GRAVITY, CALCIUM, AND BONE: UPDATE, 1989

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This update highlights some of the results of recent short-term flight and ground-based experiments that have contributed new insight into skeletal adaptation, calcium metabolism, and growth processes in 0 g. After 6 months in space, bone demineralization, invariably involving the os calcis (20), was found not to extend to the lumbar spine in 4 exercising cosmonauts (3). A flight experiment in the Space Shuttle crew has documented the early events in the calcium endocrine system during spaceflight (12).

On the ground, brief (<35 days) and long-term (>4 months) bed rest studies of healthy volunteers in the head-down tilt (HDT) model of weightlessness have been completed. The skeleton of the adult male responds more rapidly to unloading than previously recognized (2). Regional changes in bone density can be quantified in only 30 days, are highly individual, and follow the direction of gravitational forces in the HDT model during inactivity (1). Bone biopsy results in healthy volunteers after bed rest (11) differ from results in paraplegics from the same sampling site (21).

Flight experiments in growing rats reveal changes in the composition of bone mineral and matrix in the femur postflight that were found to be highly regional and suggestive of an effect of gravity on mineral distribution (10). These observations may be relevant to the results from an earlier Cosmos flight where artificial gravity in space was found to maintain bone strength, but not to correct the radial growth deficit (19).

Mineral in the Lumbar Spine

Ever since Krolner and Toft reported a reduction (–3.8%) in the average density of the 2nd to 4th lumbar vertebrae following therapeutic bed rest in 28 patients suffering from prolapsed intervertebral discs, there has been some concern that vertebrae in bed rest subjects and space travelers may demineralize (7). Fortunately, no significant changes were observed by Drs. Cann and Oganov, who used quantitative computer tomography to quantify the mineral content of the body of the 2nd lumbar vertebra of 4 cosmonauts before and after 6 months in space (3). These data have not completely erased the concern of osteoporosis in the lumbar spine because the Cosmonauts exercised daily.

Nevertheless, nonexercising bed rest subjects have also failed to show reduced bone density in the lumbar spine. LeBlanc et al. found no change in the density of the 2nd through 4th lumbar vertebrae in 5 of 6 subjects after 5 weeks of bed rest (horizontal); one showed a 3% decrease (8). Oganov et al. reported average increases to 12.6% in density of the spongiosa of the lumbar vertebrae of 3 bed rest subjects after 120 days in a –5° head-down tilt position (HDT) (14).

We used dual photon absorptiometry to measure the density of the 2nd through 4th lumbar vertebrae before and after 30 days HDT (–6°). Subjects were participating in a study designed to test the effect of isokinetic and isotonic exercise on orthostatic tolerance (5). Our results, shown as percent change in the histogram in Fig. 1, revealed no differences in 17 subjects, irrespective of the exercise group. Two subjects showed changes in opposite directions (–7 and +10%), well outside the error of the test.

Calcium Endocrine System

To identify factors that could have contributed to the change in lumbar spine mineral in 2 of 19 subjects, we examined the diet and a variety of parameters known to influence bone metabolism. Table I enumerates the values in these two subjects before and after bed rest. Figures 2a and 2b illustrate changes in circulating hormone concentrations that may be related to the alteration in lumbar spine density. None of the values except for serum parathyroid hormone (PTH) reached values outside the normal range. Combined increases in serum PTH and weight loss (4) favored decrease in lumbar spine mineral; the opposite changes were associated with an increase. These data are consistent with the known effects of PTH to enhance bone resorption. The precise role of serum 1, 25-dihydroxyvitamin D in the change in bone mineral content is not clear.

The early response of the calcium endocrine system in 4 astronauts was documented in serum obtained before, during, and after 7 days in space on the SL2 mission (12). The published data are summarized in Fig. 3. An increase in the vitamin D hormone, 1, 25-D, within the first 36 hours of launch was the only significant change, although trends toward increases in total serum calcium and phosphorus and decreases in bioactive PTH were present in 3 astronauts. Possible explanations for the early increase in 1, 25-D include perturbations during launch, transient lack of dietary calcium associated with space motion sickness, a nonspecific stimulation of renal 1-alpha hydroxylase connected with fluid shifts, or a specific

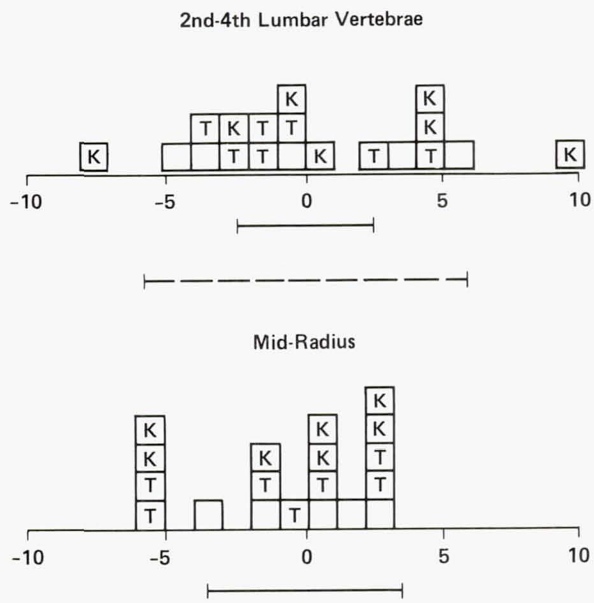

Figure 1. Percent change in density of the lumbar spine and mid-radius of 19 men referenced to each subjects basal level (0). Distribution was not affected by exercise (isotonic = T, isokinetic = K, no exercise = blank). The error of the tests are indicated by the bars, ⊢—⊣ and $2 \times \sqrt{2} \times$ coeff. of variation, by (⊢-----⊣).

Table I. Comparison of clinical data in two exercising subjects who showed opposite changes in lumbar spine density after 30 days head-down tilt bed rest.

	A			B		
Study day, from 1st bed rest day	–5	4	27	–5	4	27
Age, years	42			37		
Weight, kg	68		68.6	83.8		82
Height, cm	171			183		
Plasma volume,[a] ml/kg	46.3		40.5	48.2		39.9
Body fat,[a] %	7.7			8.0		
Serum						
Total calcium, mg/dl	9.1		8.8	9.9		9.8
Ionized calcium, mg/dl	4.20		5.00	4.88		4.96
Total protein, g/dl	7.2		7.0	7.2		7.2
Phosphorus, mg/dl	2.0		3.2	2.3		2.3
Parathyroid hormone,[b] pg/ml	20	24	17	24	59*	44
1, 25-dihydroxyvitamin D, pg/ml	16	17	37	33	28	30
Cortisol,[c] ug/dl	16.9		15.6	9.5		11.3
Testosterone,[c] total, ng/ml	882			871		
Testosterone,[c] free, ng/dl	25			22.2		
Urine						
Creatinine clearance, ml/min/1.73m^2	128		123	108		118
Calcium, mg/24 hr	229		165	182		301
Hydroxyproline, mg/24 hr	21		15	36		42
Diet during study[d]						
Calories, kCal/kg	42		45	34		36.8
Calcium, mg	1281		1398	1274		1431
Phosphorus, mg	1816		1959	1883		2020
Sodium, mg	5756		5941	5615		5976
Protein, g	117		119	114		119
Bone density[e]						
Radius, gm/cm	1.304		1.337	1.422		1.434
Lumbar spine, L2-4, gm/cm^2	1.175		1.293	1.875		1.725

Analysis of [a] by J. Greenleaf, [b] by R. Marcus, [c] by C. Wade, [d] by R. Williams, and [e] by M. Powell.
*Above the normal range.

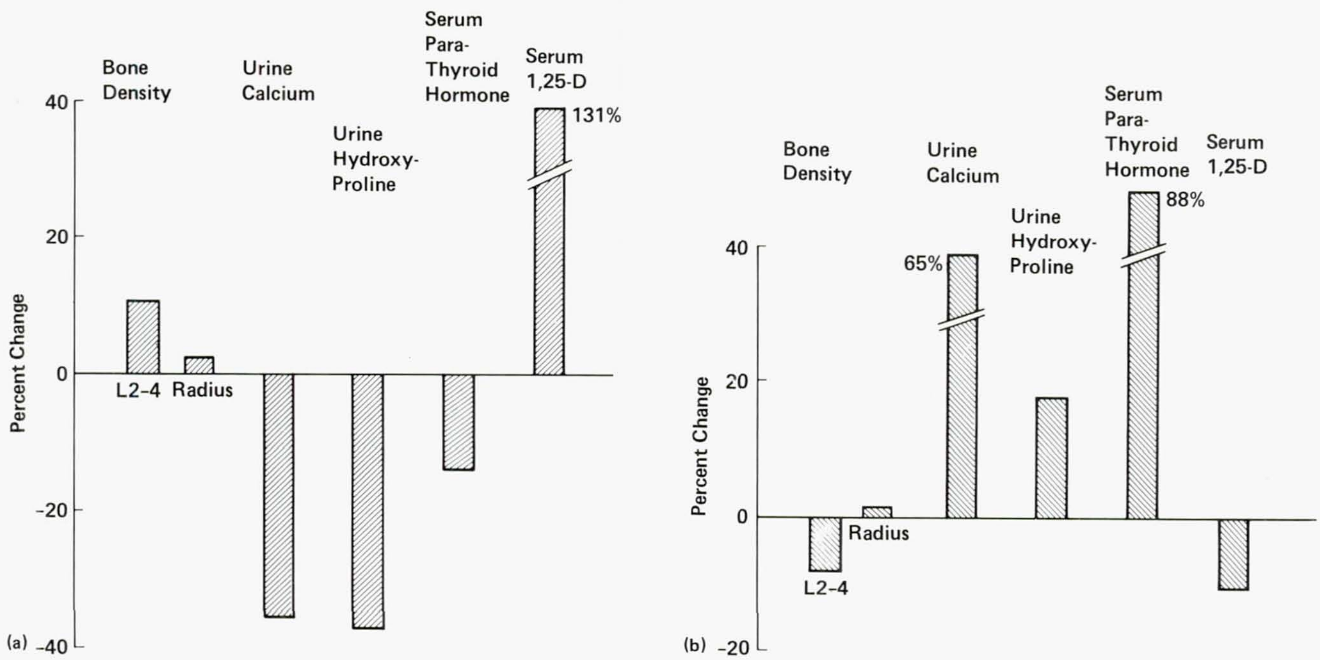

Figure 2. Changes in some parameters of calcium homeostasis, referenced to pre-bed rest levels, in subject A (a) who showed an increase, and subject B (b) who showed a decrease in lumbar spine density after 30 days bed rest. Of interest, both subjects performed isokinetic exercise for 30 min twice daily (5).

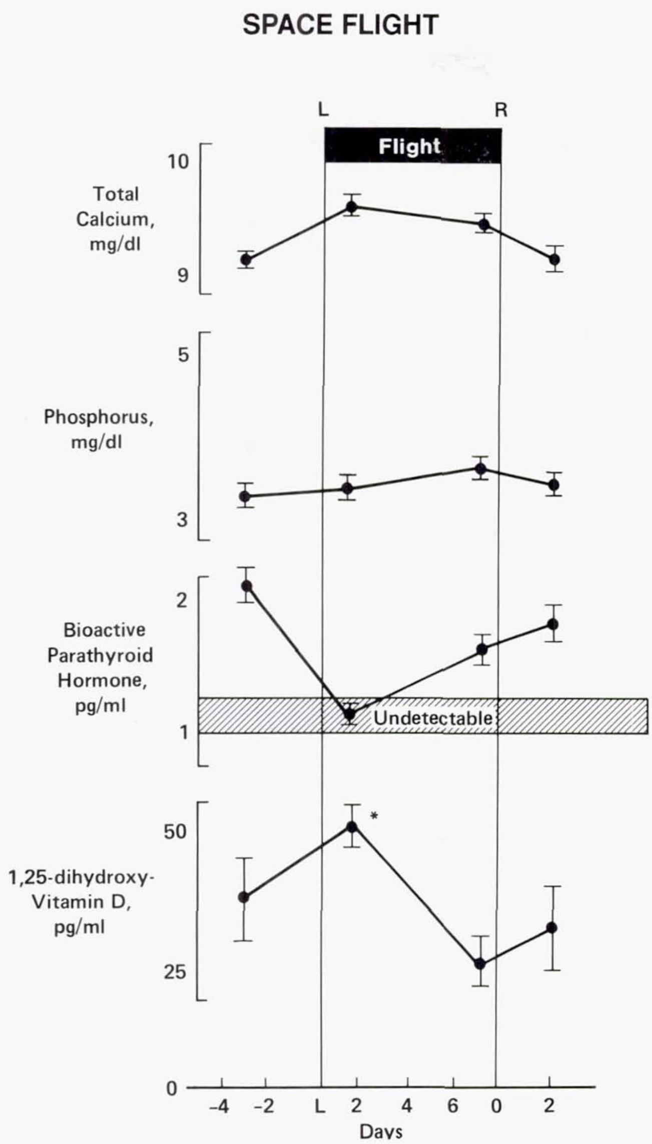

Figure 3. Mean values (±SE) in the serum of four astronauts obtained 1 week before, during, and the first week after launch (L) of a 7-day shuttle spaceflight (SL2) (data replotted from reference 13).

response in vitamin D metabolism to a change in a biomechanical stimulus originating in bone or muscle. Differences in the values during the first 24 hours did not seem to affect 7-day values, which are in the direction of being lower, but are not different from preflight values. The important contribution of these few samples taken during a flight is the preliminary knowledge that biologically active PTH, undetectable in serum after 36 hours in space, was not increased after 7 days, nor was 1, 25-D. While excesses of serum PTH cannot be responsible for the early mobilization of bone calcium, transient increase in 1, 25-D may be.

The above short-term data in flight differs from the results of a 7-day HDT study conducted at Ames Research Center, in which no changes were found during the first 36 hours. However, after 7 days, the trends to lower serum PTH and 1, 25-D in flight and on the ground were similar (11). The long-term Soviet bed rest study shows changes compatible with parathyroid hyperplasia with increases in serum calcium and PTH (especially after 49 days), suggestive of differences in early and late responses in the calcium endocrine system (13).

Of interest, in the Soviet bed rest study, were early increases in serum levels of calcitonin, an inhibitor of bone resorption, that gradually decreased to lower than basal levels after 3 months. Given the variations in both assay methods and bed rest protocols, the status of the calcium endocrine system, at least, after the first week in space or bed rest in healthy individuals, remains uncertain.

Bone Morphology

If newer concepts in the role of PTH and 1, 25-D in the processes of bone remodeling are correct, i.e., that PTH governs the differentiation and number of bone cells, and 1, 25-D, cell activity (9), the pattern of circulating hormone levels from the SL2 mission suggests the following early sequence of events: enhanced mobilization of calcium from bone related to the increase in 1, 25-D followed by suppressed mineralization in unloaded bones after a few days, with no increase in the number of osteoclasts or osteoblasts. Standard post mortem examination of some of the bones of 3 Cosmonauts after 28 days in space showed normal histology, fewer vascular channels than a control sample, and some increase in the porosity of the femoral epiphysis and diaphysis, but not in the rib, vertebrae, or calcaneus (16). Jowsey's analysis of the iliac crest of patients after 4-17 days horizontal bed rest for conditions unrelated to the skeleton, demonstrated reduced bone formation and no difference in the extent of resorption surfaces from normal in 11 of 14 patients. Cell counts are not in the report (6).

Following a 4-month period of bed rest in 3 healthy Soviet volunteers, Vico et al. found a two-fold increase in resorption surfaces, no increase in cell number, and reduced bone formation rate in specimens from the iliac crest (21). A puzzling observation was no measurable change in the volume of bone in healthy bed rest subjects, unlike patients with paraplegia (11). That the normal subject shows changes in surface morphology indicative of bone loss with no apparent diminution in volume at the two-dimensional level, suggests some form of compensation in microarchitecture. Either standard measurements may not be sensitive enough to detect losses in volume or other measurements involving the three-dimensional structure of bone, not usually done, may be needed to show how normal subjects maintain bone volume.

Gravity-Dependent Gradients of Mineralization

Comparison of the increments in whole-body calcium of rats exposed to 0, 1, and 2 g reveals accumulation of bone mineral directly related to the gravitational force (15). The mechanism of this acquisition of skeletal mineral must involve systemic as well as local processes. The cardiovascular system, whose general structure is oriented in the direction of gravity and where blood vessels, flow, and volume are known to differ at the local bone level in active and inactive individuals, is the most obvious candidate to influence bone mass.

Until recently, however, there were no data that suggested that there was a generalized cardiovascular effect on bone or that a shift of the hydrostatic column of pressure with changes in position, was associated with changes in bone mineral. In the tail-suspended rat, Roer and Dillaman found the expected decrease in ash in the bones of the unloaded lower extremities, no change in the humerus and ulna, and importantly, an increase in bone ash in the skull (17). By dual photon absorptiometry, the density of the head region of adult bed rest subjects was found to be increased an average of 10% after a 30-day HDT study (1). These two studies suggest a gravity-dependent distribution of mineral in the whole skeleton, which may be a function of changing pressures, fluid flow, or volume in the cardiovascular system in response to change in position.

During the Cosmos 936 mission, centrifugation in orbit permitted comparison of the effects of gravity on the strength and growth of the femur of young rats in space (19). Rats treated with artificial gravity showed the same increases in density and strength during the 18.5-day flight as ground controls; however, the growth defect was not improved. Spengler et al. attributed the growth deficit to poor adaptation of the rats to the short-arm radius centrifuge and concluded that centrifugation normalized material properties, i.e., quality, but not the quantity of the femur. These paradoxical findings following artificial gravity could be explained by the recently observed linear gradients of mineralization in the diaphysis of the femur (10). At 1 g bone mineral concentration was lower in the distal than in the proximal diaphysis of the femur of the 14-week old rat, a disparity that persists in flight, but tends to disappear by 16 weeks on Earth. Because of the logistical problems connected with the 1887 flight where these diaphyseal gradients of mineralization were observed, and because the results differ from our expectation that mineral deposition proceeds from the center of a growing bone proximally and distally, confirmation of this observation is needed. Collectively, all of the above studies reveal an important connecting link between gravity, per se, and bone mineral distribution and deposition, most likely related to the cardiovascular system. The interaction of what appear to be gravity-dependent gradients visible at the whole-body and organ level, with the highly regulated processes that change bone structure at the local tissue level in response to biomechanical forces is not now apparent.

In summary: Advances in recent years have enabled us to recognize that two principal components of calcium metabolism, the calcium endocrine system and bone, respond promptly (within days), to changes in body position and weightlessness. The vitamin D hormone may be the best candidate for mobilizing bone mineral early, and newly identified gravity-dependent gradients, probably involving the cardiovascular system, may have a significant role in its distribution at the whole-body level. These observations have given us a new perspective on the results of balance studies in healthy subjects and astronauts (18,22). During inactivity or weightlessness, negative balances in bone minerals may be more directly a reflection of diets, and alterations in the function of the gastrointestinal tract and kidney that parallel, but do not necessarily derive from the highly localized activities concerned with the restructuring of bone and redistribution of bone mineral to meet new functional requirements. These studies imply that bone biomechanics are more severely affected by spaceflight than bone mass.

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References

1. Arnaud SB, MR Powell, J Vernikos-Danellis, et al. *J. Bone Mineral Res.* 3:S119, 1988.
2. Arnaud SB, DJ Sherrard, N Maloney, et al. *J. Bone Mineral Res.* 4:S233, 1989.
3. Cann CE. Technology assessment: Calcium homeostasis and bone demineralization research. *In: USRA Proceedings: Calcium Science Working Group.* Sept. 1987, p. 97.
4. Greenleaf JE, EM Bernauer, LT Juhos, et al. Effects of exercise on fluid exchange and body composition in man during 14-day bed rest. *J. Appl. Physiol*: Respirat. Environ. Exercise Physiol. 43(1):126-1432, 1977.
5. Greenleaf JE, CE Wade, G Leftheriotis. *Aviat. Space Environ. Med.* 60: 537-42, 1989.
6. Jowsey J. Bone at the cellular level: the effects of inactivity. *In: Hypogravic and Hypodynamic Environments*, ed. RH Murray and M McCally. NASA SP-269, 1971, p. 111-119.
7. Krolner B, B Toft. *Clin. Sci.* 64:537-540, 1983.
8. LeBlanc A, V Schneider, J Krebs, et al. *Calcif. Tissue Int.* 41:259-261, 1987.
9. Malluche HH, C Matthews, M Faugere, et al. *Endocrinology* 119:1298-1304, 1986.
10. Mechanic GL, SB Arnaud, A Boyde, et al., Regional distribution of mineral and matrix in the femurs of rats flown on Cosmos 1887 biosatellite. *Faseb J.*, in press.
11. Minaire P, P Meunier, C Edouard, et al. *Calcif. Tissue Res.* 17:57-73, 1974.
12. Morey-Holton ER, HK Schnoes, HF DeLuca, et al. *Aviat. Space Environ. Med.* 59:1038-41, 1988.
13. Morukov BV, OI Orlov, AI Grigoriev. *The Physiologist* 32:S37-S40, 1989.
14 Oganov VS, AS Rakhmanov, BV Morukov, et al. *Moscow Kosm. Biol. Aviakosm. Med.* 22:30-33, 1988.
15. Pace N. AH Smith, DF Rahlman. *The Physiologist* 28:S17-S20, 1985.
16. Prokhonchukov AA, NA Zhizhina, RA Tigranyan. Homeostasis of bone tissue under normal and at extremal action. *In: Problems in Space Biology*, ed., PD Gorizontov, Nauka Press, Moscow, 1984, p. 152-165.
17. Roer R, R. Dillaman. Bone growth and calcium balance during simulated weightlessness in the rat. *J. Appl. Physiol.*, in press.
18. Schneider VS, J McDonald. *Calcif. Tissue Int.* 36:S151-S154, 1984.
19. Spengler DM, ER Morey, DR Carter, et al. *Proc. Soc. Exp. Biol. Med.* 174:224-228, 1983.
20. Stupakov GP, VS Kazeykin, AP Kozlovskiy, et al. *Space Biol. Med.* 18:42-47, 1984.
21. Vico L, D Chappard, C Alexandre, et al. *Bone Mineral* 2:383-394, 1987.
22. Whedon GD, L Lutwak, PC Rambaut, et al. Mineral and nitrogen metabolic studies, experiment MO71. *In: Biomedical Results from Skylab*, ed. R Johnston and L Dietlein. NASA SP-377, 1977, p. 164-174.