived signals were calibrated against blood pressure measured oscillographically with a pressure cuff on the left upper arm. Heart rate and blood pressure were continuously displayed on a monitor visible to the staff but not to the subjects (Figure 4).

Subjects were instructed to immediately report any experience of nausea, dizziness or blurriness of vision. In addition to these subjective criteria, a systolic blood pressure of less than 80 mmHg, heart rate greater than 140 beats per minute, pallor, and profuse sweating were also defined as presyncopal symptoms, subsequent to which, the tilt table test was terminated immediately. The tilt table test was conducted with the following protocol: after an initial rest period in the horizontal position for 15 minutes, the tilt table was then raised progressively to 10°, 20°, 30°, 40°, 50° and 60° with 5 minutes spent at each level.

If any of the defined presyncopal symptoms occurred, the tilt table was immediately returned to the horizontal position and 500 ml saline solution was rapidly infused via the venous cannula over 15 minutes. This was followed by slow (500 ml/h) infusion of further saline solution and a modified tilt table protocol: the tilt table was now raised to 20°, 40°, and 60° for 5

minutes each. To increase venous return, the subjects were instructed to activate the muscle pump by slowly shifting their body weight between both legs during the 40° and 60° tilts.

Once the subject successfully reambulated, he was assisted to step off the tilt table and sit in a wheel chair and was then observed for 15 minutes by medical and nursing staff whilst receiving intravenous fluids.

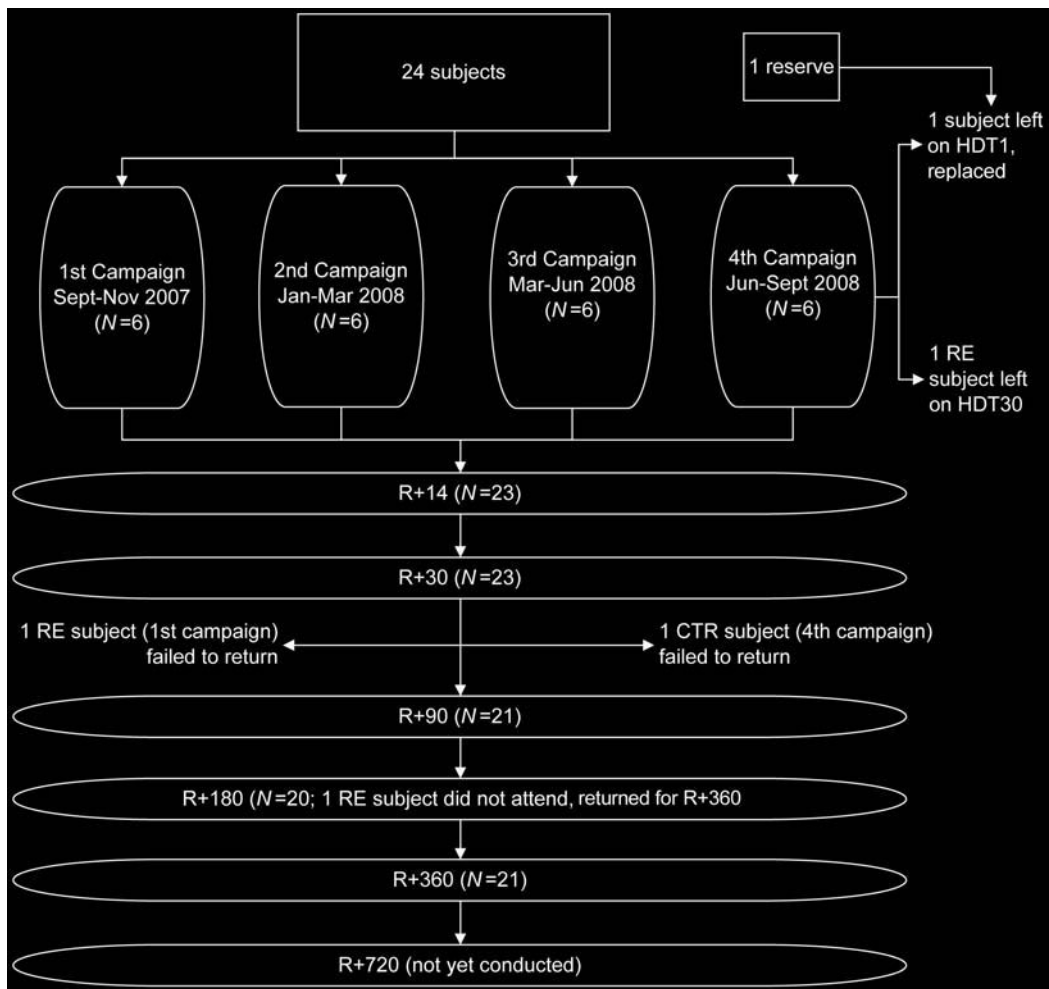
### *Statistical analyses*

Changes in daily body weight, urine output, fluid intake and body temperature with respect to subject group (RVE, RE and CTR), study date (with each day from the BDC phase up until R+7 factorised) and a group × study date interaction were assessed with linear mixed effects models<sup>16</sup>. Where necessary, allowances for heterogeneity of variance according to study date and/or subject group were applied. Random effects for each subject were permitted. An  $\alpha$  of 0.05 was taken for statistical significance on analysis of variance. Where significant effects were seen, subsequent analyses determined which study days differed from baseline (BDC). As measurements were conducted on multiple study days on the same subjects, a Bonferroni adjustment was not performed for posthoc analyses, rather we looked for consistent significant differences across time points. All analyses were performed in the “R” statistical environment (version 2.4.1, [www.rproject.org](http://www.rproject.org)).

## **Results**

### *Important events during the study*

Not all aspects of the study occurred as planned (Figure 5). One subject (RVE) chose to leave the study on HDT1 and was replaced with a standby subject (not all research groups were able to attain new baseline data from this standby subject).



**Figure 5.** Flow chart of events in the 2<sup>nd</sup> Berlin BedRest Study. CTR: control group, RE: resistive exercise only group, RVE: resistive exercise with wholebody vibration group. R+: indicated number of days of recovery after bedrest.

Group	Age (years)	Weight (kg)	Height (cm)	Fluid intake/day (ml)	Urine output/day (ml)	Temperature (°C)
CTR	33.1(7.8)	80.6(5.2)	181.3(6.0)	2406(345)	2503(688)	35.7(0.4)
RE	31.1(5.1)	75.0(12.8)	179.3(7.7)	2170(681)	2159(508)	35.8(0.3)
VRE	32.2(10.4)	81.5(6.2)	179.6(5.8)	2900(755)	2782(988)	36(0.3)

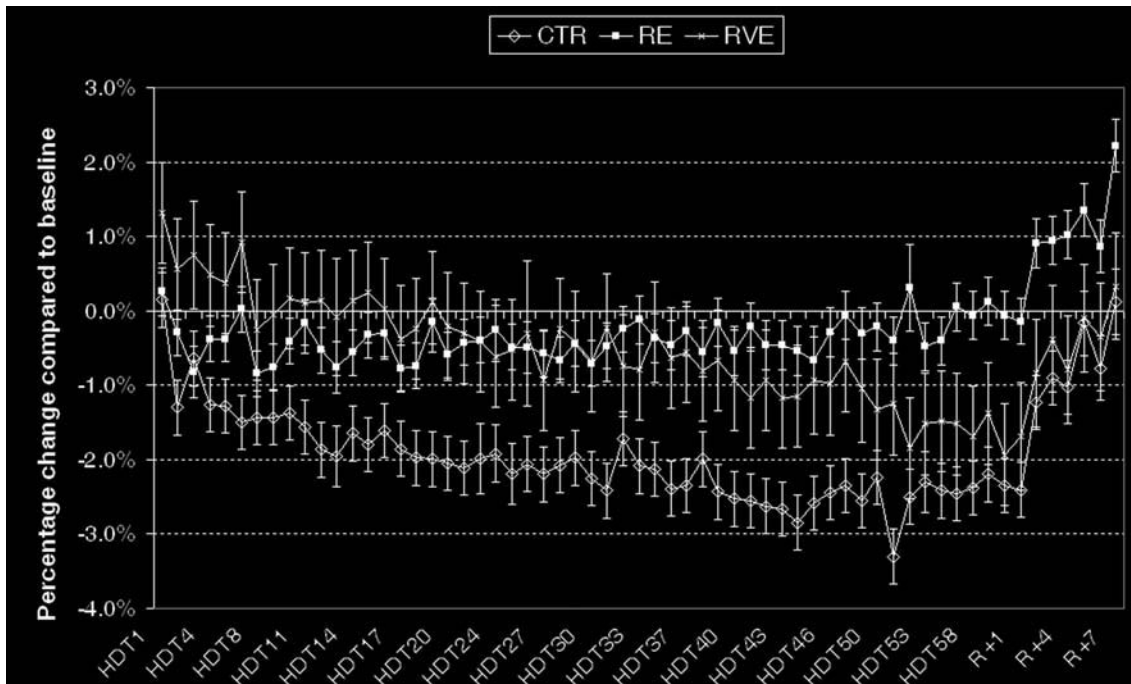
Values are mean (SD). There were no differences between groups for any of these variables ( $F_{all} \leq 1.8$ ,  $p \geq .20$ ).

**Table 1.** Baseline (BDCphase) characteristics.

This standby subject underwent the same duration of bedrest and recovery as the other subjects, but his bedrest phase began four days after the other subjects in his campaign. Another subject, whilst able to perform the familiarisation exercises in the ambulatory BDCphase, was randomised to the RVE group, but was unable to perform the exercise programme once in headdown tilt due to severe exercise induced headache. An independent medical consultant assessed the subject and stipulated that the subject could not continue in the RVEgroup,

but could remain in the study as a CTR subject. Another subject (REgroup) withdrew from the study at HDT30 for medical reasons. Thus, 9 subjects comprised the CTRgroup, 8 in the REgroup (until HDT30; n=7 beyond this) and 7 in the RVEgroup. The baseline characteristics of these subjects are given in Table 1. Two subjects (one RE, one CTR), did not attend followup examinations from R+90 and beyond. One further subject (REgroup) did not attend the R+180 examination, but returned subsequently.





**Figure 6.** Changes in subject bodyweight over the course of the study. Values are mean(SEM) percentage change in bodyweight compared to baseline (average of BDCphase). Data from HDT7, HDT21, HDT35, HDT49, HDT54 and HDT55 were not included in analyses as a number of subjects could not be weighed on these days. During bedrest, a decrease in bodyweight ( $p < .01$ ) in the control (CTR) group is seen from HDT4 onwards. In the resistive exercise only (RE) group, only marginal decreases in bodyweight were observed, and in the resistive exercise with wholebody vibration (RVE) group, no significant change in body weight was seen up until HDT51 ( $p \geq .051$ ) beyond which a marginal decrease in bodyweight was observed ( $p \leq .042$ ). After reambulation (R+1 and beyond), body weight increases in all groups.

### Muscle biopsy

Both baseline and end bedrest muscle biopsies from m. vastus lateralis and m. soleus of the right leg of the bed rest volunteers amounted to between 250400mg wet weight. These samples could be separated into several smaller specimens to serve the needs of at least four different research groups. During preparation, connective tissue, fascia and blood were removed from the harvested biopsy before freezing. In the replacement subject (RVEgroup), muscle biopsy was performed on the 1<sup>st</sup> day of bedrest with the endbed rest biopsy conducted on HDT57 (as opposed to HDT58 in the other subjects, due to availability of the surgical theatre and surgical staff). The REgroup subject who left bedrest on HDT30 did not complete the endbedrest biopsy procedure. Otherwise, all subjects completed the biopsy procedure according to the planned protocol.

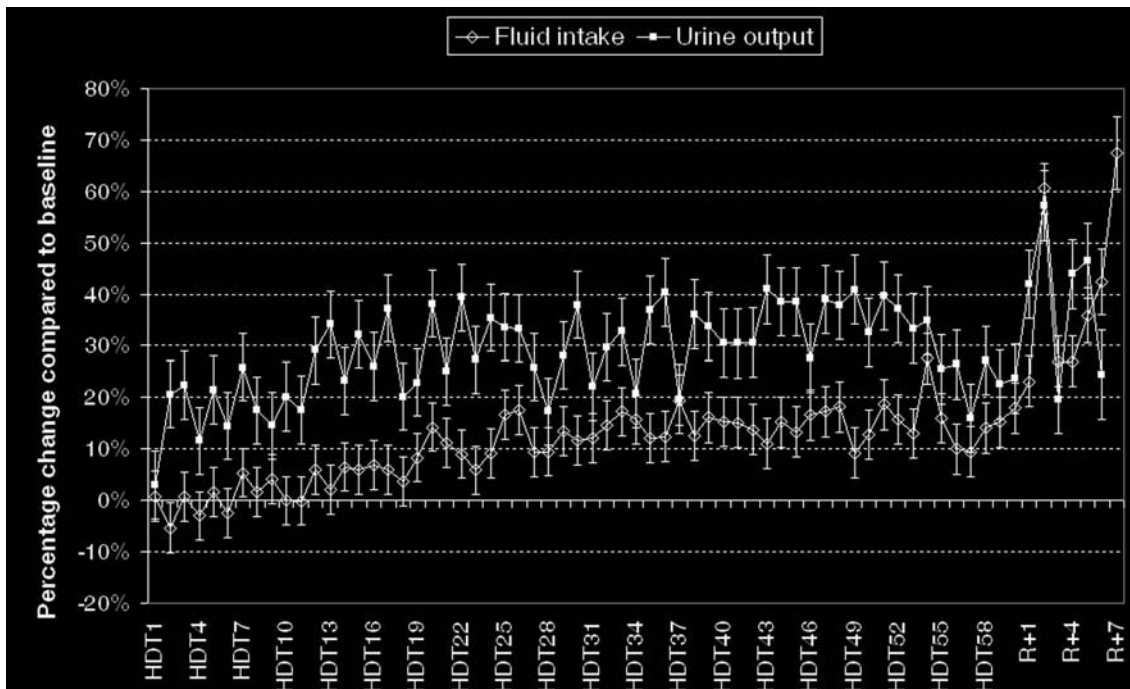
In three subjects, in the weeks following the biopsy procedure (two endbedrest m. vastus lateralis biopsy, one baseline m. vastus lateralis biopsy), it was found that not all of the stitches used in closing the wound had been completely removed. One of these subjects reported persistent pain at the biopsy site until removal of the remaining stitch, the second reported persistent pain and swelling until removal of the remaining stitch and the third reported no adverse symptoms. For the m. vastus lateralis biopsy in all remaining subjects and for all m. soleus biopsies, no other adverse events (such as indications of superficial nerve damage) were reported or noted in the patient chart.

### Conduct of countermeasure exercise

According to scheduling, 25 exercise sessions were planned per subject. Nine subjects (4 RVE, 5 RE) completed all sessions. One session was missed in three subjects (2 RVE, 1 RE) due to trainer absence. A further subject (RE) missed two sessions due to headache on one day and trainer absence on another. The standby subject (RVE) did not conduct the familiarisation sessions and began training later due to the conduct of muscle biopsy procedure on the 1<sup>st</sup> day of bedrest and hence completed 21 full exercise sessions in total.

### Daily measurements

Daily measurements of bodyweight, urine output, fluid intake and body temperature were conducted. Evidence existed for significant changes in all these parameters over the course of the study ( $F \geq 2.2$ ,  $p < .0001$ ). Bodyweight was the only parameter that displayed evidence for a different response between the three subjectgroups ( $F = 1.94$ ,  $p < .0001$ , otherwise  $F \leq 1.1$ ,  $p \geq 0.17$ ) with the CTRgroup demonstrating decreases in body weight throughout the bedrest phase, the REgroup showing only marginal decreases in bodyweight, and the RVEgroup displaying significant decreases in bodyweight beyond HDT51 (Figure 6). Fluid intake and urine output both increased during bedrest (Figure 7) with particularly strong



**Figure 7.** Changes in fluid intake and urine output over the course of the study. Values are mean(SEM) percentage change compared to baseline (average of BDC phase). No evidence ( $F \leq 1.0$ ,  $p \geq .38$ ) existed for a different response between groups, therefore data have been pooled. Fluid intake comprised volume of drinks only. Fluid intake was consistently increased ( $p < .05$ ) from HDT25 onwards and urine output from HDT2 onwards.

increases seen in these parameters upon reambulation (R+1 and beyond). Body temperature was generally higher during bedrest and recovery than before bedrest (Figure 8).

#### Reambulation

Twelve (of twentythree) subjects completed the first tilt table test without suffering presyncopal symptoms. In six subjects, heart rate increased above 140 beats per minute, three subjects reported nausea or dizziness, one subject's systolic blood pressure decreased below 80 mmHg and one subject experienced profuse sweating. All these subjects successfully completed the second modified tilt table protocol without any presyncopal symptoms and were able to step off the tilt table as described above.

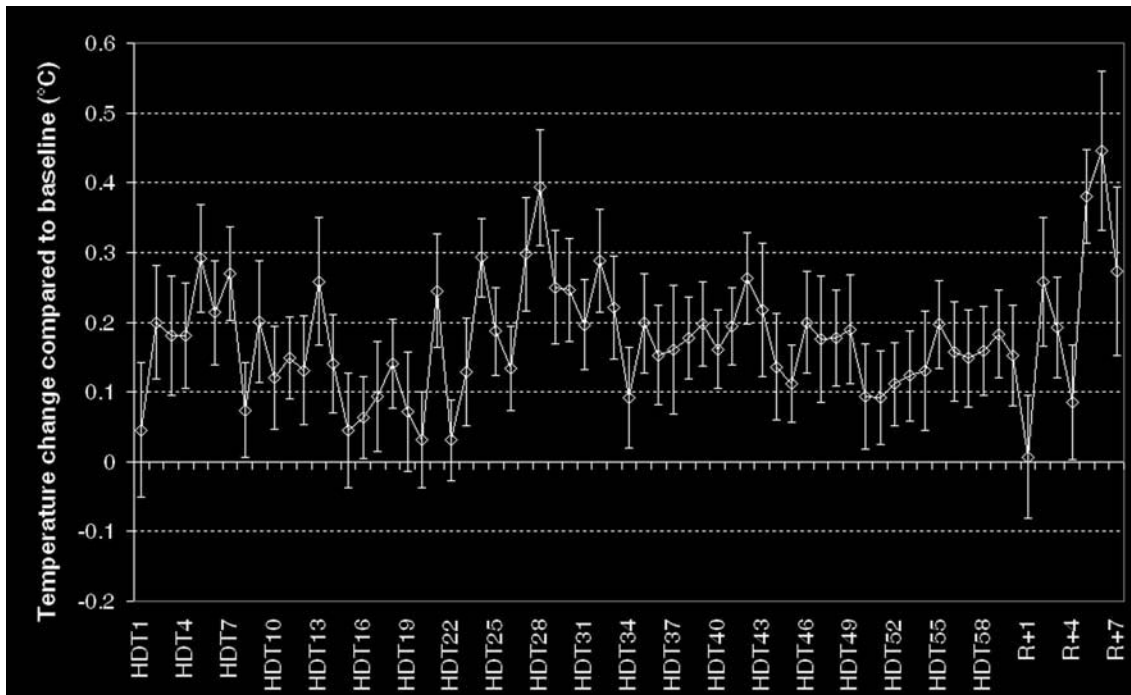
## Discussion

The methodology of prolonged bedrest for the simulation of spaceflight is a challenging undertaking. The complexity of the enterprise associated with the logistics of housing and caring for subjects over a period of many weeks and the integration of several, at times conflicting, experiments is, to a certain extent, one of the easier problems to deal with when undertaking such research. It is, however, the unpredictable human element of bedrest which represents the largest challenge of all. A good example is if, despite the supportive efforts of carers, study management and consulting psychologist, a subject de-

cidates to leave the study, this represents a loss for all concerned. In addition to the loss of potential data, the withdrawal of a subject creates the logistical difficulty of activating the stand by subject, organising baseline examinations for this person and then having to stagger this subjects within bed rest examinations and procedures for the entirety of this phase to factor in their delayed start. Ultimately, the "best laid plans" of such an undertaking cannot control the human element, but can at least create contingencies to deal with such events.

Based on our current experience, we would recommend other bedrest facilities ensure, as a standard approach, that at least one standby subject is available for the study. This is currently not part of ESA's bedrest standardisation plans<sup>14</sup>. In the current study, the inclusion of a replacement subject prevented the loss of data for a number of research groups for the RVE group. With an N of seven in the RVE group, data from each subject is critical. It was, however, not feasible to include a replacement subject when the RE group subject left after 30 days of bedrest. In longterm (60day) bedrest we feel that if a subject successfully completes the first two weeks of bed rest, then there is little risk of this subject deciding to leave the study. Medical events and events affecting the subject's relatives and loved ones outside of the study can, however, occur at any time.

The recruitment of subjects for the summer (June-August 2008) campaign was difficult. For this campaign, four candidates withdrew their interest after being invited to the medical



**Figure 8.** Changes in axillary temperature over the course of the study. Values are mean(SEM) change in degrees Celsius compared to baseline (average of BDCphase). No evidence ( $F=1.1$ ,  $p=.17$ ) existed for a different response between groups, therefore data have been pooled. Axillary temperature was higher ( $0.00001 < p < .048$ ) than before bedrest on fortyfive of the sixtyseven measurement days during bedrest and recovery. on HDT7, HDT21, HDT35, HDT49, HDT54 and HDT55 a number of subjects could not be weighed due to other experiments being performed.

screening step of recruitment. This compares to only one candidate withdrawing their interest (after being invited to medical screening) for the all other three campaigns in the autumn, winter and spring months. In parts of the world where good weather is less common, such as Berlin, we would advise against the conduct of bedrest campaigns in the summer months. This can also provide a break for the fatigued study and research team to recover. As part of subject recruitment, we were surprised by the detected prevalence of genetic mutations associated with blood clotting disorders. Five candidates (12.8% of all candidates attending medical screening) were excluded from the study on these criteria. This contrasts with published data suggesting a prevalence of 6% or less<sup>17,18</sup>.

For the first time in ESA sponsored bedrest studies, an open incision muscle biopsy procedure was implemented instead of the Bergström needle technique as routinely implemented previously. We propose to consider the open incision biopsy technique performed by a welltrained surgeon as an alternative to obtain high quality biopsy samples at sufficient amounts. In needle biopsies, experience has shown that sometimes multiple attempts are needed to attain sufficient tissue, which causes additional stress for the subject. Also, using needle biopsy, superficial nerves cannot be identified and sectioned as in a surgical approach and hence are more liable to be injured, although such side effects are typically not reported. (In prior work<sup>11</sup>, one subject reported symptoms of superficial nerve

damage.) We feel the open incision biopsy approach, should be considered in future bedrest studies, particularly those, as in the current work with access to surgical facilities.

The reambulation protocol was designed to achieve two different goals: firstly to assess orthostatic tolerance after bed rest and record the accompanying haemodynamic responses and secondly, to achieve a rapid and safe reambulation in subjects who suffered presyncopal symptoms during the first attempt. The stepwise tilt table protocol was applied because advising critical care physicians from our hospital deemed an immediate tilt from 0° to 60° after 60 days of bedrest to be too challenging for the subjects. Despite this, it is our view that this protocol presents a similar level of challenge as an immediate tilt to 60°. This view is supported by the finding that in 11 of 23 subjects the stepwise tilt table protocol had to be ceased due to presyncopal symptoms, which is similar to other works in bedrest (e.g. where 9 of 18 subjects could not complete a tilttest protocol comprising immediate tilt to 80 degrees after 90days bedrest)<sup>19</sup>. The measures employed to ameliorate orthostatic intolerance on the second attempt of reambulation (i.e. increase of blood volume by rapid infusion of saline and reduction of venous blood pooling by activation of the muscle pump) were selected as they are easy to apply and constitute no risk for the subjects. This combination was successful in achieving reambulation of all subjects who had suffered presyncopal symptoms during the first attempt.

ESA has completed its plans for further standardisation of bedrest studies and these will be published in the near future<sup>4</sup>. Standardisation of bedrest studies is important not only for our ability to compare data between studies across facilities within Europe, but also with bedrest studies conducted in other parts of the world. In light of our experience with the current and prior<sup>5</sup> longterm bedrest studies, we feel that the majority of these plans can be implemented without major difficulty, although the monetary cost for a bedrest study will of course increase. We feel, however a number of timelines suggested as part of standardisation<sup>4</sup> (e.g. 10 months between start of planning and start of study or that a meeting of all researchers 6 months after the end of bedrest) could be set more flexibly, and are more ambitious than the reality of the current study or other studies currently being organised. Aside from this, we would recommend that an external consulting physician be available to assess the subjects and advise the study management. Whilst the head medical doctor is not involved in the experiments being conducted, he or she is nonetheless integrated in the study management and may not, particularly from the subject's point of view, be seen as completely independent. Having another independent medical doctor to consult with the subjects proved important in the current study.

In summary, the conduct of bedrest studies is a challenging, but rewarding, undertaking. Bedrest studies provide not only a simulation platform for testing potential countermeasures for implementation in spaceflight but also provide an approach that can deepen our understanding of the human body. The standardisation of bedrest studies is an important endeavour to ensure comparability between studies and facilities. The experience of the 2<sup>nd</sup> Berlin BedRest Study provided an opportunity to assess current plans for the standardisation of bedrest studies. Based on this experience, we have provided recommendations for future work.

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#### References

1. White RJ. Weightlessness and the human body. *Sci Am* 1998;279(3):5863.
2. Nicogossian AE, Dietlein LF. Microgravity Simulation and Analogues. In: Nicogossian AE, editor. *Space physiology and Medicine*. Philadelphia: Lea & Febiger; 1982, p. 240248.
3. PavyLe Traon A, Heer M, Narici MV, Rittweger J, Vernikos J. From space to Earth: advances in human physiology from 20 years of bed rest studies (19862006). *Eur J Appl Physiol* 2007;101(2):14394.
4. McPhee J, Larina I, Heer M. Life sciences research standardization. *J Gravit Physiol* 2006;13(2):5971.
5. Rittweger J, Belavý DL, Hunek P, Gast U, Boerst H, Feilcke B, et al. Highly demanding resistive vibration exercise program is tolerated during 56 days of strict bedrest. *Int J Sport Med* 2006;27(7):5539.
6. Rittweger J, Beller G, Armbrrecht G, Mulder E, Buehring B, Gast U, et al. Prevention of bone loss during 56 days of strict bed rest by sidealternating resistive vibration exercise. *Bone* 2010;46(1):13747.
7. Armbrrecht G, Belavý DL, Gast G, Bongrazio M, Touby F, Beller G, et al. Resistive vibration exercise attenuates bone and muscle atrophy in 56 days of bed rest: biochemical markers of bone metabolism. *Osteoporos Int* 2010;21(4):597607.
8. Belavý DL, Miokovic T, Armbrrecht G, Rittweger J, Felsenberg D. Resistive vibration exercise reduces lower limb muscle atrophy during 56day bedrest. *J Musculoskeletal Neuronal Interact* 2009;9(4):22535.
9. Mulder ER, Stegeman DF, Gerrits KH, Paalman MI, Rittweger J, Felsenberg D, et al. Strength, size and activation of knee extensors followed during 8 weeks of horizontal bed rest and the influence of a countermeasure. *Eur J Appl Physiol* 2006;97(6):70615.
10. Belavý DL, Hides JA, Wilson SJ, Stanton W, Dimeo FC, Rittweger J, et al. Resistive simulated weightbearing exercise with whole body vibration reduces lumbar spine deconditioning in bedrest. *Spine* 2008;33(5):E121E131.
11. Blottner D, Salanova M, Puttmann B, Schiffel G, Felsenberg D, Buehring B, et al. Human skeletal muscle struc

- ture and function preserved by vibration muscle exercise following 55 days of bed rest. *Eur J Appl Physiol* 2006;97(3):26171.
12. Salanova M, Schiffel G, Puttmann B, Schoser BG, Blottner D. Molecular biomarkers monitoring human skeletal muscle fibres and microvasculature following longterm bed rest with and without countermeasures. *J Anat* 2008;212(3):30618.
  13. Mulder ER, Gerrits KH, Kleine BU, Rittweger J, Felsenberg D, de Haan A, et al. Highdensity surface EMG study on the time course of central nervous and peripheral neuromuscular changes during 8 weeks of bed rest with or without resistive vibration exercise. *J Electromyogr Kinesiol* 2009;19(2):20818.
  14. Heer M, Liphardt AM, FringsMeuthen P. Standardization of bed rest study conditions. Cologne: Deutsches Zentrum für Luft und Raumfahrt; 2010. Report No.: ESTEC CONTRACT Number 20187/06/NL/VJ.
  15. Fahrenberg J, Hampel R, Seig H. Freiburger Persönlichkeitsinventar Testanweisung; 2001.
  16. Pinheiro JC, Bates DM. Mixedeffects models in S and SPLUS. 1st ed. Berlin: Springer; 2000.
  17. Ridker PM, Miletich JP, Hennekens CH, Buring JE. Ethnic distribution of factor V Leiden in 4047 men and women. Implications for venous thromboembolism screening. *Jama* 1997;277(16):13057.
  18. Herrmann FH, Koesling M, Schroder W, Altman R, Jimenez Bonilla R, Lopaciuk S, et al. Prevalence of factor V Leiden mutation in various populations. *Genet Epidemiol* 1997;14(4):40311.
  19. Belin de Chantemele E, Blanc S, Pellet N, Duvareille M, Ferretti G, GauquelinKoch G, et al. Does resistance exercise prevent body fluid changes after a 90day bed rest? *Eur J Appl Physiol* 2004;92(45):55564.