Skeletal Effects of Long-Duration Head-Down Bed Rest

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Introduction: Skeletal unloading during spaceflight causes regional loss of bone mineral density (BMD), primarily in the spine and lower body regions. This loss of skeletal mass could adversely affect crew health during and after spaceflight and jeopardize mission success. Bed rest has long been used as a spaceflight analog to study the effects of disuse on many body systems, including the skeleton. This study was undertaken by the NASA Flight Analogs Project (FAP) to collect control data for upcoming countermeasure studies. ***Methods:*** There were 13 subjects who participated in 42, 44, 49, 52, 60, or 90 d of continuous, head-down bed rest. DXA scans (dual-energy X-ray absorptiometry) were obtained before and after bed rest to measure changes in BMD of the whole body, lumbar spine, hip, heel, and wrist; the 90-d subjects were also scanned at the 60-d time point. Follow-up DXA scans were performed after 6 mo and 12 mo of reambulation to assess BMD recovery. ***Results:*** BMD changes were consistent with earlier bed rest and spaceflight studies, with statistically significant losses averaging 1% per month in the hip, pelvis, and heel. Recovery data were also consistent with data obtained after spaceflight. Bone biomarker data are described, and support the findings of previous studies. Specifically, the process of normal bone remodeling is uncoupled: increased bone resorption with no concomitant change in bone formation. ***Conclusion:*** The FAP appears to be a valid test bed for skeletal disuse studies, and should provide a useful research platform for evaluating countermeasures to spaceflight-induced bone loss.

Keywords: microgravity, disuse, bone mineral density, BMD recovery, bone resorption, bone formation.

BONE ATROPHY is a well-established adaptation of the skeleton to the weightlessness of spaceflight. The reduced bone mineral density in the calcaneus (16) and the increased urinary and fecal excretion of calcium (17,19) detected in the Skylab crews have historically characterized the skeleton's adaptation to its reduced mechanical function in space. Data from crewmembers on long-duration spaceflights substantiate how the hypokinesia and microgravity of space occupation induce atrophy in those regions experiencing the greatest reduction in strain (5,7,15). In other words, the reduction in bone mass occurs at skeletal sites that no longer require the structural competence to bear the mechanical loads normally experienced in Earth's gravity. Skeletal atrophy is also driven by the reduction in muscular forces as muscle atrophy is also induced by microgravity (7). It is the accelerated loss of bone mass and the potential deterioration of microarchitecture that highlights premature osteoporosis as an occupational health risk in long-duration crewmembers. Spaceflight-induced loss of bone mass may increase the risk of skeletal fractures and of renal stone formation, while the uncoupled response of bone cells during remodeling could impair fracture healing. These skeletal risks could adversely affect the ability of spaceflight crewmembers to perform an emergency egress, reduce human performance because of injury or pain, or increase the risk for fracture because of a reduction in skeletal integrity. Moreover, incomplete recovery of bone mass and irreversible changes to geometry and microarchitecture following prolonged spaceflight exposure may induce a premature onset of age-related osteoporosis in crewmembers after return.

Bone loss countermeasures are needed to prevent deleterious skeletal effects and to maintain skeletal integrity to meet the performance demands of space exploration missions. Thus, there is considerable value in developing a ground-based test bed with which to evaluate the efficacy of skeletal countermeasures for flight-induced skeletal atrophy. This paper is one of a series of reports on the NASA Flight Analogs Project (FAP), which is designed to lay the groundwork for a standard bed rest protocol. In this paper, we report skeletal data [changes in regional bone mineral density (BMD) and in biomarkers of bone turnover in response to bed rest] that were collected in the subjects participating in the FAP. These FAP data are compared to data collected in crewmembers after flight as well as with data generated from previous bed rest studies.

METHODS

Flight Analogs Project

The conditions and methods by which the FAP applies prolonged bed rest to mimic gravitational unloading of spaceflight are as described by Meck et al. (9). Bed rest and test protocols were reviewed and approved by the NASA Johnson Space Center (JSC) Committee for

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the Protection of Human Subjects, the University of Texas Medical Branch (UTMB) Institutional Review Board, and UTMB General Clinical Research Center Science Advisory Committee. Subjects received verbal and written explanations of the bed rest and test protocols prior to providing written informed consent.

In brief, normal, healthy male or female subjects were recruited from the general population or from a pool of human test subjects acquired by NASA JSC to participate in a single bed rest experiment. In general, subjects were as follows: a) between the ages of 25 and 55 yr; b) required to pass a modified Air Force Class III flight physical (described below) within 1 yr prior to the study; c) mentally fit for bed rest study as assessed by psychiatric interview and test; and d) not currently taking prescribed medication that would have interfered with physiological measurements, including hormonal contraceptives and bisphosphonate therapy. The Air Force Class III physical included a full physical exam and vital sign assessment, 12-lead ECG, vision test and audiometry, urine analysis, electrolyte panel, complete blood count with differential, test of liver function, drug screen, TB skin test, lipid panel, chest X-ray, and HIV/Hepatitis screen and H sensitivity C reactive protein. Moreover, the following list represents exclusionary criteria which included factors that may influence bone loss or issues that may be exacerbated by bed rest: a) recent substandard nutritional status; b) medical history of metabolic disease or endocrine perturbations; c) personal or family history of thrombosis; d) menopause status; e) positive pregnancy test for women; f) a body mass index outside of 21-30; g) abnormal blood or urine values; h) smoked within the previous 6 mo; and i) history of renal stones.

After admission to the Bed Rest Facility, subjects were allowed to engage in activity ad lib during the 11–14 d ambulatory period. During the periods of bed rest, subjects were in the supine position with a 6° head-down tilt for consecutive days. While in bed or on a wheeled stretcher, subjects were carefully monitored to ensure consistency and compliance to the bed rest protocol. Freedom of horizontal movement in bed was permitted, and subjects were allowed to raise their body on one elbow while eating or reading. However, subjects were not allowed to sit up or dangle their legs over the bed. Nursing staff supervised subject activity levels. Urination and bowel movements, as well as showers, were all performed while supine.

Subjects were required to consume meals in their entirety during this phase and throughout the study. During reambulation the subjects were allowed to resume activities ad lib, but due to marked tenderness of soles of the feet, many subjects were cautioned to avoid excessive walking during the first several days. Subjects remained at the facility during this ambulatory recovery period, which included post-bed rest testing and data collection.

The JSC FAP carefully controlled dietary and liquid consumption by subjects to ensure that a consistent bodyweight was maintained throughout the bed rest experiment. Reports on the FAP conditions for diet and nutrition are included in this issue (4,20). In brief, all food and fluid intake were recorded, monitored by dietary staff, and adjusted as required to avoid changes in bodyweight. Baseline caloric intake was 35.7 kcal $\cdot$ kg^{-1} bodyweight with a fluid intake of 28.5 ml $\cdot$ kg^{-1} of bodyweight. Subjects were restricted from consuming caffeine, cocoa, chocolate, tea, or herbal beverages. Other dietary intakes included: a) carbohydrate/fat/protein ratio of 55:30:15; b) sodium $<$ 3500 mg $\cdot$ d^{-1}; c) potassium 3000–3500 mg $\cdot$ d^{-1}; d) calcium 1000 mg $\cdot$ d^{-1}; and e) phosphorous 1400 mg $\cdot$ d^{-1}.

The 13 subjects whose data are reported herein had been recruited at different times and were skeletally unloaded for varying durations of bed rest. The initial experiment for the FAP unloaded three test subjects for 60 d. Subsequent to that experiment, it was recognized that the evaluation of countermeasures for bone loss required the flight analog to induce biologically meaningful skeletal changes. For this reason, the duration of bed rest unloading was increased to 90 d to induce BMD losses that were more likely to exceed the least significant change in BMD, as predetermined by the standardized procedures for evaluating the measurement precision for a DXA machine and the technologists (2). With this modification, it was also decided that all standard measures would be conducted at day 60 of a 90-d experiment, thereby increasing data collection for the evaluation of the experiment protocol. The total number of subjects that were skeletally unloaded for a specific duration of bed rest and the sequence by which the bed rest experiments were conducted are tabulated below. As previously mentioned, six subjects (#4–7, 12, and 13) participated in an extra DXA session at the intermediate time point of 60 d of bed rest. Also indicated in the table are four subjects (#8–11) who were skeletally unloaded for only ~47 d. The approach of Hurricane Rita in September 2005 elicited a mandatory evacuation of the Galveston region, resulting in a premature termination of the bed rest experiment; DXA measurements, however, were conducted in these four subjects prior to discharge. Subjects #8–11 were scanned after 42, 44, 49, and 52 d of bed rest (denoted in **Table I** as ~47 d) and these data for the shorter duration of unloading were also evaluated.

Skeletal Evaluations

All BMD measurements were obtained by DXA using either a Hologic QDR 4500 (Bedford, MA; subjects 1–3) or a Hologic Discovery Unit (subjects 4-13) whole body densitometer. DXA scans were performed once during the pre-bed rest period to provide a baseline assessment and again within 2 d after the end of the bed rest period.

TABLE I. SUBJECT NUMBERS AND BED REST DURATIONS.

Subject #	Duration of Bed Rest (days)
1-3	60
4-7	90
8-11	~47
12,13	90

At each testing session, a series of scans was performed including the whole body, lumbar spine, left and right hips, heel, and forearm. BMD measurements for each subject were the mean of triplicate scans (with repositioning between scans) except for subjects 8–11, who were scanned in duplicate. Left and right hip values for each subject were averaged. All DXA scans were performed and analyzed by a single operator to reduce inter-operator variation.

To ensure the protection of subject health and safety, at the completion of the bed rest study subjects were asked to return for follow-up DXA scans to monitor the recovery of bone loss. Only single DXA scans were performed in returning subjects. Follow-up heel scans were not obtained on the subjects scanned at the ~47-d time point.

Assays for Bone Turnover Markers

Biomarkers for bone formation and resorption were assayed in urine and serum specimens to provide an index of bone turnover. The increased production of bone-specific alkaline phosphatase (BSAP) and osteocalcin in serum by the bone-forming osteoblasts serve as biomarkers of bone formation while the change in N-telopeptide (NTX) and deoxypyridinoline (DPD) in urine were used monitor the effects of bed rest on bone resorption. The biochemical analyses were performed by personnel at the NASA Johnson Space Center Nutritional Biochemistry Laboratory with standard commercial techniques as described by Zwart et al. (20).

Statistical Analysis

The difference between pre- and post-bed rest measurements of BMD was calculated and expressed as percentage of pre-bed rest BMD. The following evaluations were conducted on the reported data where effects were considered significant at $P < 0.05$:

1) Effect of bed rest duration: changes in BMD per skeletal region were expressed as group means ± SD for each duration of bed rest unloading (i.e., ~47, 60, or 90 d) and one-tailed paired Student's *t*-tests were performed on the absolute BMD data to determine a significant effect of bed rest unloading on regional BMD—except for the forearm where a two-tailed Student's *t*-test was used.
2) Effect of bed rest duration on serial measurements: changes in BMD at 60 and 90 d of bed rest were compared by two-tailed paired Student's *t*-tests on the absolute BMD data of the six subjects in whom repeated measures were performed.
3) Effect of bed rest-induced BMD changes with space-induced changes: total BMD changes were averaged over the total months of bed rest (percent change per month) and compared to calculated monthly BMD changes in crewmembers after long-duration spaceflight missions.
4) Effect of bed rest duration on biomarkers of bone turnover: data for bone biomarkers were generated as described in detail by Zwart et al. (20). The bone biomarker data in this report, however, were expressed as a percent change from pre-bed rest values and graphed as a function of bed rest duration; one-tailed *t*-tests were used for resorption markers with two-tailed *t*-tests for the formation markers based upon a priori expectations of biomarker changes after bed rest.
5) Effect of reambulation on BMD recovery: BMD recovery after bed rest was evaluated in terms of whether BMD changes exceeded LSC values established for each bone region at the 95% confidence level. Group mean BMD values after 6 mo reambulation were also compared back to BMD values at the end of the bed rest period by one-tailed Student *t*-test.

RESULTS

Bed Rest Subject Demographics

The bed rest subjects were 8 men and 5 women with a mean age of 35.5 ± 9.6 yr. The mean values for bodyweight and height of subjects were 72.6 ± 16.2 kg and 168.2 ± 9.1 cm, respectively, with a mean BMI of 25.4 ± 3.9 kg · cm^{-2}.

Bed Rest-Induced Changes in BMD

Fig. 1 depicts the mean BMD changes (with standard deviation error bars), per skeletal region for the various durations of bed rest (~47, 60, or 90 d). The forearm failed to show any significant losses in BMD at any duration of bed rest. For all other skeletal regions: after ~47 d of bed rest there were significant decrements in BMD for the trochanter, total hip, and heel; after 60 d of bed rest, all sites showed significant changes in BMD except the heel; after 90 d of bed rest, all sites showed significant losses in BMD except for the femoral neck, lumbar spine, and the heel.

60 vs. 90 d of Bed Rest Changes

Differences between serial BMD measurements at 60 d ($N = 6$) vs. 90 d ($N = 6$) were evident for the lumbar spine (gain) and the heel (loss) only. The change in BMD for lumbar spine exceeded the least significant change of 0.035 g · cm^{-12} in only one of six subjects after 90 d compared to seven of nine subjects after 60 d. For the total hip, there were more subjects that displayed biologically significant decrements after 90 d (four of six) vs. 60 d (three of nine).

Bed Rest-Induced Changes in Bone Biomarker Levels

Fig. 2 depicts the changes in bone biomarkers during varying durations of bed rest and the response of biomarkers 1 wk following bed rest. These measurements

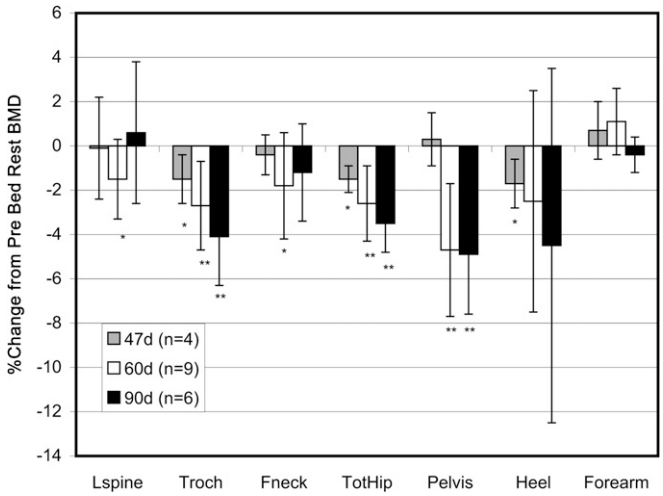

Fig. 1. Change in bone mineral density (BMD) after 47, 60, or 90 d of bed rest. Changes are expressed as group means ± SD for 3 durations of bed rest in 13 subjects. Significant changes from pre-study measures are denoted by $*P < 0.05$ and $**P < 0.01$. Abbreviations: Lspine = lumbar spine; Troch = trochanter; Fneck = femoral neck; TotHip = total hip.

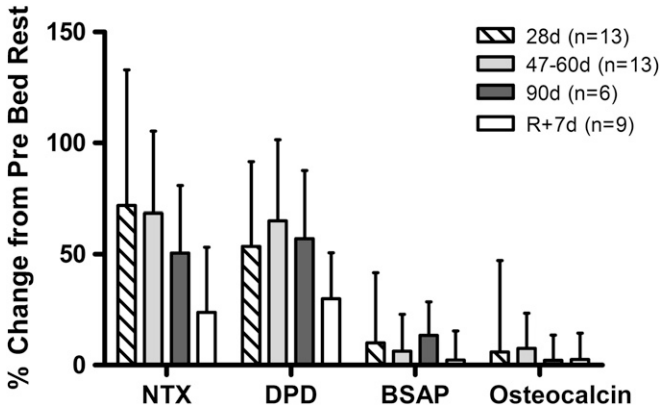

Fig. 2. Change in bone biomarkers after 28 and 90 d of bed rest and 7 d of recovery. Changes were calculated as percent change from pre-study assays of markers. Significant changes from pre-study measures are denoted by $*P < 0.05$ and $**P < 0.01$. Abbreviations; NTx = N-telopeptide; DPD = deoxypyridinoline; BSAP = bone-specific alkaline phosphatase.

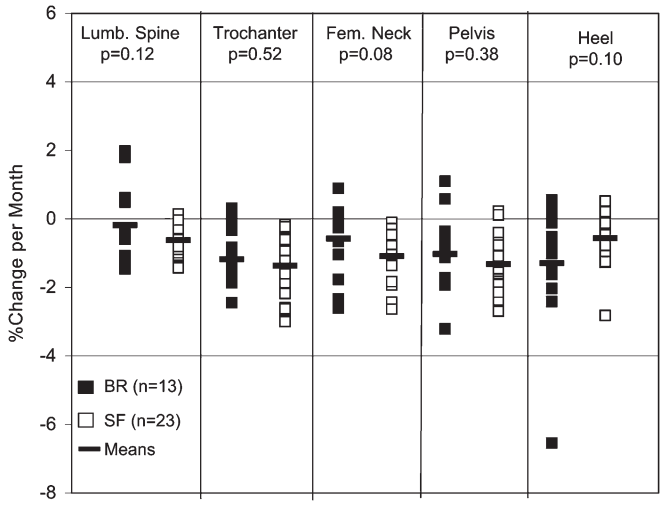

Fig. 3. Changes in bone mineral density (BMD) after bed rest (BR) and spaceflight (SF). *P*-values based on two-tailed Student's *t*-tests assuming equal variances, BR vs. SF; SF subjects are 23 U.S. astronauts from Mir and ISS spaceflights; BR subjects are 13 controls from NASA Flight Analogs Project (FAP) bed rest studies. [Reprinted with kind permission from the publisher, Springer Science + Business Media (13)].

are reported in greater detail by Zwart et al. (20), but are included here to evaluate changes in bone turnover in conjunction with changes in bone mineral density. Bone resorption markers (DPD, NTX) showed significant elevations above baseline at 28, 47–60, and 90 d of bed rest and into the first week of recovery. Bone formation markers were generally unchanged from pre-bed rest except for BSAP at 90 d of unloading.

Bed Rest-Induced Changes Compared to Spaceflight-Induced Changes

As depicted in **Fig. 3**, there were no significant differences between averaged BMD change per month as determined in bed rest subjects and crewmembers after long-duration flights in the lumbar spine, hip, pelvis, and heel.

BMD Recovery After Bed Rest

Of the 13 subjects, 10 returned after 6 mo of reambulation and 6 of those 10 also returned at 12 mo. There was minimal power to evaluate an effect of bed rest duration on BMD recovery. The group mean BMD, however, increased significantly during the 6 mo of reambulation for the trochanter, total hip, pelvis, and heel ($P < 0.01$), but not for the femoral neck and lumbar spine. On an individual basis (LSC), significant BMD decrements persisted in 3 of 10 subjects in at least one site after 6 mo of ambulation. Significant BMD decrements persisted after 12 mo for ambulation in two of the six subjects.

DISCUSSION

Changes in BMD and in biomarkers of bone turnover, measured over ~47 to 90 d of bed rest, closely model the skeletal changes that have been documented by LeBlanc and colleagues in long-duration crewmembers (astronauts and cosmonauts) who flew on the Mir and ISS space stations (126–428 d) (7,12). These changes also compare favorably with flight data from 23 astronauts presented in this report (Fig. 3). Consistent with these spaceflight changes, BMD losses induced by bed rest were most pronounced in the regions of the lower half of the skeleton and in normally weight-bearing sites, i.e., hip, pelvis, and heel. There were minimal losses in the forearm which were consistent with flight data (7), but for which we had no a priori expectation from bed rest studies reported in the literature.

For flight data, we report total changes in BMD as the mean loss per month in order to normalize BMD data of crewmembers serving on different durations of spaceflight missions. Upon comparison, the mean losses observed in the bed rest subjects are consistent with mean 1–1.5% per month losses for the hip and pelvis in crewmembers. Incidentally, the BMD deficits detected in the heel of crewmembers after spaceflight have been smaller than those of subjects after bed rest, possibly due to the direct impact of ground reaction forces during treadmill running, a mainstay of in-flight exercise regimens.

Furthermore, the BMD changes documented in bed rest subjects participating in the JSC Flight Analogs Project are similar in terms of magnitude and site-specificity to those reported in previous long-duration bed rest studies conducted at other institutions and centers (6,8,10,11,17). The failure to detect significant changes at some of the sites with even longer periods of bed rest appears to be attributed to the highly variable responses in BMD. Large variability in the lumbar spine observed in this study was evident in previous long-duration studies (6,8). In this report, five subjects had positive changes in lumbar spine BMD (with a nearly 6% increase in one subject) at the end of bed rest; the average $+0.2 \pm 1.1\%$ per month loss in the lumbar spine of bed rest subjects contrasted with changes of $-0.8 \pm 0.6\%$ per month after spaceflight, suggesting the possible influence of muscular forces in the back. The +6% BMD outlier in the bed rest

subjects, for example, had numerous warnings of protocol violations because of excessive in-bed physical activity. Thus the ability of the bed rest analog to model skeletal unloading with spaceflight underscores strict subject compliance to the bed rest protocol. Likewise, the large variability of the heel BMD to bed rest unloading may reflect the different levels of daily physical activity in the test subjects prior to pre-bed, particularly since the heel is susceptible to varying levels of ground reaction forces during ambulation. In general the skeletal sites that display greater atrophy are the sites that had the greater weight-bearing function during ambulation. However, unlike these aforementioned sites, the BMD variation in the femoral neck can also be attributed to the larger scanning error due to subject positioning of the hip.

The bone biomarker data from these bed rest subjects are similar to biomarker data obtained from previous spaceflight and bed rest studies (14,20). Mechanical unloading of the skeleton stimulates bone resorption (increased excretion of collagen degradation products) while having little effect on bone formation (the release of osteoblast-specific proteins). Increased bone resorption occurs early in bed rest and spaceflight and remains elevated throughout the period of mechanical unloading until the period of reambulation (3,14). Thus, not only do the biomarker data suggest a stimulation of bone resorption, they also suggest a net loss of bone. While circulating levels of bone turnover markers cannot be used for the diagnosis of osteoporosis, the changes in biomarkers can reflect the rate of turnover (an index of "bone quality"), can predict the corresponding changes in bone mass, and thus can be useful for monitoring the skeletal response to therapies (1,18).

Although this study provided cross-sectional DXA BMD data for different bed rest durations, the limited number of subjects and variability in the measurements did not provide sufficient power to analyze a temporal response of BMD nor could we evaluate the impact of bed rest duration with BMD recovery after reambulation. Even with the repeated measures of subjects, the data to date do not enable us to conclude that 90 d can consistently induce meaningful decrements in BMD compared to 60 d. This information is critical for designing a cost-effective test bed to evaluate the efficacy of countermeasures. Thus, there are no overt modifications to the design of the bed rest protocol by the JSC FAP at this time.

Although the restoration of BMD after bed rest is not the thesis of this paper, the recovery of BMD after bed rest displays some similarity with the restoration of BMD after long-duration spaceflight (12). BMD restoration exhibits substantial inter- and intrasubject variability and occurs over a longer period than the time to incur the loss (12). Similarly, not all bed rest subjects recovered BMD completely in all regions within the 12-mo time frame and some subjects showed minimal recovery, if any; this is not unexpected since subject selection criteria do not control for genetics, which could account for 50–80% of the variability in intersubject BMD (12). Moreover, aside from the measurement of BMD at 6 mo and 12 mo after reambulation, there is no evaluation or physiological assessment of subjects beyond the 2-wk post-bed rest rehabilitation period

The skeletal unloading by bed rest, as implemented by the JSC FAP, induced DXA BMD changes in 13 subjects who were skeletally unloaded for at least 60 d of bed rest. These changes were consistent with BMD changes documented in earlier bed rest and in spaceflight studies, with statistically significant losses occurring in the hip, pelvis, and heel. Bone biomarker data are also consistent with previous spaceflight and bed rest findings, demonstrating an increase in bone resorption with minimal change in formation. The variability in BMD responses suggest that the FAP needs to control in-bed activity of subjects, to consider the pre-study physical activity levels of subjects, and to monitor post-study rehabilitation of subjects to reduce potential skeletal health risks. Finally, preliminary results regarding recovery of BMD after head-down bed rest appear similar to observations of spaceflight BMD recovery. The bone measurements obtained from this first series of studies indicate that the NASA Flight Analogs Project integrated bed rest protocol is a valid test bed for skeletal disuse studies and should provide a useful research platform for evaluating countermeasures to spaceflight-induced bone loss.

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