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The 2nd Berlin BedRest Study: protocol and implementation


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Abstract

Long-term bed-rest is used to simulate the effect of spaceflight on the human body and test different kinds of countermeasures. The 2nd Berlin BedRest Study (BBR2-2) tested the efficacy of whole-body vibration in addition to high-load resistance exercise in preventing bone loss during bed-rest. Here we present the protocol of the study and discuss its implementation. Twenty-four male subjects underwent 60-days of six-degree head down tilt bed-rest and were randomised to an inactive control group (CTR), a high-load resistive exercise group (RE) or a high-load resistive exercise with whole-body vibration group (RVE). Subsequent to events in the course of the study (e.g. subject withdrawal), 9 subjects participated in the CTR-group, 7 in the RVE-group and 8 (7 beyond bed-rest day-30) in the RE-group. Fluid intake, urine output and axillary temperature increased during bed-rest (p<0.001), though similarly in all groups (p≥.17). Body weight changes differed between groups (p<0.001) with decreases in the CTR-group, marginal decreases in the RE-group and the RVE-group displaying significant decreases in body-weight beyond bed-rest day-51 only. In light of events and experiences of the current study, recommendations on various aspects of bed-rest 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 body1. 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 Earth-based methodology. Prolonged, strict bed-rest, particularly in a head-down tilt (HDT) position, has established an established spaceflight analog methodology for testing countermeasures for spaceflight on Earth1-5.

Whilst using a ground-based methodology such as bed-rest to test these countermeasures is more cost effective and easier than doing so in a weightless environment, the costs of bed-rest studies typically run into millions of euros and the complex nature of these studies can be challenging. Consequently, the number
of subjects participating in bed-rest 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 bed-rest on a particular body system. To alleviate this problem, one may attempt to pool data from a series of bed-rest studies, but this can be hampered by differences in protocol between studies. These differences in protocols can also impair our ability to compare the effectiveness of different countermeasures across studies. For these underlying reasons, the European Space Agency (ESA) has begun to standardize the conditions of the bed-rest studies it sponsors across Europe.

One recent ESA sponsored bed-rest study, the 1st Berlin BedRest Study, focused on the effects of a high-load resistive exercise with whole-body vibration (RVE) countermeasure, on bone and muscle adaptations in 56-days of horizontal bed-rest. The findings of this study showed that the countermeasure exercise programme was able to ameliorate bone loss and changes in bone metabolism, loss of muscle mass, and muscle fibre adaptations and molecular signalling, and impairment of muscle contractile capacity and motor control. One important question that remained unanswered after this study was whether whole-body vibration during high-load resistive exercise provided an additional stimulus above that of resistive exercise (RE) alone, for the retention of muscle and bone during bed-rest. For that reason, the 2nd Berlin BedRest Study was initially planned to compare RVE and RE during 56-days of horizontal bed-rest with 10 subjects in each group and with a view to using data from the 1st Berlin BedRest Study for the inactive control group. However, as part of standardisation of bed-rest studies across Europe, the study organisers were requested by ESA to implement 60-days of six-degree head-down tilt bed-rest with lengthier pre-bed-rest baseline data collection and post-bed-rest recovery periods (with the remaining aspects of standardisation, such as specific outcome measure protocols, more precise physical fitness assessment, amongst other procedures, 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 2nd Berlin BedRest Study including some specific aspects of the study protocol (e.g. open muscle biopsy, remobilisation), 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 bed-rest study standardisation and recommendations on other aspects of bed-rest methodology. Detailed data on the main outcome measures (i.e. bone and muscle changes) from the 2nd Berlin BedRest Study are not presented here, but will be the subject of future publications.

Materials and Methods

Study characteristics

The 2nd 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 bed-rest study 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.

Twenty-four male subjects were recruited. The bed-rest 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 bed-rest, one that performed the same resistive exercises but with whole-body 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 (BDC-9 to BDC-1) and 60-days of six-degree head-down tilt bed-rest (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 two-year (R+720) follow-ups still underway. For logistical reasons, R+14 was performed on days 13, 14, and 15 post-bed-rest (two subjects per day), R+30 on the 29th and 30th day post-bed-rest 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 follow-up examinations up to 2 years after bed-rest. The exclusion criteria were as follows: any addictions (alcohol, drug or medication), regular medical treatment or long-term 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 non-bacterial 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 <8 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 X-ray absorptiometry of the lumbar spine and hip <1.5 SD, or trabecular density of the lumbar spine via
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 Persönlichkeits-Inventar [FPI] 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 one-week 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 stress-cardiogram was performed by a sports physician and an additional echo-cardiogram 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 Bed-Rest Conditions

Whilst attending the bed-rest facility, subjects received 24-hour nursing supervision.

Medical consultations: were conducted on a daily basis and a medical doctor was on-call 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 bed-rest (BDC-2) and then on days 5, 12, 19, 26, 33, 40, 47, 54, 60 of head down tilt bed-rest as well as immediately prior to reanimation (R+1) and on post bed-rest recovery days 3, 6, 30, 90 and 180.

Body-weight, urine output, fluid intake and body temperature: Between 6.30 and 7.30 am every day, subjects were weighed* (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

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* on HDT7, HDT21, HDT35, HDT49, HDT54 and HDT55 a number of subjects could not be weighed due to other experiments being performed.
(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 bed-rest conditions:* Subjects followed a day-night cycle of 7am wake-up and lights-out at 11pm. Showering was performed in a dedicated head-down tilt shower-bed. Subjects were instructed that during bed-rest 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 side-lying 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 bed-rest (BDC-6) and shortly before the end of bed rest (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 1-2% 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