The Flight Analogs Project as a Test Platform for the Skeletal Effects of Space Flight

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Early in the space program, scientists recognized bone atrophy with exposure to space flight as best documented by reduced bone mineral density of the heel and increased urinary and fecal excretion of calcium detected in the Skylab crews. Consistent with Wolff's Law, which asserts that the skeleton will remodel its structure in an adaptive response to its physiological and mechanical functions, densitometry data from long-duration space flight crew members substantiate a demineralization of the skeleton in those regions that experience the greatest reduction in strain; i.e., weight-bearing skeletal sites. It is the accelerated atrophy, and the potential deterioration of microarchitecture, that identifies "bone loss" as a primary risk factor for human health during the occupation and exploration of space. This space-flight-induced loss of bone mass may increase the risks 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 influence the ability of space flight crew members to perform an emergency egress, or increase the risk of injury during egress. Moreover, incomplete recovery of bone mass and irreversible changes to geometry and microarchitecture may accelerate the onset of age-related osteoporosis in long-duration space flight crew members as they age back on Earth. Osteoporosis therapy on Earth focuses on prevention; thus, bone loss countermeasures that prevent or mitigate skeletal deconditioning may be required to maintain skeletal integrity and to meet the performance demands of space exploration missions. Critical to the development of these countermeasures is the ability to model the skeletal deconditioning observed in space to test countermeasure efficacy in a controlled experimental environment.

In this paper, we report changes in regional bone mineral density (BMD), along with serum and urinary biomarkers of bone turnover, measured in test subjects skeletally unloaded in a flight analog of prolonged bedrest. We compared bone measures, conducted at the Johnson Space Center Flight Analog Facility (long-duration head-down bedrest), located at the University of Texas Medical Branch in Galveston, to data from previously reported bedrest studies and to data from crew members after long-duration space flight.

Regional BMD measurements were obtained by dual-energy x-ray absorptiometry (DXA) using either a Hologic QDR 4500 (subjects 1–3) or a Hologic Discovery (subjects 4–13) whole body densitometer. We performed DXA scans once during the pre-bedrest period to provide a baseline assessment and again within 2 days after the end of the bedrest period. In addition, two subjects (12 and 13) participated in an extra DXA session at the intermediate time point of 60 days of bedrest. At each testing session, we performed a series of scans to include the whole body, lumbar spine, left and right hips, heel, and forearm. Subjects were multiply scanned to generate triplicate BMD measures of each region. (However, we performed duplicate rather than triplicate scans on subjects 8–11, who were scanned at bedrest days 42, 44, 49, or 52 (referred to here as ~47d) due to the urgency of the Hurricane Ike evacuation.) Left and right hip values for each subject were averaged to improve measurement precision of hip BMD as were triplicate (or duplicate) BMD values per region for each test session. A single operator performed and analyzed all DXA scans to reduce inter-operator variation. After completion of the bedrest study, we requested that subjects return for follow-up DXA scans (DXA series performed only once per follow-up) to evaluate bone recovery. Of the 13 subjects, 10 returned for follow-up testing at 6 months of reambulation (R+6mo), and six of those 10 returned also at 12 months (R+12mo). Follow-up heel scans were not obtained on the subjects scanned at the approximately 47d time point.

We assayed biomarkers for bone turnover in urine and serum specimens to provide a corroborating index, at the whole tissue level, by which to monitor the relative effects of bedrest on bone resorption and bone formation. We also assayed N-telopeptide (NTX) and deoxypyridinoline (DPD) from 24-hour urine collections as biomarkers of bone resorption. Levels of serum bone-specific alkaline phosphatase and osteocalcin served as markers of bone formation. Other bone-related assays included urinary calcium, serum calcium, pyridinium crosslinks, alkaline phosphatase, intact parathyroid hormone, 1,25 dihydroxyvitamin D, and 25-hydroxyvitamin D, using standard commercial techniques.

We calculated and expressed the difference between pre- and post-bedrest measurements of BMD as a percentage

of pre-bedrest BMD with regional BMD changes as group means ± SD for each time period of bedrest (i.e., ~47, 60, or 90d). Total BMD changes were also averaged over the total months of bedrest (% change per month) for comparison to calculated monthly BMD changes after space flight missions. To determine a significant effect of bedrest on BMD for each bedrest duration, we performed one-tailed, paired Student's t-tests on the absolute BMD data; significant effects were designated at $P < 0.05$. The one exception to this approach was for the forearm (two-tailed) because there was no a priori expectation of a change in BMD, based on previous bedrest and space flight studies. To maintain an approach consistent with the BMD data, we expressed the bone biomarker data as a percent change from pre-bedrest values; we used Student's t-tests (two-tailed, paired, based on the absolute BMD values) to determine statistical significance for the pre- vs. post-bedrest changes. BMD recovery after bedrest was concluded if increased BMD with re-ambulation exceeded the measurement error ("Least Significant Change") established for that skeletal site at the 95% confidence level.

Figure 1 shows the average BMD changes, per skeletal region, for the various durations of bedrest. The figure also provides the calculated *average* monthly rate of BMD changes for each region. We noted statistically significant changes from pre-bedrest BMD in the lumbar spine, hip, pelvis, and heel, although not for every duration of bedrest. Mean BMD values of the total hip, hip trochanter, and pelvis were significantly decreased at all durations of bedrest. We detected significant loss in the heel only at the approximately 47d bedrest time point, and loss in the lumbar spine only at 60d of bedrest. No significant changes were noted in the forearm (serving as an "internal" negative control) for any periods of bedrest. The team analyzed paired, longitudinal BMD measurements for the six subjects (subjects 4–7, 12, and 13) who had DXA scans both at 60d and 90d of bedrest (figure 2). Statistically significant differences between the 60d and 90d measurements for the lumbar spine and heel were recorded.

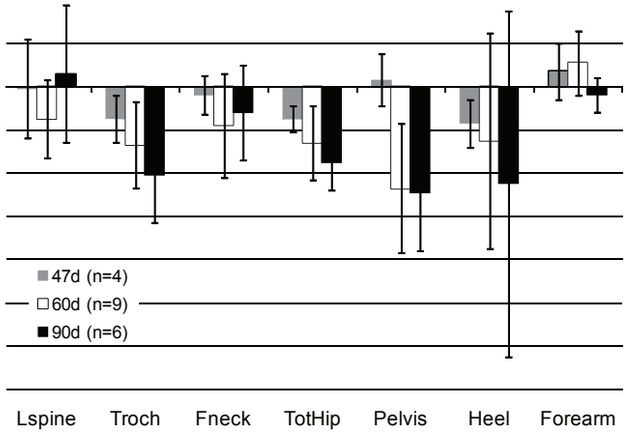

Fig. 1. *Percent change from pre-bedrest BMD.*

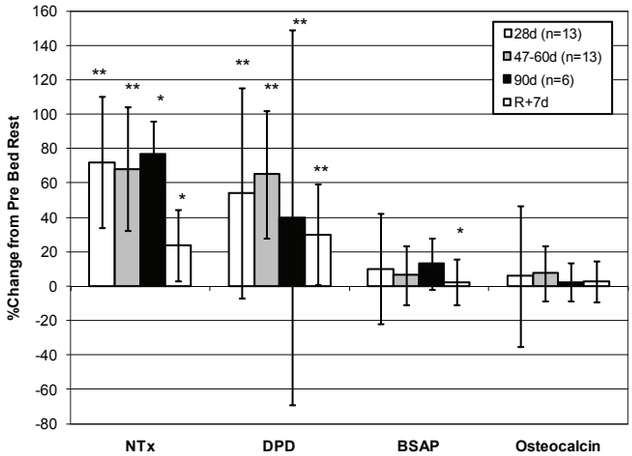

Fig. 2. *Percent change from pre-bedrest N-telopeptide, deoxypyridinoline, bone-specific alkaline phosphatase.*

Three of the 10 subjects who participated in follow-up DXA scans still had BMD deficits in at least one bone site after 6 months of re-ambulation (BR+6mo). Some bone regions in these individuals exhibited no significant increase in BMD from the immediate post-bedrest measurement; i.e., BR+0 values. Of the six subjects who had follow-up DXA scans at BR+12mo (three 60d subjects, two 90d subjects, one ~47d subject), two subjects still had BMD losses in at least one bone site that were greater than the least significant change value. Furthermore, these deficits were equal to or even greater than those documented at the BR+0 and BR+6mo time points, although we did not test these differences statistically. We examined the recovery data for group changes as well.

For the nine subjects who had recovery measurements at BR+6mo, t-tests indicated that mean BMD increases from BR+0 were statistically significant ($P < 0.01$) for the trochanter, total hip, pelvis, and heel. We noted no significant increase for the femoral neck or lumbar spine (the latter of which showed no measurable decrease from baseline at BR+0 and therefore would not be expected to show "recovery"). In the six subjects who had recovery measurements at both BR+6mo and BR+12mo, no statistically significant differences were noted for any bone region between the BR+6mo and BR+12mo time points.

Figure 3 outlines the changes in bone biomarkers during and after bedrest. Bone resorption markers (DPD, NTX) showed significant elevations above baseline at 28 days of bedrest, and these changes persisted through bedrest days 60 and 90 and into the first week of recovery. Bone formation markers were generally unchanged from pre-bedrest.

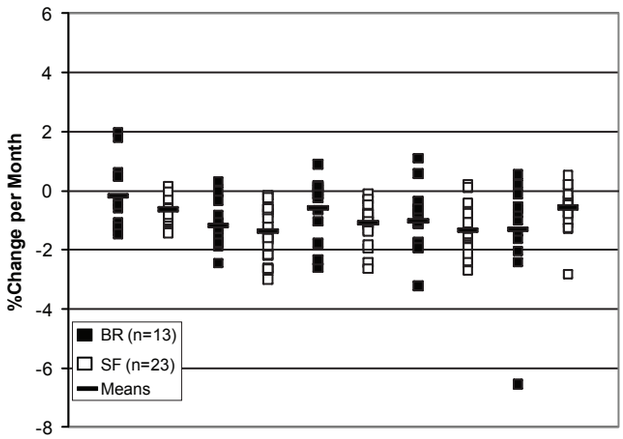

Fig. 3. *Percent change per month. Comparison of bedrest (BR) and space flight (SF) studies.*

DXA BMD changes measured in the 13 subjects reported here are consistent with BMD changes documented in earlier bedrest and space flight studies, with statistically significant losses occurring in the hip, pelvis, and heel. The lumbar spine results suggest the possibility that subject positioning in the axial direction and in-bed activity during bedrest might affect regional BMD results. Bone biomarker data are also consistent with previous space flight and bedrest findings, and demonstrate an increase in bone resorption with no significant change in formation. Finally, preliminary results regarding recovery of BMD following head-down tilt bedrest appear similar to observations of space flight BMD recovery, which include the considerable BMD variability between site-specific measurements within one individual and between several individuals, as well as the prolonged period of recovery relative to period of disuse. Based on bone measurements obtained from this first series of studies, the NASA Flight Analogs Project integrated bedrest protocol appears to be a valid testbed for skeletal disuse studies, and should provide a useful research platform for evaluating countermeasures to space-flight-induced bone loss.

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