Long-duration bed rest as an analog to microgravity

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Hargens AR, Vico L. Long-duration bed rest as an analog to microgravity. J Appl Physiol 120: 891–903, 2016. First published February 18, 2016; doi:10.1152/japplphysiol.00935.2015.—Long-duration bed rest is widely employed to simulate the effects of microgravity on various physiological systems, especially for studies of bone, muscle, and the cardiovascular system. This microgravity analog is also extensively used to develop and test countermeasures to microgravity-altered adaptations to Earth gravity. Initial investigations of bone loss used horizontal bed rest with the view that this model represented the closest approximation to inactivity and minimization of hydrostatic effects, but all Earth-based analogs must contend with the constant force of gravity by adjustment of the G vector. Later concerns about the lack of similarity between headward fluid shifts in space and those with horizontal bed rest encouraged the use of 6 degree head-down tilt (HDT) bed rest as pioneered by Russian investigators. Headward fluid shifts in space may redistribute bone from the legs to the head. At present, HDT bed rest with normal volunteers is the most common analog for microgravity simulation and to test countermeasures for bone loss, muscle and cardiac atrophy, orthostatic intolerance, and reduced muscle strength/exercise capacity. Also, current physiologic countermeasures are focused on long-duration missions such as Mars, so in this review we emphasize HDT bed rest studies with durations of 30 days and longer. However, recent results suggest that the HDT bed rest analog is less representative as an analog for other important physiological problems of long-duration space flight such as fluid shifts, spinal dysfunction and radiation hazards.

bed rest; bone; muscle; simulated microgravity; space flight

BACKGROUND

LONG-DURATION, HEAD-DOWN TILT (HDT) bed rest studies have provided unique insights into the physiology of non-weight bearing and deconditioning associated with space flight. Previously the physiology of bed rest and the usefulness of this microgravity analog were extensively reviewed by Fortney and colleagues (34) and more recently by Pavy-Le Traon and associates (82). Because normal space flight operations are not duplicated in bed rest studies and astronauts are required to participate in space flight countermeasures, bed rest is probably not a true simulation of space flight. However, bed rest does represent a model of unloading that is less confounded by inflight crew activities. Also, because of the lack of flight opportunities and the high cost thereof, bed rest simulations of microgravity are useful to understand better the mechanisms and rates of adaptation between tissues and between physiological systems during space flight. In this regard, the underlying mechanisms of adaptation to microgravity include loss of body weight, tissue fluid redistribution, loss of hydrostatic pressures (Fig. 1A), and decreased sensory inputs within the body (42). Horizontal and HDT bed rest models may simulate actual microgravity in terms of arterial pressures and fluid shifts, but weight bearing, tissue fluid redistributions, and skin surface areas of compression in bed are very different from those in actual microgravity (Fig. 1B). In all bed rest models, body weight is not lost, but rather the gravity force vector is transferred from the head to feet direction to the chest to back direction (Fig. 2).

It is noteworthy that normal daily activity on Earth involves about 16 h of upright activity, which includes mostly sitting, walking, standing, and various forms of exercise. The remaining part of the 24-h day is approximately 8 h of sleeping without axial loading. Unfortunately the horizontal or HDT bed rest position does not exactly reproduce the weightlessness of space flight. For example, more of the surface area of the body is compressed than in space because the greater contact of the body with the bed during the entire 24-h bed rest period. In
actual microgravity, external compression of all body surface areas is minimal, whereas bed rest generates more compression of tissues over a greater surface area of body area. This greater compression increases tissue pressures and probably dehydrates areas of weight bearing because of greater interstitial flow into the microcirculation (43). Another feature of bed rest is that volunteers are allowed to prop themselves up on one elbow to eat/drink, which alters the neck and head loading from both space and upright posture on Earth. Moreover, exercise, showering, and body waste management activities must be performed in a strict horizontal or HDT posture to maintain better the fidelity of the simulation to the microgravity of space. In the horizontal or HDT posture, bones, most muscles, and the heart work much less against the force of gravity in the vertical direction (Gz) compared with normal upright activity on Earth.

Long-duration HDT bed rest studies for countermeasure testing should be designed to increase statistical power while reducing numbers of human subjects. Usually one group of volunteers is the countermeasure group, such as an exercise cohort, whereas the other group is a pure microgravity, control group. Both groups must be exposed to the same conditions of HDT bed rest (diet, activities, and environmental conditions) so that the only difference between the two groups is the experimental countermeasure. Currently, the most common design is to include different subjects in each group. Two possible strategies to improve statistical power are possible. The first is to use the same subjects in two separate bed rest campaigns, separated by sufficient time so that the subjects recover completely from the first bed rest study before starting the second bed rest study. In this regard, bone studies should be separated by as long as 1 to 2 years for complete recovery to occur. An even more powerful strategy is to use identical twins so that environmental and genetic conditions for the twins will be nearly identical, except that one twin will undertake the countermeasure while her/his sibling will serve as the control subject (40). Under these conditions, the identical twin pair can be housed in the same room so that environmental conditions are also identical except for the countermeasure treatment.

Future long-duration bed rest studies should emphasize international cooperation and development and testing of integrated countermeasures for deep space missions. International bed rest studies should be encouraged to reduce costs and promote international collaborations and cooperation, espe-

Fig. 1. Hypothetical arterial blood pressures (mmHg) and tissue fluid accumulations on Earth and in actual microgravity, horizontal bed rest and 6 degrees head-down-tilt bed rest. A: while upright on Earth (left) and during microgravity (right). B: while during horizontal bed rest (top) and 6 degrees head-down-tilt bed rest (bottom).

Fig. 2. Gravity vectors on Earth, in HDT bed rest, and in space. A: upright posture on Earth provides a 1 Gz vector from head to feet with about 10% body weight (BW) at neck level, about 50% BW at the center of mass (lumbar spine) and 100% BW at the bottom of the feet. B: in 6 degrees head-down-tilt bed rest, the Gz vector is mostly lost, so that the body adapts to a Gx vector from anterior to posterior when supine. C: astronauts experience no significant gravity vector and the body adapts to microgravity conditions.
cially for costly space missions. International bed rest studies may offer opportunities to optimize pre- and post-bed rest testing of functionally important parameters as well as conditions of selection, nutrition, pre-bed rest activities, and general care of volunteers. Future countermeasures for long-duration missions, for example an international mission to Mars, will probably require an integrated and international approach to protect many physiological systems concurrently. Development and testing of physiologic countermeasures will be aided by long-duration bed rest studies through evaluation of devices and strategies that protect multiple physiologic systems simultaneously. In this regard, criteria for such evaluation should include crew member safety and use of devices with low mass, low volume, low power, minimal crew time, and low nutritional cost.

Tests used to evaluate countermeasures depend on the investigative team’s preferences, but the selection and order of tests is critical to the success of the HDT bed rest study. Pre- and post-bed rest tests should be scheduled so that the tests match the importance of functional parameters postflight. For example, an orthostatic tolerance test should precede a test of bone integrity because the former parameter recovers more rapidly than the latter. HDT bed rest studies must also avoid confounding effects of one test affecting the outcome of another test. For example, the collection of a leg muscle biopsy may adversely affect the outcome of an aerobic capacity or other performance test for one or more days.

In this review, we provide a critical assessment of long-duration HDT bed rest (1 mo and longer) as an analog to microgravity. It is important to note that physiologic systems such as bone, muscle, and cardiovascular are not independent and affect each other. For example, microcirculatory flow is critical to musculoskeletal growth, function, and repair and skeletal muscle tension maintains bone remodeling and the tendon insertion site. Many previous HDT bed-rest studies have investigated bone adaptation and bone countermeasures to simulated microgravity, and therefore our review will emphasize this dynamic tissue that is well-adapted to weight bearing on Earth. Studying bone adaptations in bed rest is also important for clinical cases of osteoporosis and aging research (119). In addition to bone, we also briefly review other important microgravity-related systems and parameters such as skeletal muscle, headward fluid shifts, spine, and physical performance. Finally, we include discussion of countermeasure effectiveness and recovery capacity after bed rest.

### Bone Changes during and after Long-Duration Bed Rest

Initial investigations of bone loss used horizontal bed rest with the view that this model represented the closest approximation to inactivity and minimization of hydrostatic effects for a ground-based analog of microgravity (96). Most subsequent studies employ HDT bed rest to allow simultaneous studies of cardiovascular parameters such as central venous pressure, orthostatic tolerance, and headward fluid shifts. However, as emphasized later in this review, that some features of vascular pressures, fluid shifts, and transcapillary fluid exchange in actual microgravity are probably not well stimulated in the HDT bed rest analog (42).

### Bone Mass and Structural Parameters

Although bed rest alters Gz, the microgravity of space flight totally abolishes gravitational stress. Similarities are observed between the bed rest and actual microgravity (Table 1), and both conditions are characterized by diminished bone mineral density (BMD), alteration in bone architecture as measured by quantitative computed tomography (QCT), increased calcium in urine, and increased risk of renal stone formation, even if reported changes are often more rapid during space sojourns than with bed rest (82). However, the bed rest model has obvious relevance for studies of bone alteration and provides the most robust analytical measurement techniques for the quantification of bone changes not only before and after bed rest but also while lying in bed. As for spaceflights, these

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### Table 1. Comparison of osteoporosis with elderly as a reference, and bone alterations due to long-term bed rest (≥1 mo) and long-term spaceflights (4–6 mo)

<table>
<thead>
<tr>
<th>Geriatric ages</th>
<th>Bone Adaptation to Bed Rest</th>
<th>Bone Adaptation to Space*</th>
</tr>
</thead>
<tbody>
<tr>
<td>35–55 yr</td>
<td>Men = Women †</td>
<td>35–55 yr</td>
</tr>
<tr>
<td>More serious in women</td>
<td>Trabecular adaptation might differ</td>
<td>Men = Women†</td>
</tr>
<tr>
<td>Systemic</td>
<td>Not systemic</td>
<td>Not systemic</td>
</tr>
<tr>
<td>↓ aBMD (DXA)</td>
<td>↓ ↓ aBMD (DXA)</td>
<td>↓ ↓ aBMD (DXA)</td>
</tr>
<tr>
<td>approximately 1%/yr</td>
<td>approximately 0.3 to 1%/mo</td>
<td>approximately 0.4 to 3%/mo</td>
</tr>
<tr>
<td>↑ Trabecular density</td>
<td>↑ ↓ Trabecular density</td>
<td>↑ ↓ Trabecular density</td>
</tr>
<tr>
<td>↑ Anisotropy of trabeculae according to g vector</td>
<td>↑ ↓ Cortical density and thickness</td>
<td>↑ ↓ Cortical density and thickness</td>
</tr>
<tr>
<td>↓ Cortical density, area and thickness</td>
<td>- Increased resorption</td>
<td>- Increased resorption</td>
</tr>
<tr>
<td>↑ Cortical porosity</td>
<td>- Unchanged or slightly decreased bone formation</td>
<td>- Unchanged or slightly decreased bone formation</td>
</tr>
<tr>
<td>Irreversible if not treated at trabecular and cortical compartments</td>
<td>- Tibia cortical bone recovers</td>
<td>- Femoral neck bone size recovers, but not its BMD after 1 yr</td>
</tr>
<tr>
<td>- Tibia trabecular bone does not recover after 1 yr</td>
<td>- Tibia cortical and trabecular parameters do not recover 6 mo after a 6-mo space mission</td>
<td></td>
</tr>
</tbody>
</table>

†, Lack of statistical power; ?, unknown; ↓, ↓, ↓, ↓, ↓, ↓, decrease: from slight to more severe; ↑, increase; aBMD, areal bone mineral density measured by DXA (dual energy X-ray absorptiometry). *Space missions with use of the Advanced Resistive Exercise Device or with bisphosphonate therapy are not considered.
measurements are also feasible before and after flight, but often not during the mission because of obvious technical constraints. The ultimate goal of quantitative bone analyses is the assessment of the recovery of bone loss and its possible combination with age-related loss that should prematurely increase risk fracture in later life. If BMD accounts for 65% of the variation in bone strength, inclusion of indexes of bone geometry and microarchitecture improves this prediction of bone strength to 94% (22).

In the 1980s, iliac crest bone biopsies were performed, allowing investigation of bone structural and bone remodeling cellular parameters (Fig. 3). The first study of iliac crest biopsies from male volunteers before and after 120 days of HDT bed rest in USSR (120) included five groups: control ambulatory, continuous bed rest, bed rest with physical training, bed rest with bisphosphonate, and bisphosphonate plus physical exercise. Trabecular bone volume remained constant in each group. Cellular activities showed that in continuous bed rest, the osteoblastic mineral apposition rate decreased and osteoclastic resorption increased. Later it was shown, in the same study, that although cortical and trabecular bone volumes were unchanged in volunteers without any countermeasures, the bone trabeculae were less numerous but the remaining ones were thicker, suggesting that bone architecture is affected earlier than bone mass (25, 80). An 84-day bed rest (127) confirmed the absence of bone volume changes at the iliac crest level by histomorphometry along with increased trabecular active osteoclastic surfaces and increased eroded surfaces at the cortex. The longest HDT bed rest experiment ever conducted lasted 370 days and involved eight men (111). Three subjects were treated with bisphosphonate combined with exercise for the entire study period and underwent transiliac bone biopsies at baseline and at the end of the bed rest. The other five subjects experienced 120 days (7 wk) of bed rest without countermeasures, followed by 250 days of bed rest with the exercise regimen. These subjects had biopsies at baseline, day 116, and day 366 at alternating sides of the ileum. The group with 120 days of HDT bed rest without countermeasures had decreased bone volume (−6.3%, \( P = 0.046 \)) and a trend to lower trabecular number (−10.2%, \( P = 0.080 \)). Taken together, these histomorphometric papers suggest that trabecular bone changes at the iliac crest are visible between 3 and 4 mo of continuous bed rest in healthy men. It is plausible that changes in bone microarchitecture such as a trabecular perforation occur, compensated by a later increase in trabecular thickness that somehow preserves bone mass but not bone strength (1). Moreover, only one histomorphometric study (25) investigated cortical bone, showing no alteration in thickness and porosity after 120 days.

Most skeletal changes during bed rest are assessed by noninvasive techniques, the most popular being dual X-ray absorptiometry (DXA), the standard for assessing fracture risk and diagnosing osteoporosis through the measure of areal bone mineral density (aBMD, g/cm²) (Fig. 3). In experiments of either 20 days horizontal bed rest (109) or 35 days (57), no DXA changes were reported, whereas muscles had already deteriorated. In a 42-day antiorthostatic bed rest study (116), no significant alteration of aBMD was detected, although trends toward decreases in lumbar vertebrae and lower extremities were seen. As a part of the NASA Flight Analogs Project,

![Fig. 3. Proposed synthesis of bone changes after 2–3 months bed rest duration (except when known). A: DXA areal bone mineral density (aBMD, mg/cm²) changes versus baseline (59, 105, 116, 127). B: histomorphometry of iliac crest biopsies (111, 120, 127). C top: quantitative computed tomography, peripheral (pQCT, BMC in mg) (87); C, bottom: high-resolution-pQCT (HR-pQCT, BMD, bone mineral density in mg/cm³) (3, 15).](http://jap.physiology.org/)
13 healthy volunteers (8 men and 5 women, aged 35.5 ± 9.6 yr) participated in a 6-day HDT bed rest for periods of 47, 60, or 90 days (105). No changes at the radius were seen whatever the time point, but decrements in aBMD were observed beginning at 47 days at the hip and later at the pelvis, both worsening with time up to the 90-day end point, thus indicating that in the same subjects measured changes are seen in long-duration bed rest. In an American 120-day horizontal bed rest study, LeBlanc and coworkers (59) showed that aBMD decreased with a gradient from −1.4 at the lumbar spine to −10% at the calcaneus compared with baseline. In the 84-day bed rest study, Zerwekh and coauthors (127) found that in addition to histomorphometric results, DXA revealed a significant aBMD decline at the hip trochanter (−3.8%). The upper extremities again do not display bone loss, and there are reports of increased BMD in the skull (59, 116). For example, mice display increased parietal bone volume after 15 days in space (128). Possible reasons may include increased microvascular flow to the skull, redistribution of minerals from the lower to the upper body because of a headward fluid shift and elevated intracranial pressure.

To reconcile these observations with the trabecular bone volume decrease in iliac crest biopsies seen beyond 84 days, it can be argued that iliac crest has a limited weight bearing function compared with the proximal femur. During the last two decades, HDT bed rest studies have also used peripheral quantitative computed tomography (pQCT) to evaluate the tibia from its distal region (mainly composed of trabecular bone) to the proximal diaphysis (19, 85, 87, 88) with assessment of bone area, bone mineral content, and volumetric density (Fig. 3C). Bed rest studies of 60 and 90 days showed that the mean pQCT bone mineral content decrease was systematically greater in diastolic trabecular bone than in cortical diaphysis with cortical loss starting from the beginning of bed rest and being mostly linear over time. On the other hand, trabecular loss is initially delayed but becomes aggravated with longer immobilization (24).

Two 60-day HDT bed rest studies, one in men (15) and one in women (3), offered the opportunity to investigate changes in radius and tibia bone structure with high resolution pQCT (HR-pQCT, 82 μm isotopic voxel size) (Fig. 3C). In both sexes, the strongest effects were seen at the distal tibia with expected losses in volumetric BMD as well as reductions in cortical area and thickness. At the tibia trabecular compartment, men and women displayed reduced trabecular number, but increases (in men) and no change (in women) in trabecular thickness were reported. This finding in men is similar to that made by histomorphometry at the iliac crest (80). Whereas no change in radius aBMD is previously reported (59), HR-pQCT shows deterioration of the radius at the cortical level in men (15) and at the trabecular level in women (3), suggesting a risk for non-weight bearing bones. At this stage we need to take great care in the interpretation of these sex-specific differences, because individual variability exceeds the variability between sexes (3, 83). Furthermore, by combining data from five HDT bed rest studies (n = 50 men, 24 women) of 14-90 days, Morgan and coworkers (72) report that as far as aBMD, bone biochemistry, and renal stone risk are concerned, men and women do not have different skeletal responses with bed rest.

In summary, we can make the following observations: 1) at weight-bearing sites, aBMD loss gradually increases in the following ascending order: lumbar spine, pelvis, and lower legs, and thus these sites are more at risk when unloaded than upper extremities and thoracocephalic areas; 2) bone alterations are detectable at the hip as early as 47 days of bed rest and these alterations worsen with time; 3) both leg cortical and trabecular bone compartments are altered but not in the same way or with the same kinetics; and 4) analysis of non-weight bearing bones (radius) is necessary during long-term bed rest studies because HR-pQCT detects anomalies (3, 15), perhaps related to the relative inactivity of arms during bed rest compared with greater arm activity during actual microgravity. Overall, the long-term bed rest analog is a required model to evaluate determinants of bone loss during the supine and HDT postures that can help to predict/quantify and understand changes in bone that might occur with space flight. Such investigations are difficult with spaceflight using current technology, and inflight resources limits them to pre- and post-flight measures, except in the case of studying calcium kinetics and biomarkers. It is possible that such alterations occur sooner inflight (121), despite the fact that crewmembers are participating in countermeasures (all astronauts and cosmonauts currently are required to participate in some form of exercise).

Bone Cellular Activities during Bed Rest

Most HDT bed rest studies have detected an increase in osteoclastic activity, directly in iliac crest biopsies (120, 127) or indirectly from an increase in calcium urinary excretion and diminished intestinal calcium absorption. Recently, such changes were monitored using urinary markers (pyridinolines, representative of bone and cartilage collagens), and more specific bone markers either in urine or serum (deoxypyridinoline, DPD; C- or N-terminal telopeptide, CTX or NTX; tartrate resistant acid phosphatase, TRAP5b). Urinary calcium excretion increases as early as the first day of bed rest, and urinary CTX and NTX excretion significantly increases on the second day of bed rest (6, 44). Such increases in urinary calcium along with diminished urine volume (38, 58, 72) bring concerns about the risk of renal stone formation (47, 69, 79, 122, 126). It is not yet clear whether bone resorption activity increases with time of inactivity (72, 116) or whether a plateau is reached around the 20th day of bed rest at 50–150% above pre-bed rest values (48, 101). Of note, urine phosphorus excretion follows a similar trend as calcium but to a lesser extent (29, 127). Mineral intakes are typically included in the recommended diet intakes (82); thus it is believed that inactivity-induced bone loss is the main factor responsible for stimulation of bone resorption. In contrast, bone formation markers (osteocalcin, bone ALP, type I procollagen propeptide) display either no changes or slight decreases (3, 12, 48, 64, 116). However, it should be noted that osteoblastic activity (57, 120, 127) is decreased in the iliac crest. The release of calcium from bone is expected to suppress parathyroid hormone (PTH), which in turn is associated with a drop in circulating 1,25-dihydroxyvitamin D. This is indeed what occurs in some but not all bed rest studies (6, 59, 72, 100, 102, 103, 116, 118). When present, these hormonal changes are likely to occur in response to bone cellular activity alterations. Recently a new technique that measures the ratios of natural calcium (Ca) isotopes has been used during bed rest (73, 93, 99). Bone has a natural Ca isotope composition that differs from the other organs of the body, i.e., depleted in the $^{44}$Ca isotope relative to the $^{42}$Ca isotope. When
the bed rest-induced bone mineral balance is negative, the blood, and ultimately the urine, both have a bone-like isotopic signature where the Ca isotope ratio shifts in parallel with NTX changes (101). Ultimately, the added value of Ca stable isotopes should be linked to the hypothesis that their variations precede that of other biomarkers, such as NTX.

The activity of osteoblasts and osteoclasts are orchestrated by osteocytes, the terminal differentiation of osteoblasts and the most abundant cells embedded in the bone matrix (76) that produce key molecules, such as bone-formation inhibitor sclerostin and dickkopf-1 (DKK1) (7, 12, 117) and receptor activation of nuclear factor-kB ligand (RANKL) to support osteoclastogenesis (124). Recently it was reported that sclerostin and DKK1, whose production is inhibited in mechanically stimulated bone (89), are elevated during 60 and 90 days of bed rest (104). In the same study (104) no change was seen in serum levels of RANKL, nor of osteoprotegerin (OPG), the soluble receptor of RANKL. However, the RANKL/OPG pathway primarily affects the cellular level, which may not be adequately reflected in serum (124).

In summary, bone resorption markers are elevated early and continuously during bed rest. Bone formation markers display mild or no changes with long-duration bed rest, suggesting that bone formation activity is either unaffected or is bone site-specific and thus, does not produce major changes at systemic levels. Furthermore, the osteocyte’s canaliculi communicate with cells at the bone surface and vascular capillaries, providing a mechanism to ensure mechanotransduction and endocrine functions, still to be investigated in bed rest. Indeed, it is now established that bone cells are involved in the regulation of energy metabolism. For example, the osteoblast-produced hormone osteocalcin, a vitamin K-dependent carboxylated protein stored in the bone matrix and released by bone resorption in its undercarboxylated form, the active hormonal form (97), is able to increase insulin sensitivity of muscle and white adipose tissue to promote glucose homeostasis and is also able to favor testosterone biosynthesis (51). No striking changes occur in undercarboxylated osteocalcin nor in vitamin K status during bed rest in any of the three studies reanalyzed by Zwart and associates (132). However, a recent study supports osteocalcin-mediated crosstalk between the skeleton and energy metabolism because 60-day HDT bed rest causes a subclinical “diabetes-like” state, and plasma osteocalcin is significantly associated with serum insulin, low-density lipoprotein, and leptin (125).

The Forgotten Recovery Period for Bone: Facts, Efficiency of Countermeasures, and Reversibility

As with exposure to space flight, the recovery process from bone loss is much longer than the time in bed rest (Fig. 4) and estimated as 5 to 6 times longer than the rate of bone loss (60). In the Women’s International Space Simulation for Exploration (WISE) female study (19), a 3.5% decrease in total hip aBMD seen after 60 days HDT bed rest in the control group progressively recovered over the 1-year follow up. However, the distal tibia trabecular BMD, which diminished by 3% during bed rest, did not recover during this time period. On the other hand, calf muscle cross-sectional area, which declined by more than 20% at the completion of bed rest, recovered fully by the third month of reambulation. A relatively rapid return of muscle strength, coupled with continued depression of bone density is also seen after hindlimb unloading in mature male rats (2). It is assumed that the discordant kinetics between leg muscle and bone increases the risk for skeletal injury during recovery. In the WISE female study, distal tibia HR-pQCT (3) showed that the 2.4% lower trabecular BMD at the end of bed rest displayed two distinct phases during the reambulation period. First, there is a recovery trend up to 6-mo of reambulation (up to −1.2% vs. baseline). Then from 6 mo to 1 year the slope reverted and BMD decreased to −2%. In a 60-day HDT bed rest study of men (15), HR-pQCT data profiles were similar to those for the 60-day HDT bed rest study of women, i.e., trabecular BMD diminished by 1% at the end of the bed rest and linearly increased after bed rest for 3 mo when full recovery was attained. However, from 3 mo up to 2 years post bed rest (measurement points at 90, 180, 360, and 720 days), HR-pQCT decreased again, reaching −1% at 720 days. In both men (15) and women (3), tibia cortical thickness began to recover after 90 days and was back to pre-bed rest values between 180 and 360 days. In contrast to women who displayed no alteration in tibia cortical BMD, significant reductions post bed rest persisted for up until 180 days in men (15). The study of Cervinka and coworkers (24) that reanalyzed pQCT bone data from three previous bed rest studies and one unilateral lower limb suspension study showed that distal tibia deterioration at cortical and to a lesser extent, trabecular bone continues after reambulation, culminating at the end of the first month. Consistent with HR-pQCT data, trabecular density does not return to pre-bed rest values 1 year after reambulation.

Overall, tibia changes upon reambulation can be categorized by two phases. In cortical bone, the early first-month phase is marked by further deterioration. Then recovery begins and seems fully completed within a year. In trabecular bone, an early and slow bone loss is followed by a recovery (15, 85) that is seen within the first months. Surprisingly a third phase is observed with HR-pQCT, characterized by a slope reversal reaching significant bone loss after 3 mo in men (15) and 6 mo in women (3). These results cannot be attributed to age-related bone loss in view of the age of the volunteers (20–45 yr old for men, 25–40 yr old for women).

This general picture should be further investigated by more frequent and longer time-course monitoring to (I) define better the time lag before the recovery process becomes effective and
how kinetics between cortical and trabecular compartments differ, and 2) check whether the lack of full recovery or even the late bone loss seen by HR-pQCT in both men and women is an established fact. Lastly, we acknowledge that all these assertions originate from a relatively small sample size of bed rest subjects where great interindividual variations are present. Because large interindividual variations occur in space crewmembers as well, the reasons for these variations still remain to be elucidated.

Regarding bone remodeling, remeasurement reverses the effects of bed rest on bone resorption indexes within the first month, whereas bone formation markers, only slightly affected during bed rest, are elevated 1 to 3 mo after bed rest (12, 58, 74, 86). An increase in PTH along with a slight decrease of serum calcium (slightly but significantly increased during bed rest) up to the third month post bed rest is reported (12, 86). Very few studies have investigated these markers beyond 3 mo. The calculation of an uncoupling index shows that bone resorption and formation activities are balanced before bed rest, unbalanced in favor of resorption during bed rest and unbalanced in favor of bone formation during 20-days of recovery (116). The bone remodeling period is about 17 wk (resorption 2 wk, reversal 2 wk, and formation 13 wk) (28), and this timeframe is compatible with the lag of full recovery of bone 3–6 mo after remeasurement. However, the third phase, characterized by new radius or tibia trabecular bone loss, remains unexplained.

Countermeasures Efficiency for Bone

Different types of countermeasures have been tested such as antiresorptive pharmaceutical treatments, mechanical challenges, or dietary manipulations. Considering the rapid elevation of bone resorption, it appears possible to consider short-term bed rest studies as a first step for the evaluation of countermeasures effectiveness using biomarkers (44). After 90 days of bed rest, a single injection of 60 mg pamidronate 14 days before the start of bed rest almost completely prevented the reduction in BMD of the proximal femur (122) and mitigated tibia diaphyseal BMC reduction (87). Bed rest also promotes crystallization of Ca salts and may induce renal stone formation (47). Ruml and coworkers (91) report that alendronate prevents hypercalciuria and crystallization of Ca salts induced by a 3 wk of bed rest, and Zerwekh and associates (126) document lower renal stone risk by potassium-magnesium citrate during 5 wk of bed rest. Antiresorptives also appear to prevent the development of renal stones during spaceflight (78). In the USSR 120-day bed rest study with iliac crest biopsies, etidronate treatment decreased bone osteoclastic and osteoblastic activities (120). Furthermore, if etidronate normalizes osteoclast number in the trabecular compartment of the iliac crest, osteoclasts in the cortex are unaffected (25). Given the uncoupled bone remodeling, redifining the dose or use of new antiresorptive drugs (92) to avoid inhibition of bone formation (63) should be studied. In addition to suppression of bone turnover, other side effects included gastroesophageal irritation as seen in 2 of 10 crewmembers under weekly alendronate (56). Vascular complications in the oral cavity using bisphosphonates during bed rest should be considered in the safety evaluation for potent antiresorptives (84). Finally, osteanabolic drugs such as Teriparatide and monoclonal antibodies against sclerostin prevent bone loss in immobilized or unloaded animal models. It is possible that these anabolic agents will be good alternatives for immobilization osteoporosis. However, taking drugs might not please all bed rest volunteers or crewmembers. Interest in the use of mechanical strategies that benefit all body systems and target specific bone regions are still of high priority in future long-term bed rest and spaceflight studies.

The effects of bed rest on bone are region specific with weight-bearing regions (e.g., hip, tibia) more affected than lower weight-bearing regions (e.g., arms). Thus a supine exercise regimen during bed rest should be designed to address these regional differences. Various aerobic exercises [e.g., treadmill running within lower body negative pressure (LBNP) or cycle ergometry], resistive exercise, or a combination of both have been tested (98, 102). Resistive exercises are also performed with feet placed on vertical leg press or on an ergometer such as the flywheel. LBNPex alone (100) and LBNPex with resistive exercise (102) both help maintain bone. However, most aerobic exercises do not mitigate bone loss, whereas resistance exercises appear more efficient in preventing bone loss fully or partially, depending on the regimen load and bone site (52, 87, 98). This beneficial effect is associated with increased bone formation, whereas bone resorption remains elevated. The same is found when resistive and aerobic exercises are combined (102). Recent studies performed during 56 days or 60 days of bed rest (4, 14, 85) have added side alternative, whole body vibration to resistive exercise. This combined resistive and vibration exercise regimen reduced bone loss better than resistive exercise alone at least at the distal tibia (14). Interestingly, this combination enhances bone recovery for up to 3 mo after bed rest (14). In the 60-day bed rest period, an increase in vertebral bone marrow adiposity is documented by MRI in men (115) and women, where it persists 1 year after cessation of the inactivity (55). This increase is attenuated in men but not in women who undertake resistance exercises with or without whole body vibration (115). Of note, the fat increases in hematopoietic bone marrow may have important physiological implications not only on bone but also on hematopoietic metabolism, immunity/inflammation, as well as on energy metabolism and thermogenesis (55). We thus have evidence that combining high-load resistive exercise with whole body vibration are beneficial for bone and bone marrow. Nevertheless, further optimization and investigation are needed to improve exercise parameters and training such as frequency of loading, work load, rate, rest period, and specific exercise "dose" for each individual.

Intuitively, restoring appropriate g-vector through artificial gravity may offer the best long-term approach. However, technical challenges and safety concerns must be taken into account. Use of a short-arm centrifuge must overcome several negative side effects, mainly Coriolis effects, motion sickness, dizziness, inability to exercise normally, and possible effects on cognitive or motor function. Use of a long-arm centrifuge may obviate many of these concerns, but long-arm centrifuges are difficult to implement in spacecraft (60). In humans, only daily short-arm centrifugation for 1 h at 2.5 g (Gz at the feet) has been tested during 20 days bed rest and does not prevent bone loss (103), even when combined with ergometric exercises (49). In HDT bed rest, there is a headward fluid shift (81, 118), although the fluid shift is not exactly the same as in...
Review

Long-Duration Bed Rest • Hargens AR et al.

microgravity (42). Thus hypergravity gradients probably induce blood pooling in lower extremities that might contribute to increased dependent vessel volume. Furthermore, higher capillary density and enhanced microcirculation are reported after “gravitational therapy” with a short-arm centrifuge at 1.5–3 Gz in humans with fractures or osteomyelitis (53). In rats, 1 h of daily centrifugation at 1.5 or 2.6 G does not prevent tail suspension-induced bone loss (130). In mice, 21 days of continuous hypergravity targets trabecular bone and its vascularization with a threshold benefit to bone at 2 G and with deleterious effects at 3 G (37). Thus hypergravity holds promise but an effective intermittent regimen of centrifugation (magnitude of G-load and exercise workload) is still unknown.

If mechanical loading is a key mechanism of bone adaptation, a balanced diet, including key ingredients such as proteins, calcium, vitamin D, and various potent micronutrients or specific fatty acids, seems essential (110). Whether an increase in calcium intake has a beneficial effect during short-term bed rest has been investigated in a metabolic ward under tightly controlled study conditions (5). Results indicated no prevention of increased bone resorption. Dietary protein supplementation did not appear to impact bone status (3) in bed rest. The efficiency of such countermeasures for preventing bone loss might be questionable when the baseline diet nutrient intake is already adequate (106). Because space crewmembers have high sodium intake with concomitant low-grade metabolic acidosis, Frings-Meuthen and coworkers (35) compared one group with high NaCl intake with another group having low NaCl intake on calcium and bone metabolism during 14 days bed rest. They found that high NaCl intake enforces disuse-induced bone and muscle losses by causing further protein wasting and increased bone resorption.

Pharmaceutical or resistance exercise coupled or not with other types of interventions (i.e., vibration, nutrition) have led to progress in the prevention of bone loss during bed rest. However, optimization is still required to ascertain that all bone sites can be preserved. Logical next steps will test a combination of effective therapeutic exercise paradigms, nutritional supplementation, and pharmacological agents to prevent bone loss. A more frequent and long-term, post-bed rest follow-up period is necessary because different phases of skeletal alteration are observed with trabecular loss present even after 2 years. Lack of full trabecular BMD recovery at the hip is also seen in 8 astronauts, 2–4.5 yr after 6-mo spaceflights (23). Thus 60–90 days of long-term bed rest act as useful analog for 6 mo spaceflight, being able to replicate bone deterioration sufficiently to study bone recovery.

Bone Responses to the Analog of Long-Term Bed Rest and Future Directions

The temptation to link bone deterioration to the sole stimulation of bone resorption and thus to believe that blocking this stimulation will prevent any further bone loss seems too simplistic. We still have only a partial understanding of the kinetics of bone cellular activities (osteoclasts, osteoblasts, osteocytes) during and after the bed rest and their relationship with other physiology systems. Such knowledge is needed to help future studies using pharmaceutical or other intervention approaches to define a therapeutic course with clear outcomes. Such studies will also help to decipher how long bed rest duration is required. However, failure of recovery or even late post-bed rest bone loss poses safety concerns for elevated long-term health risks associated with an altered or accelerated trajectory of bone loss with aging. These concerns strongly suggest the need for long-term bed rest studies and long-term post bed rest follow up.

Skeletal Muscle Changes during Long-Duration Bed Rest Compared with Actual Microgravity

As with bone, skeletal muscle is another, but often secondary, focus of space flight and analog bed rest studies. The first muscle biopsies for actual microgravity were obtained from vastus lateralis before and after Space Shuttle flights of 5- and 11-day durations. In one study by Zhou and associates (131), the results were highly specific to individual crew members and suggested a rapid transformation of Type I (slow) to Type II (fast) fibers. In a second study, Edgerton and coworkers (31) documented that some fast fibers were more susceptible to fatigue after microgravity exposure. Furthermore, capillary densities were 24% lower after flight. Overall, these biopsy findings indicate that fiber adaptations for metabolic properties, size, and vascularity occur rapidly in microgravity and that these adaptations are similar to those in rodents after actual microgravity or hindlimb suspension. For longer duration missions of about 6 mo on the International Space Station, Fitts and coworkers (33) documented major losses of fiber mass, force, and power in the following order: soleus Type I > soleus Type II > gastrocnemius Type I > gastrocnemius Type II. These fiber type transformations associated with actual space flight are confirmed by the same and other investigators for short- and long-duration HDT bed rest studies (21, 54, 77, 90, 114).

Short-term bed rest provides interesting insights into muscle physiology and possible effects of fluid shifts on skeletal muscle morphology. For example, even 24 h of HDT bed rest increases neck muscle cross-sectional area, whereas calf muscle cross-sectional area is decreased (27), but these alterations are seemingly are not related to muscle contractions or changes in water content. Morphological features of skeletal muscle atrophy occur more rapidly than bone loss during short-term HDT bed rest, so bed rest periods as short as 10 days to 2 wk are conducive to studies of skeletal muscle adaptation to non-weight bearing and countermeasure development. It is beyond the scope of this minireview to cover the fields of skeletal muscle-bone interactions and skeletal-muscle adaptations to bed rest and microgravity comprehensively, so readers are referred to recent reviews of these topics (32, 33, 110, 119).

Shorter-duration HDT bed rest also offers the opportunity to test hypotheses and countermeasures for skeletal muscle before they are ready for a space flight experiment. For example, sex differences related to muscle atrophy and countermeasure efficacy can be explored comparing male and female subjects with adequate sample size during bed rest. Again, this is important because it is difficult to get high numbers of crew members in flight experiments and even more difficult to compare women to men in flight because women in all international space agencies comprise only 10–20% of space flight crews. The 60-day HDT bed rest study (WISE), although a longer-term, was unique in terms of a collaboration between multiple international space agencies to study muscle loss and...
to test a nutrition protocol versus the combination of an aerobic and resistive exercise countermeasure (30, 39, 62, 95, 102, 107). The WISE study also allowed comprehensive mRNA analyses of skeletal muscle by Chopard and colleagues (26) of exercise, nutrition, and control women (pure HDT bed rest) groups, a precursor to future space flight genomic investigations. Moreover, innovative single muscle fiber function tests by Trappe and coworkers (113) found that the combination of flywheel resistive exercise and treadmill exercise within lower body negative pressure was a highly effective countermeasure, whereas a nutritional intervention was not. The WISE study of women also allowed comparisons to many other studies of men for HDT bed rest periods of 60 days and more (4, 11, 18, 20, 68, 75).

Headward Fluid Shifts and Vision Impairment during Long-Duration Bed Rest compared with Actual Microgravity

Much attention has recently focused on headward fluid shifts and their possible contribution to vision impairment observed in crew members exposed to prolonged space flight (66, 129). The importance of the loss of hydrostatic blood pressures and tissue weight in microgravity is gaining increasing importance in understanding microcirculatory Starling pressures (81), transcapillary fluid shifts, facial edema, and possible elevation of intracranial pressure. In microgravity, loss of tissue weight in the brain probably alters the Starling-Landis equilibrium toward greater fluid flow from blood into tissue. This transcapillary fluid filtration along with the loss of gravitational emptying of cranial and jugular veins may cause a postulated increase in intracranial pressure (129). Because loss of tissue weight seen in space does not occur in any microgravity analog on Earth, long-duration HDT bed rest is probably not a good analog for studies of vision impairment. For example, no long-duration HDT bed rest study has reported impairment of vision to our knowledge. On the other hand, short-duration HDT bed rest studies with elevated CO₂ as well as tests of intracranial and intraocular pressures are worthwhile to develop and test countermeasures such as lower body negative pressure (65) and Braslets to sequester blood and tissue fluids in the lower extremities. In fact, current studies on the International Space Station are using the Chibis Suit to generate lower body negative pressure in an attempt to counteract the headward fluid shift and possible elevations in intracranial pressure.

Spine and Intervertebral Disc Changes during Long-Duration Bed Rest compared with Actual Microgravity

Short- and long-duration HDT bed rest studies have examined the cause of low back pain in space and the higher incidence of cervical and lumbar disc herniation post-flight (9–11, 13, 16, 17, 50, 94). Although back pain is commonly observed in early phases of HDT bed rest and appears similar to that in microgravity (46), there are substantial differences between the changes present with bed rest and microgravity. Even spinal traction was used in an unsuccessful attempt to elongate the body and spine to the same degree as reported for actual microgravity (108). Recommendations for understanding the high incidence of intervertebral disc herniation post-flight and future research were recently reviewed by Belavy and colleagues (8). Previous findings from bed rest document that lumbar intervertebral discs swell and increase in height, but recent in-flight ultrasound results and pre- and postflight MRIs show no increase in lumbar disc heights (S. Dulchavsky, D. Ebert, K. Garcia, A. Sargsyan, J. Lotz, D. Chang; personal communications). In fact, cervical discs seem to lose height during microgravity exposure. On the other hand, the muscle atrophy and delayed recovery of paraspinal muscles in space is well documented in long-duration HDT bed rest studies (9, 11).

Reduced Work Capacity and Other Performance Parameters during Long-Duration Bed Rest compared with Actual Microgravity

Exercise is the most important countermeasure for maintaining health and preventing deconditioning of crew members during prolonged spaceflight. However, the physiology of exercise performed in a microgravity environment is not well understood. A detailed understanding of the mode, volume, frequency, and intensity of microgravity exercise necessary to maintain health and fitness in a microgravity environment is important. Previously, aerobic exercise in space has employed cycle ergometers, rowers, and treadmills. Aerobic exercise alone, without some form of artificial gravity, does not maintain anaerobic threshold, upright exercise capacity, orthostatic tolerance, carotid arterial elasticity, or musculoskeletal mass and function after spaceflight (45, 70, 71, 112) or bed rest (39, 41, 61, 123). To date the cardiovascular benefits and impact loading of bungee-cord treadmill exercise in space are physiologically insufficient because of the lack of hydrostatic pressures in blood vessels of the body and only 70% body weight loading for the musculoskeletal system (36, 41, 67).

When applied individually, few countermeasures employed to date have been thoroughly documented as effective in preventing deconditioning during long-duration spaceflight. Recent long-duration HDT bed rest studies suggest that to prevent deconditioning in microgravity, it is necessary to perform exercise countermeasures within some form of artificial gravity to induce footward loading and to restore circulatory hydrostatic pressures. Aerobic exercise within a centrifuge restores cardiovascular function and, separately, aerobic exercise within lower body negative pressure maintains exercise capacity, cardiovascular function, and helps protect the musculoskeletal system (41). Thus long-duration HDT bed rest studies have and will continue to contribute important insights into the development and testing of exercise countermeasures to maintain work capacity and other important performance parameters for deep space missions such as to Mars.

Summary

Long-duration bed rest is widely employed to simulate the effects of microgravity on various physiological systems, especially for studies of the musculoskeletal and cardiovascular systems. Importantly, the musculoskeletal and cardiovascular systems interact with each other and should not be studied without considering this interaction. The microgravity analog of bed rest has made great strides in the development and testing of countermeasures for maintaining the health and well-being of space crews. Unfortunately, all Earth-based analogs must contend with the constant force of gravity by adjustment of the G vector. At present, HDT bed rest with normal volunteers is the most common analog for microgravity
simulation and to test countermeasures for bone loss, muscle and cardiac atrophy, orthostatic intolerance, and reduced muscle strength/exercise capacity. However, recent results suggest that the HDT bed rest analog is less reliable as an analog for other important physiological problems of long-duration space flight such as fluid shifts, spinal dysfunction and radiation hazards.

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