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Skeletal responses to space flight and the bed rest analog: A review

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Abstract

The potential for loss of bone mineral mass due to space flight was recognized by space scientists even before man's first venture into micro-gravity. Early life science studies in both the U.S. and Russian space programs attempted to measure the effects of reduced gravity on skeletal homeostasis, and these measurements have become more sophisticated with time. Bone-related measurements have typically included: bone mineral density measured by X-ray absorptiometry and more recently CT scanning; bone-related hormones and other biochemical markers of bone turnover; and calcium excretion and balance. These measurements, conducted over the last 4 decades, have shed light on the nature of disuse bone loss and have provided preliminary information regarding bone recovery. Ground-based analog (bed rest) studies have provided information complementary to the space flight data and have allowed the testing of various countermeasures to bone loss. In spite of the wealth of knowledge obtained thus far, many questions remain regarding bone loss, bone recovery, and the factors affecting these skeletal processes. This paper will summarize the skeletal data obtained to date by the U.S. and Russian space programs and in ground-based disuse studies. In addition, related body composition data will be briefly discussed, as will possible countermeasures to space flight-induced bone loss.

Keywords: Musculoskeletal, Space Flight, Bed Rest, Bone Mineral Density, Bone Markers, Bone Remodeling

Introduction

Prior to the beginning of manned space flight, the potential problems related to reduced gravitational forces on the musculoskeletal system were recognized if not fully understood. Therefore, NASA researchers and managers of the nascent American and Russian space agencies began to devise studies to determine the extent of and potential problems associated with bone changes that might occur during space flight.

The American human space flight program began in 1962 with the Mercury program and the launch into Earth orbit of a one-astronaut capsule. Five additional one-man Mercury flights took place, ending in 1963. Between 1965 and 1966, the Gemini program launched 10 two-man flights. The Apollo program followed, with 11 three-man missions taking place between 1968 and 1972; the final 7 of these missions involved 2 of the 3 crewmembers traveling to the lunar surface. After the successful completion of the Apollo program, NASA launched the Skylab space station. Between 1973 and 1974, three 3-man crews occupied Skylab on missions of 29, 59, and 84 days. The Skylab missions focused extensively on life science objectives, including several related to the musculoskeletal system. In 1975, the space programs of the U.S. and the U.S.S.R. conducted a 9-day joint mission involving the docking of Apollo and Soyuz space capsules. In 1981, the Shuttle flight program began and continues today. At the time of this writing, 114 Shuttle flights have been completed, with typical mission durations of approximately 1-2 weeks.

The Russian space program followed a direction similar to that of the American program, except that more emphasis was placed on building large space stations culminating in the completion of the Mir space station in 1986. The Mir space station was continuously manned until 2000, when the space station

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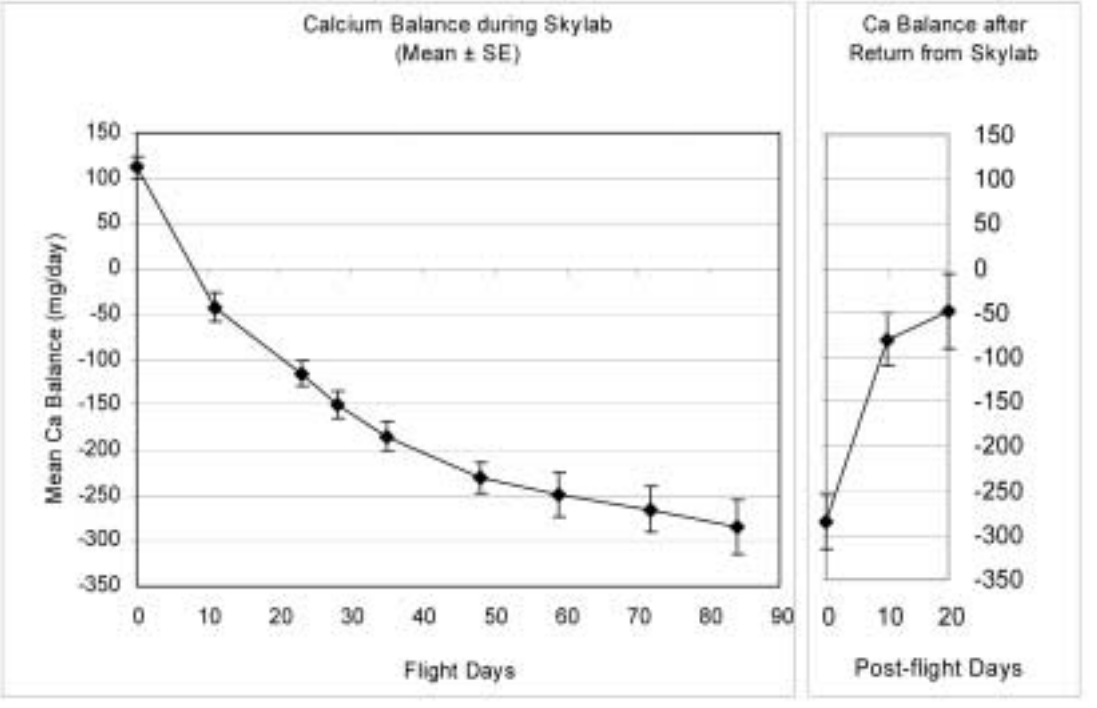

Figure 1. Calcium balance during and after Skylab missions. Adapted from Rambaut and Johnston[8].

was decommissioned. A period of U.S. and Russian collaboration began in the early 1990s, resulting in a series of visits to Mir using the Shuttle; these missions are referred to in this report as Shuttle/Mir. There were a total of 7 Shuttle/Mir missions, during which 7 U.S. astronauts and 14 Russian cosmonauts each spent 4-9 months aboard the Mir space station. The Mir and Shuttle/Mir flights were important in that the extent and location of bone loss was verified using dual X-ray absorptiometry (DXA) technology. The building of the International Space Station (ISS) began in 1999 and continues to this day. The ISS is envisioned to be a test bed for long duration missions and a model for international co-operation for future flights to the planets. In the area of bone research, the ISS flights are noted for the implementation of QCT measurements that documented the compartmental nature of bone loss, long speculated but not heretofore proven. The U.S. and Russian manned space flight programs are summarized in Table 1.

Against the backdrop of these U.S. and Russian space missions, this review article will summarize the human data developed to date regarding microgravity-induced bone loss and recovery. In addition, relevant findings from ground-based analog (e.g., bed rest) studies will be summarized. A brief discussion of potential countermeasures to space flight-induced bone loss will also be discussed. Due to limited space in this review paper, only selected data (primarily from published studies) will be presented; the interested reader is referred to the original publications for more detailed information. The history of animal bone research in space will be covered in a companion paper in this issue.

U.S.		**Russian**	
Mercury	1961 - 1963	Vostok	1961 - 1963
Gemini	1965 - 1966	Voskhod	1964 - 1965
Apollo	1968 - 1972	Soyuz	1967 - 1973
Skylab	1973 - 1974	Soyuz/Salyut	1971 - 1985
Shuttle	1981 - present	Mir	1986 – 2000
Joint U.S.-Russian			
Apollo-Soyuz		1975	
Shuttle/Mir		1995 - 1998	
International Space Station		1999 - present	

Table 1. U.S. and Russian manned space programs.

Space flight bone studies

Gemini and Apollo

During the Gemini missions, bone changes in the hand and foot were measured using X-ray photodensitometry[1]. Although these flights were less than 2 weeks in duration, the measurements demonstrated large losses of roentgenographic bone mineral density ranging from 3 to 23%. These results, however, were later determined to be inaccurate because of technical problems with the methodology[2].

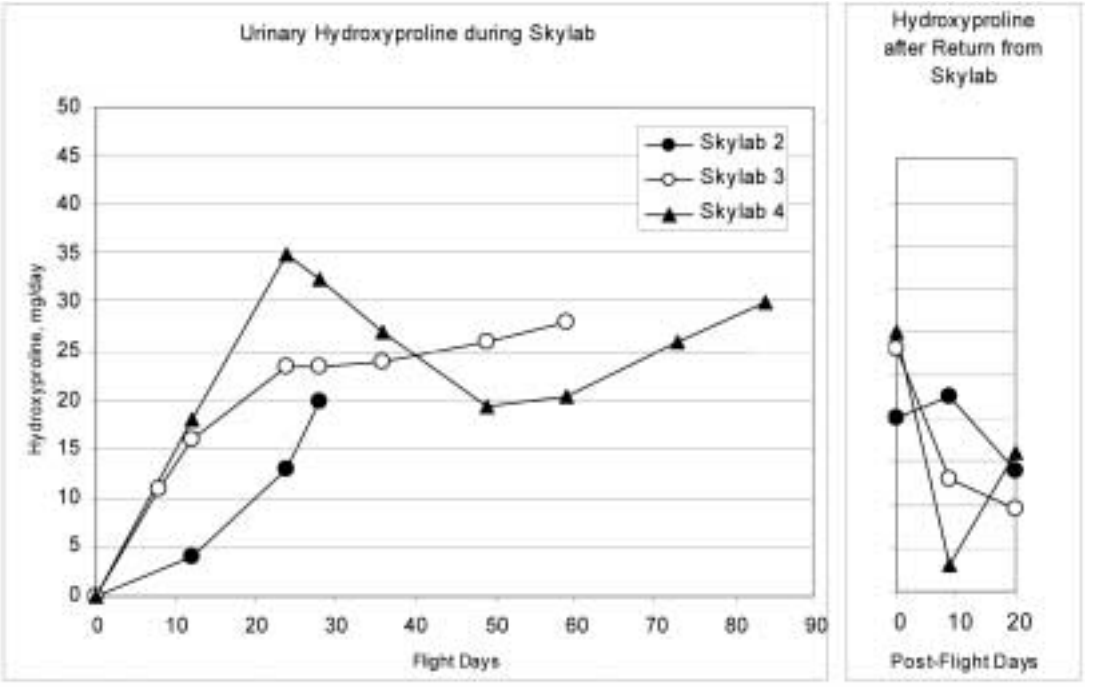

Figure 2. Urinary hydroxyproline during and after Skylab missions. Adapted from Rambaut and Johnson[8].

During the 14-day Gemini VII flight, an approximate metabolic balance study was attempted that suggested a decreased calcium (Ca) balance during flight[3].

During the 3-man Apollo missions (Apollo 7, 8, 9, 11), attempts were again made to measure Ca balance; the results indicated that significant negative balance may have occurred during flight[4]. During the Apollo 17 mission, a more controlled metabolic balance study was conducted showing a negative Ca balance of 137 mg/day. In addition, regional bone density measurements were made using a device designed and built for these missions[5]. This device was one of the first single-photon densitometers used to measure the bone density of the wrist and heel. Measurements were made using this device on Apollo 14, 15, and 16[6]. No changes in the wrist were reported in the nine crewmembers; however losses of 5 and 6% were reported in the calcanei of two of the three crewmembers of Apollo 15. The bone studies conducted during Apollo confirmed that bone loss might be substantial on long-duration flights, but probably not of concern for missions on the order of weeks in duration.

Skylab

During the 3 manned Skylab flights (lasting 29, 59 and 84 days), a comprehensive series of biochemical measurements was made. For bone, these measurements included: single photon densitometry of the wrist and heel; urine and fecal Ca excretion; Ca balance; and assays of the bone resorption marker urinary hydroxyproline. Ca balance was negative for all 3 flights and during the 84-day Skylab IV flight, Ca balance averaged -200mg/day[7-9]. Ca balance for all 3 flights as function of elapsed time in space is shown in Figure 1.

Accompanying the negative calcium balance, urinary hydroxyproline levels were elevated above baseline (preflight levels) throughout all 3 missions, indicating dramatically increased bone resorption[8,9]. These results are shown in Figure 2.

Densitometry of the calcaneus indicated a tendency for increased bone mineral loss with length of flight. There were no significant losses observed in the three crewmembers of Skylab 2 (29 day flight); one crewmember from Skylab 3 had a significant loss, -7.4%, (59-day flight); and two crewmembers from Skylab 4 (84-day flight) had significant losses, -4.5% and -7.9%[8]. Figure 3 shows the calcaneus data for these flights - the first data point is from the short duration Apollo 14-16 flights.

While three of the nine Skylab crewmembers lost statistically significant bone mineral from the calcaneus, there were no losses seen in the wrist. These early experiments reconfirmed that: bone loss might indeed be a problem for long-duration space flight; bone loss was inhomogeneous, i.e., no loss occurred in the upper extremities, while there were significant losses in the calcaneus; and the regional losses on a percentage basis could be much greater than percentage changes in total body bone mineral (based on Ca balance, Figure 1).

More recently, urine specimens that had been saved and

BMD and Body Composition Changes after 4-14.4 Months of Space Flight			
Variable	**N**	**% / Month Change**	**SD**
BMD Lumbar Spine	18	-1.06*	0.63
BMD Femoral Neck	18	-1.15*	0.84
BMD Trochanter	18	-1.56*	0.99
BMD Total Body	17	-0.35*	0.25
BMD Pelvis	17	-1.35*	0.54
BMD Arm	17	-0.04	0.88
BMD Leg	16	-0.34*	0.33

Table 2. Mir BMD changes. *$p<0.01$, significantly different from baseline. Adapted from LeBlanc et al.[22].

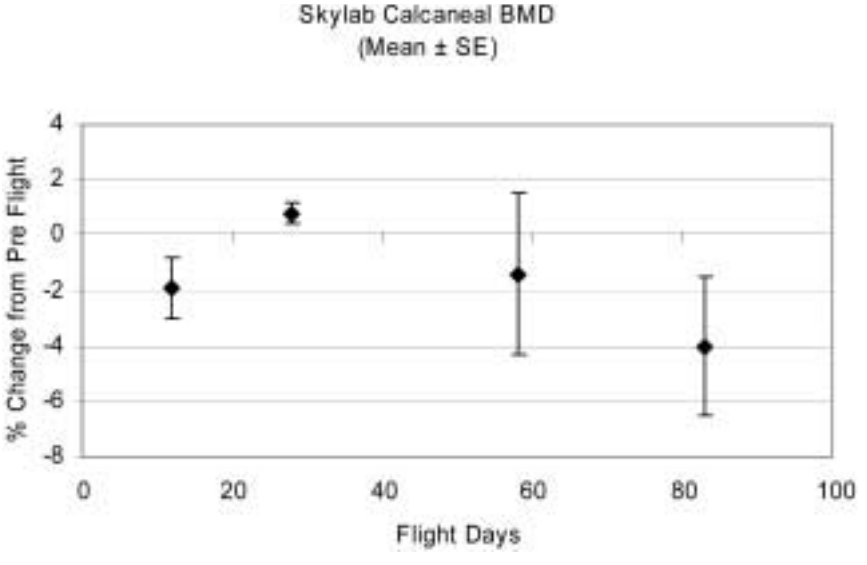

Figure 3. Calcaneal BMD during Skylab missions. Adapted from Rambaut and Johnson[8]. Left-most data point is from Apollo 14, 15 and 16.

frozen from the Skylab crewmembers (Skylab II, III, IV) were analyzed for NTX, a bone collagen breakdown product that is bone-specific (as opposed to the non-bone-specific hydroxyproline measured in the 1970s). These data showed in-flight increases in NTX of approximately 100-150% above pre-flight levels[10]. Similar changes, although of lesser magnitude, have been documented during bed rest[11-15]. These and other bed rest data will be described in more detail later. Formation markers have also been measured more recently during the Mir and Shuttle/Mir flights and will also be discussed later.

Soyuz and Mir

Russian scientists were making bone measurements similar to those of the American program and were obtaining similar findings[16,17]. The Soviets measured calcaneal bone density in cosmonauts before and after missions lasting 75 to 184 days and demonstrated losses ranging from -0.9% to -19.8%[18]. Computed tomography was used to estimate bone mineral density (BMD) changes in four cosmonauts after Salyut missions from five to seven months in length[19,20]. All four lost vertebral bone mineral density (-6.1%,-0.3%,-2.3%,-10.8%) in the lumbar spine.

In 1989, the American and Russian space agencies embarked on a collaborative endeavor to comprehensively measure the regional bone mineral changes of cosmonauts after long-duration space flight. In January 1990, a dual photon device (Hologic 1000W) was obtained and sent to the Cosmonaut Training Center in Star City, Russia, to measure bone mineral density before and after long-duration flights aboard the Mir space station. The scanning protocol included the lumbar spine, left hip, left proximal tibia, and whole body. The data from the first 7 cosmonauts were published[21]

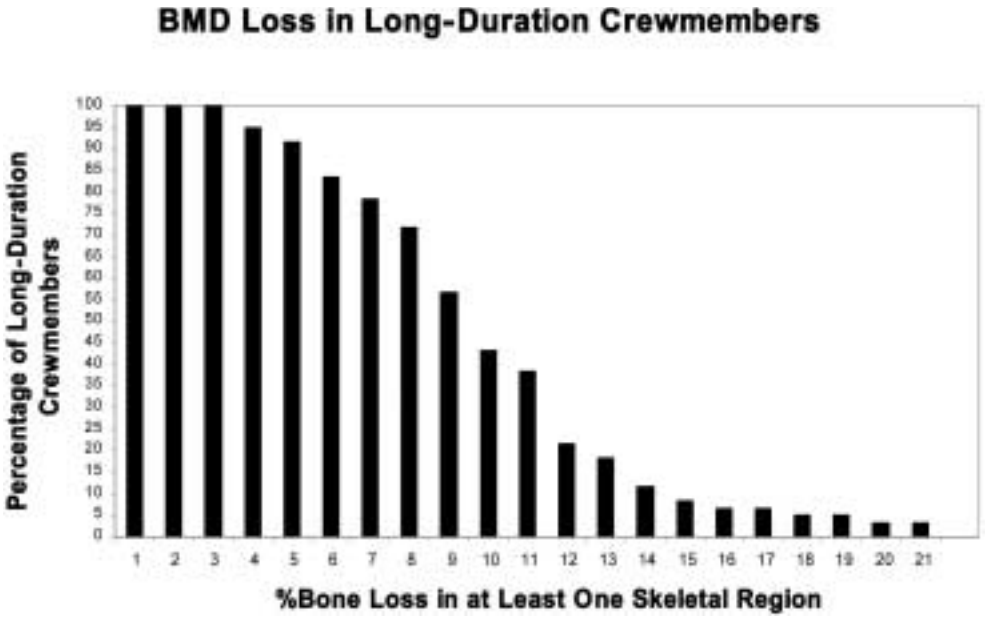

Figure 4. BMD loss in long-duration crewmembers (Mir and ISS).

and, more recently, data from 18 of the cosmonauts were published[22]. Table 2 is from the latter publication showing significant monthly BMD losses in the lumbar spine, femur neck, trochanter, pelvis, legs and whole body of 1.1%, 1.2%, 1.6%, 1.4%, 0.3%, and 0.4%, respectively. The 0.4%/month whole body BMD change is consistent with the reported average Skylab IV Ca loss of 0.5%/month calculated from Ca balance[8]. It is important to realize that although the flight changes in BMD were normalized by the time in space and therefore expressed as percent change per month, the actual rate of bone loss is not linear as indicated from Skylab Ca balance; this will also be demonstrated in bed rest studies. Large differences in BMD loss were observed between individuals and between bone sites in a given individual, a pattern that is also seen in bed rested subjects[23].

In spite of this large inter- and intra-subject variability, measurable bone loss in at least one skeletal site is seen in nearly all long-duration crewmembers. Among astronauts and cosmonauts who participated in long-duration flights aboard Mir and ISS (n=60, average duration 176 days±45 days), 92% showed a minimum 5% loss in at least one skeletal site and 43% of the crewmembers experienced a 10% or greater loss in at least one skeletal site (Figure 4). In the subset of Mir cosmonauts, 20% experienced a 14-20% loss in at least one site[24].

These losses occurred in spite of the crewmembers' participation in the Mir and ISS exercise regimens. Since most of these flights lasted 4-6.5 months, the data are inadequate to reliably determine the ultimate extent of bone loss with longer-duration flights. This is an area that still requires further data.

The Mir missions also afforded an opportunity to measure bone markers in flight. Figures 5 and 6 show published data from 4-6 month Mir missions (6 male crewmembers) for the bone resorption marker n-telopeptide (NTX) and the bone formation marker osteocalcin[25]. The data show that, as in Skylab, resorption of bone is elevated, but contrary to what early animal studies had suggested, bone formation appears to be minimally affected.

In addition to the bone marker studies described above, calcium metabolism studies were also conducted on Mir flights[26]. Calcium balance studies demonstrated a mean calcium balance of -234 mg/d, very similar to the results obtained during the earlier Skylab studies.

International Space Station (ISS)

In the year 2000, NASA funded an investigation to examine the possible compartmental changes in bone mass using quantitative computerized tomography (QCT) of the hip and spine. These studies were in addition to the routine DXA measurements performed before and after each ISS mission. High-resolution QCT scanning and DXA of the hip and spine were performed on 16 ISS crewmembers before and after 4-6-month flights. The results on the first 14 have been published[27]. Table 3 shows the published changes in integral (cortical+trabecular), cortical-only and trabecular-only volumetric bone mineral density (vBMD) in the spine and hip. Integral vBMD of the spine showed an average loss of 0.9%/mo, similar to DXA measured BMD of 0.8%/mo.

Loss of DXA BMD in the hip was similar to that of integral vBMD (-1.5%/mo) and similar also to published Mir

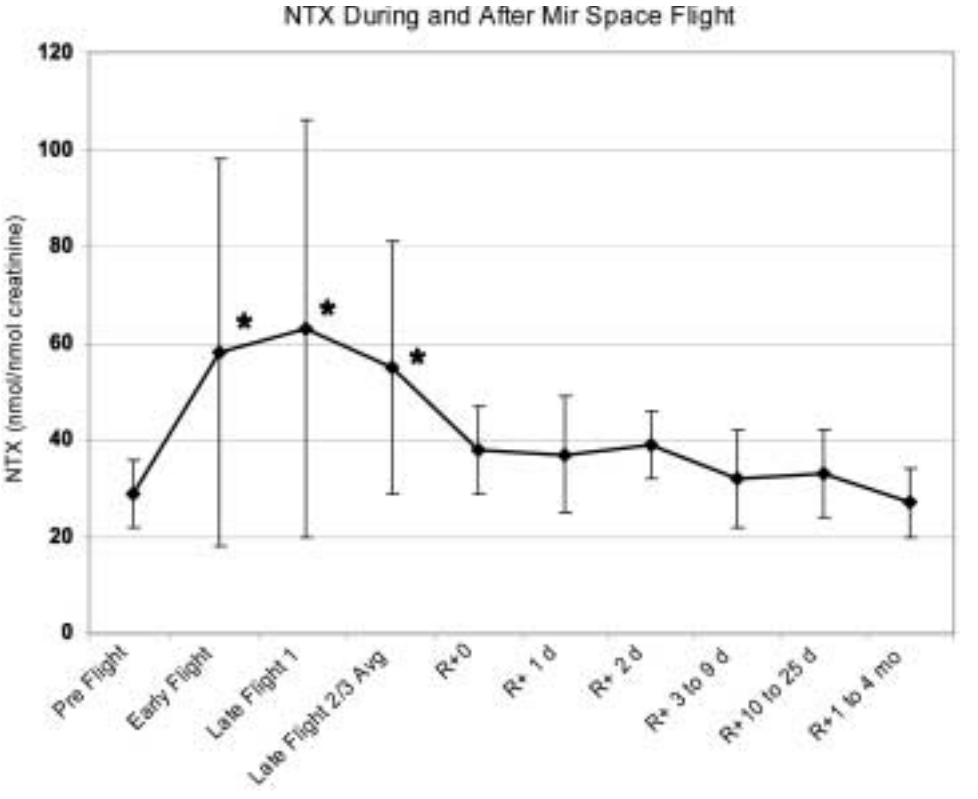

Figure 5. N-Telopeptide, Mir Space Flight. *$p<0.05$ vs. pre flight. Adapted from Smith et al.[26].

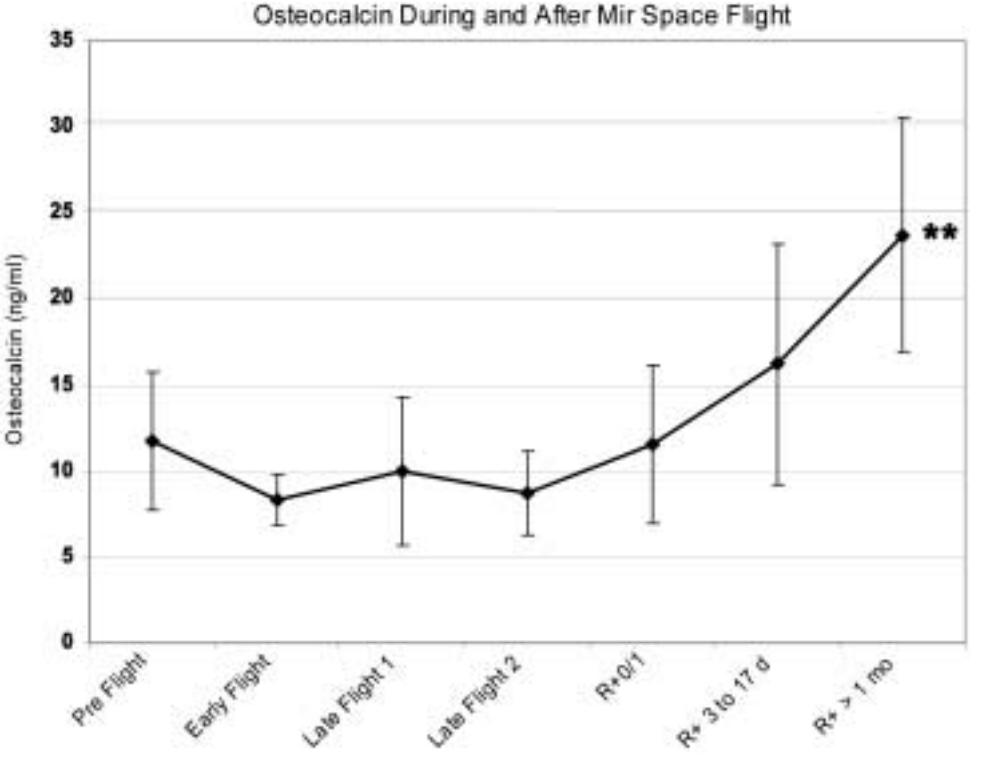

Figure 6. Osteocalcin, Mir Space Flight. **$p<0.01$ vs. pre flight. Adapted from Smith et al.[26].

results[21,22]. Trabecular bone loss of the hip measured by QCT, however, was considerably greater, -2.3%/mo. Trabecular bone losses from the hip were about 50% greater than the integral changes while compartmental differences were not significant in the spine. In addition, the QCT data demonstrated cortical thinning in the femoral neck, trochanter, and total hip, where cortical volume decreased 1.2-1.3%/mo, $p<0.05$. The loss of cortical bone appeared to take place from the endocortical side, indicating a reduction in bending strength of the femoral neck. These site-specific, compartmental changes in bone geometry are of great importance in attempting to quantify changes in bone strength, and underscore the utility of measurements such as CT (compared to standard DXA and biochemical measurements) to provide a higher-resolution view of skeletal disuse changes. These results indicate that (at least for the hip) trabecular vBMD by QCT is more sensitive than DXA for measuring space flight induced bone loss.

Measurements of the calcaneus using DXA and ultrasound in this same study[27] demonstrated that these peripheral measures were NOT useful predictors of the changes occurring in the hip or spine. Furthermore, these studies indicated that the enhanced exercise program as conducted on ISS, possibly because of exercise equipment unreliability, did not appear to mitigate bone loss during flight.

QCT Changes in BMD in 14 ISS Crewmembers (%/Month±SD)	
Lumbar Spine, Integral	-0.9±0.5**
Lumbar Spine, Trabecular	-0.7±0.6*
Total Hip, Integral	-1.4 ± 0.8 **
Total Hip, Trabecular	-2.3±0.8**
Femoral Neck, Integral	-1.2±0.7**
Femoral Neck, Trabecular	-2.7±1.9**

Table 3. QCT Changes in BMD after long-duration space flight. $^{*}p<0.01$; $^{**}p<0.001$, significantly different from baseline. Adapted from Lang et al.[27].

Body Composition Changes after 4-14.4 Months of Space Flight			
Variable	**N**	**% / Month Change**	**SD**
Lean Total Body	17	-0.57*	0.62
Lean Leg	16	-1.00*	0.73
Lean Arm	17	0.00	0.77
Fat Total Body	17	+1.79	4.66

Table 4. Body composition after space flight. $^{*}p<0.01$, significantly different from baseline. Adapted from LeBlanc et al.[22].

Recovery of bone after flight

There are limited published data on recovery of bone mineral after long duration missions. Measurements of serum and urinary bone biomarkers in crewmembers before, during and after long-duration (4-6 mos) missions have been recently published[26]. Resorption markers, which were significantly elevated (>55%) during flight, were not measurably different from pre-flight at any measurement time point after R+2 days. At landing, bone formation markers were not different from pre-flight values, but were significantly increased ($p<0.01$) after 2-3 weeks of reambulation. Although the biomarker response to reambulation appears to be rapid, recovery of BMD, as might be expected, takes significantly longer. Two sets of recovery data using DXA and QCT have been acquired; the results are currently in press. Both studies indicate that bone recovery does occur, but at a slower rate than the rate of loss during flight and complete recovery may require 1-3 years after flight. Notwithstanding the expected publication of these two articles, research is needed to document the factors that influence individual rates of recovery post-flight and, in some individuals, the lack of recovery.

Changes in body composition during long-duration flight

Published losses in whole body mass of long-duration crewmembers are in the range of 0.5 to 5.0 kg[28,29]. Whole body DXA data from long-duration Mir missions of 4-6 months in length (n=14) show that these crewmembers lost an average of approximately 1.7 kg of body mass[29]. The whole body lean mass changes averaged approximately -2 kg and were partially offset by an increase of approximately 0.5 kg of whole body fat. Regional changes in lean tissue (fat-free, bone-free) mass were obtained by DXA on 16-17 cosmonauts after 4-14.4 months of space flight[22]. These results, shown in Table 4, reveal that significant losses occurred in the legs but not the arms. Muscle volume was measured by MRI in 16 Mir crewmembers (4 to 6-month missions) and showed decreases of approximately 15% in muscles of the back and legs, with the greatest change (on a percentage basis) in the lower leg[29]. These regional changes in muscle mirror those of bone, with significant losses occurring in the lower extremities and no significant changes measured in the upper body (e.g., arms). While muscle atrophy and bone loss follow a similar, regional pattern of loss and are presumably interrelated, the available data show no significant correlation between individual changes in bone and muscle. Such correlations are made difficult due to small subject numbers, differences in rates of loss between muscle and bone and measurement variability. In addition, no conclusions can be drawn about the relationship between in-flight exercise and lean tissue mass (or, for that matter, between in-flight exercise and bone), as no reliable data exist on individual in-flight exercise regimens for these missions. Further study is needed to elucidate the relationships among in-flight exercise, lean tissue mass, and bone.

Bed Rest	Space Flight
Urinary Ca ↑	Urinary Ca ↑
Fecal Ca ↑	Fecal Ca ↑
Ca Balance ↓	Ca Balance ↓ ↓
BMD ↓	BMD ↓ ↓
Resporption ↑	Resorption ↑ ↑
Formation ↔	Formation ↓ ↔
PTH ↓	PTH ↓
Ca Absorption ↓	Ca Absorption ↓
Ca+, Serum Ca ↔	Ca+, Serum Ca ↔

Table 5. Bone related changes: bed rest vs. space flight.

Artificial Gravity	Short or long-arm centrifuge
Exercise	Aerobic
	Resistive
Pharmacologic	Bisphosphonates
	PTH
	OPG/RANK-L
Other	Vibration

Table 6. Potential countermeasures to space flight induced bone loss.

Bed rest studies

Bed rest has long been used as an analog for space flight to study the nature of disuse bone loss and, more recently, to test possible countermeasures. Bed rest-induced changes in BMD, calcium excretion, calcium balance, and bone markers have been shown to be qualitatively similar to (although quantitatively somewhat less than) those documented in space flight[21,22,25].

Changes in BMD

Several 17-week horizontal bed rest studies were carried out by NASA over a period of 13 years. The results of the first 6 male control subjects were published in 1990[23]. The results of 18 control subjects (13 males, 5 females) were published after all the studies were completed[13]. In this series of bed rest studies, DXA was used to measure changes in BMD before and at the end of a 17-week period of continuous, horizontal bed rest. Mean±SE changes were as follows: Lumbar Spine -1.3±0.6, Femoral Neck -1.5±0.7, Trochanter -3.6±0.6, Total Hip -3.4±0.6, Pelvis -3.3±0.7. All changes except those in the femoral neck were statistically significant ($p<0.05$). As with space flight, the greatest changes were noted in the hip and pelvis. There was no change in BMD in the distal radius. These results highlight the regional nature of disuse bone loss, with the greatest losses occurring in weight-bearing skeletal sites. Zerwekh et. al., conducted a 12-week bed rest study (11 males, 2 females), with similar results, including a statistically significant change in the trochanter of -3.8%[14]. Watanabe et al., measured BMD in male subjects before and after 90 days of 6° head-down-tilt bed rest. Total hip BMD decreased ~5%, while forearm BMD remained unchanged[15].

Changes in bone markers

As in space flight, bed rest causes an increase in bone resorption, documented by histomorphometry[30] or biochemical markers[11-14,31-33]. As with space flight, bone resorption appears to increase soon after disuse (e.g., bed rest) is initiated. Urinary calcium and/or bone resorption markers have been shown to increase within the first 4-7 days after the start of bed rest[32,34,35]. During the bed rest period, subjects have shown an increase in bone resorption markers of approximately 50% compared to baseline[12,13,15,32]. In comparison, space flight appears to show a greater response[10,26]. Furthermore, bed rest subjects show essentially no change in bone formation markers[11,14,31,33], supporting the idea that musculoskeletal disuse causes an uncoupling of the normal bone remodeling cycle. Collectively, these studies support the validity of bed rest as a disuse model for space flight skeletal changes. Table 5 summarizes the changes documented in ground-based vs. space flight studies.

Recovery of bone after bed rest

There are limited data on the subject of long-term bone recovery following disuse, but the studies published to date indicate that, as with space flight, resorption markers return to baseline following reambulation and formation markers increase during reambulation[11,14]. Zerwekh et al. measured markers of bone resorption and formation after 12 days of reambulation following 12 weeks of bed rest. Serum bone formation markers osteocalcin and BSAP were statistically unchanged vs. baseline, but pro-collagen type I C-terminal peptide (PICP) showed a significant ($p<0.05$) increase of 20% over pre-bed rest values[14]. After 12 days of reambulation, urinary bone resorption markers in this study were all still significantly elevated compared to pre-bed rest, but showed a trend toward reversing from the higher levels documented during the bed rest period. LeBlanc et al., reported similar findings after 17 weeks of bed rest (8 male subjects)[11]. Bone formation markers were elevated in the 5-6 weeks following bed rest, while bone resorption markers showed a return toward baseline. In another study involving 90 days of bed rest, bone markers were measured in 9 control subjects before and during bed rest, as well as during the 6-12 months following reambulation[15]. Serum markers of bone formation showed a slight increase vs. baseline throughout both the bed

rest and recovery periods. Bone resorption markers, which were elevated approximately 60% during bed rest, returned toward baseline during the recovery period; n-telopeptides returned to within 20% of baseline after 6 months of reambulation, while serum CTX-β values showed a complete return to pre-bed rest levels.

DXA measurements were performed on six male bed rest subjects following 17-weeks of continuous bed rest[23]. Data were obtained after approximately 6 months of reambulation. These data showed a return toward baseline BMD values, but at a rate that was generally 2-3 times lower than the rate of the original bone loss. The bed rest data agree with the space flight recovery data described above, where recovery appears to take 1-3 years following 4-6-month missions and where there are significant inter-subject differences in rates of recovery. As with the space flight studies, information on the role of subjects' habits (diet, exercise) in the post-disuse period are lacking and further work is needed to elucidate the role such factors play in the rate and degree of BMD recovery following disuse.

Countermeasures to disuse bone loss

Table 6 lists the potential countermeasures that could be used to prevent space flight-induced bone loss.

Artificial gravity

The most desirable and intuitively obvious countermeasure against space flight-induced deconditioning would be to prevent the reduced physical activity and possibly fluid shifts caused by microgravity. Simply introducing gravity-like conditions, as in the movie "2001: A Space Odyssey," is a difficult and expensive engineering task. The required magnitude and duration of g-forces required is not known. Use of a short-arm centrifuge to create the g-forces would likely have to overcome several potential negative side effects, including Coriolis effects, motion sickness, dizziness and possible effects on cognitive or motor function. Use of a long-arm centrifuge would obviate many of these concerns, but would have its own drawbacks in terms of size, mass, and cost. There is a NASA program ongoing to test whether artificial gravity, produced by spinning individuals in a short-arm centrifuge, would serve this purpose. The actual prescription, e.g., the length of time per day or per week and g forces necessary to accomplish a satisfactory effect are unknown and need to be determined.

Exercise

The most frequently attempted countermeasure to date has consisted of various exercise prescriptions conducted in the microgravity environment. These have been effective to varying degrees for several physiological systems, but have not been very effective in attenuating bone loss. An important caveat to conclusions concerning effectiveness, however, is whether crew compliance with the prescription and equipment reliability in space has been adequate. For many years, cosmonauts have been required to maintain physical fitness while in microgravity using bungee cord resistive exercises, as well as bicycle ergometer and treadmill aerobic exercises[36]. In spite of this exercise countermeasure program, significant bone losses, as discussed previously, were documented in Mir and Shuttle/Mir crewmembers. More recently, these early efforts were extended to the ISS program, with added emphasis on resistive exercise. An American device called the Interim Resistive Exercise Device (iRED) was placed aboard the ISS space station in 2001 to allow greater resistive exercise for crewmembers. Even so, the ISS bone DXA and QCT bone density studies indicate that the problem of protecting bone with exercise has not been solved[27]. It is also possible that, if loading intensity can be increased sufficiently, the exercise countermeasure might have a more positive effect on bone and crew time requirements could be shortened.

Per Cent Change from Pre Bed Rest BMD			
	Controls (n=18)	**Resistive Exercise (n=9)**	**Control vs. Resistive Exercise, *p* value**
Lumbar Spine	-1.3*	3.4*	<0.01
Femoral Neck	-1.5*	0.1	NS
Trochanter	-3.6*	-2.3	NS
Pelvis	-3.3	-0.5	<0.05
Calcaneus	-9.2*	1.2	<0.05
Distal Radius	0	-1.0	NS

Table 7. Change in BMD during bed rest, with and without resistive exercise. *$p<0.05$, significantly different from baseline. Adapted from Shackelford et al.[13].

Table 7 shows the results of a 17-week bed rest study in which resistive exercise was tested as a countermeasure to bone and muscle loss[13]. In this study, 5 male and 4 female bed rest subjects participated in a strenuous resistive exercise program 6 days per week. All exercises were performed supine using an exercise device built specifically for the study.

Control subjects had an increase in resorption markers and essentially no change in bone formation markers, as demonstrated in previously mentioned studies. The exercise group, on the other hand, had an increase in both resorption and formation markers, suggesting that resistance exercise caused an increased rate of bone turnover and possibly less uncoupling. These results are shown in Figure 7 (resorption marker NTX) and Figure 8 (formation marker BSAP).

Vibration

Rubin et al., are studying the efficacy of mechanical vibration in preventing bone loss. These investigators reported on

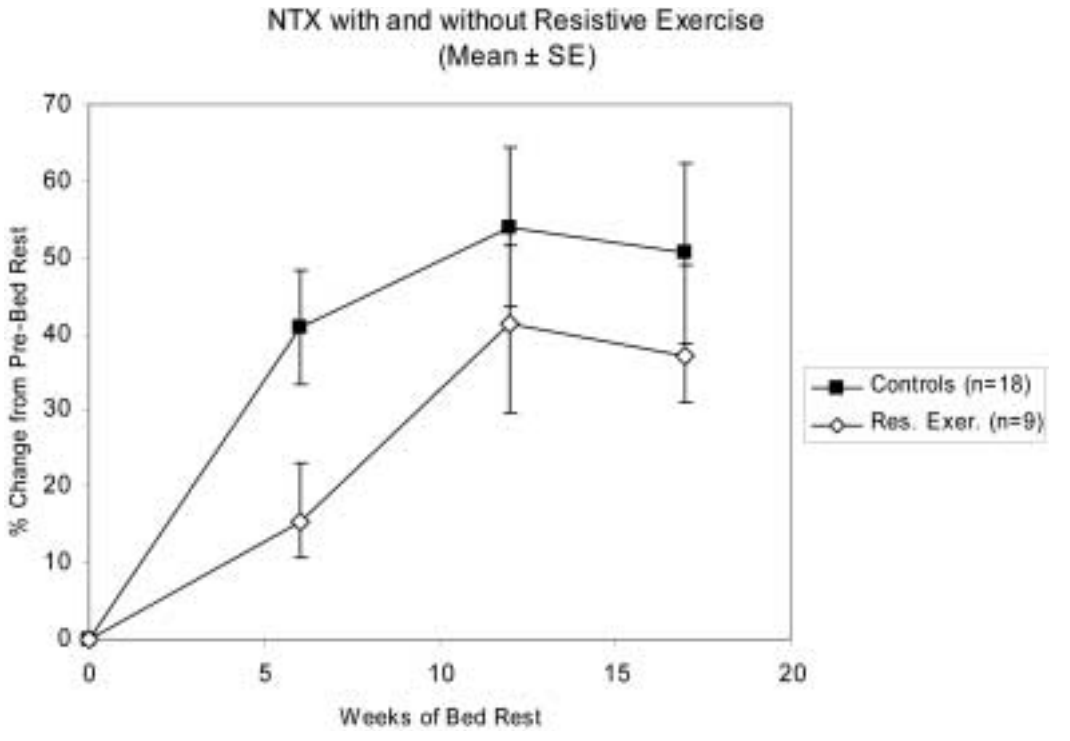

Figure 7. Change in NTX during bed rest, with and without resistive exercise. Adapted from Shackelford et al.[13].

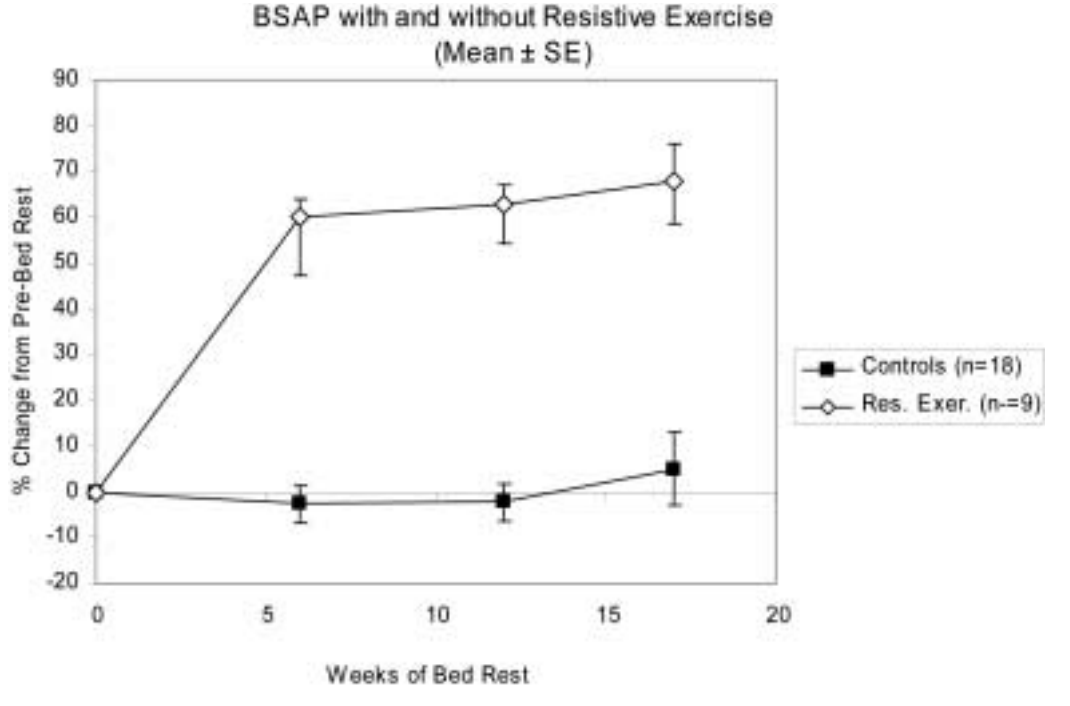

Figure 8. Change in BSAP during bed rest, with and without resistive exercise. Adapted from Shackelford et al.[13].

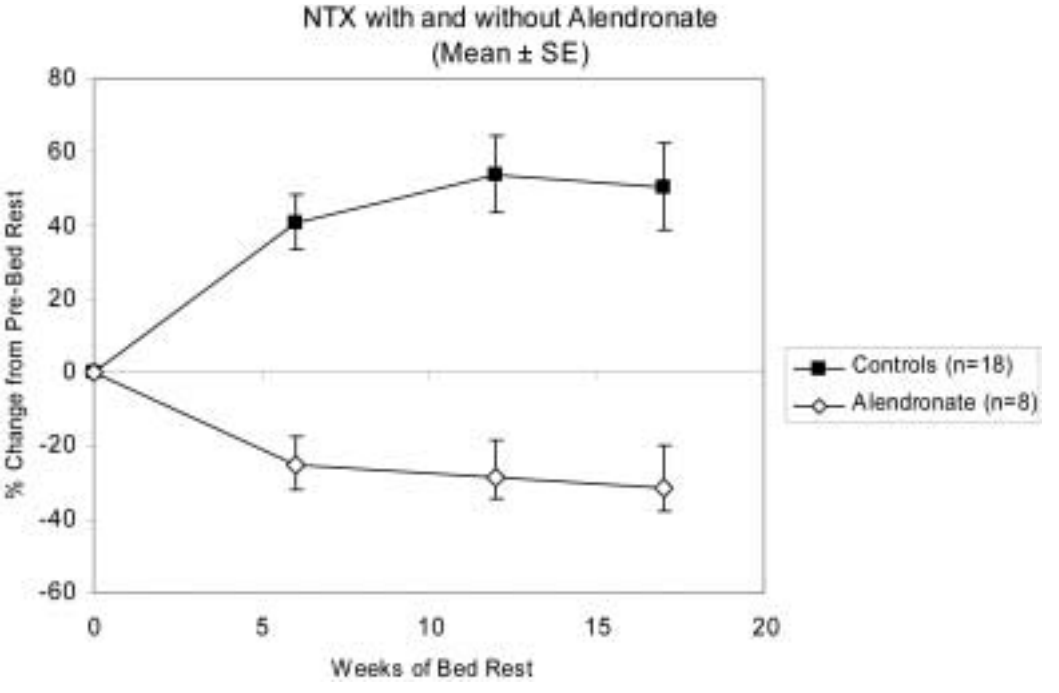

Figure 9. Change in N-Telopeptide during bed rest, with and without alendronate. Adapted from LeBlanc et al.[12].

56 women who completed 1 year of treatment with either 20 min/day of low-magnitude, high-frequency mechanical vibration or placebo[37]. While the DXA results did not show any changes in bone density based on intention-to-treat analysis, post hoc testing revealed possible protective effects in the quartile of subjects who had the highest level of compliance with the treatment. A study to examine the efficacy of this treatment during bed rest is underway.

Pharmaceuticals

Bisphosphonates

A pharmaceutical countermeasure, either alone or in combination with exercise, has several potential benefits over exercise alone. A pharmaceutical countermeasure, implemented either before flight or involving intermittent dosing during flight, would avoid many of the drawbacks of exercise countermeasures, which include concerns relating to volume, mass, energy, noise, vibration, equipment malfunction, and - above all - crew time. Exercise has benefits for other body systems besides bone, so it is not reasonable to suggest replacing exercise altogether. For the purposes of protecting bone, however, it appears that other strategies (e.g., pharmaceuticals) will need to be considered and tested. The leading candidate for a space flight countermeasure is a class of drugs called bisphosphonates. Examples of so-called third generation bisphosphonates that have been developed to treat osteoporosis and other skeletal-related disorders include alendronate, taken as an oral 70 mg/wk dose; ibandronate, taken as a monthly 150 mg oral dose; and zoledronic acid, taken via a single 4 to 5-mg I.V. infusion every 12 months.

Oral alendronate was tested in a 17-week bed rest study conducted at Baylor College of Medicine[12]. An oral weekly dose of 70 mg alendronate was tested in 8 male subjects and compared with 13 male untreated controls. Efficacy was evaluated using DXA, urinary Ca, fecal Ca, Ca balance, bone formation and resorption markers, serum Ca, ionized Ca, PTH and vitamin D. Figures 9 and 10 show the effects on NTX (a bone resorption marker) and bone specific alkaline phosphatase (a bone formation marker), respectively. The effectiveness of the bisphosphonate to reduce remodeling is evident. As with resistance exercise, alendronate positively affected BMD, as is seen in Table 8[12].

A double-blind, placebo-controlled, randomized study of 15 spinal cord injury patients given either (a) zoledronic acid 4 mg or 5 mg as a single infusion or (b) placebo within 10 weeks of injury has been completed[38]. DXA scans of the hip and lumbar spine were performed at 0, 6 and 12 months. Compared to placebo, zoledronic acid had a protective effect on the hip. BMD and cortical thickness showed reduced declines compared to placebo[39]. The treatment effects were most prominent in the femur shaft. While there have been no published results of bed rest studies employing zoledronic acid, results have been published for a bed rest study involving IV pamidronate[15]. This study tested resistive exercise and IV pamidronate in healthy male test subjects undergoing 90 days of bed rest. While exercise increased bone formation, it

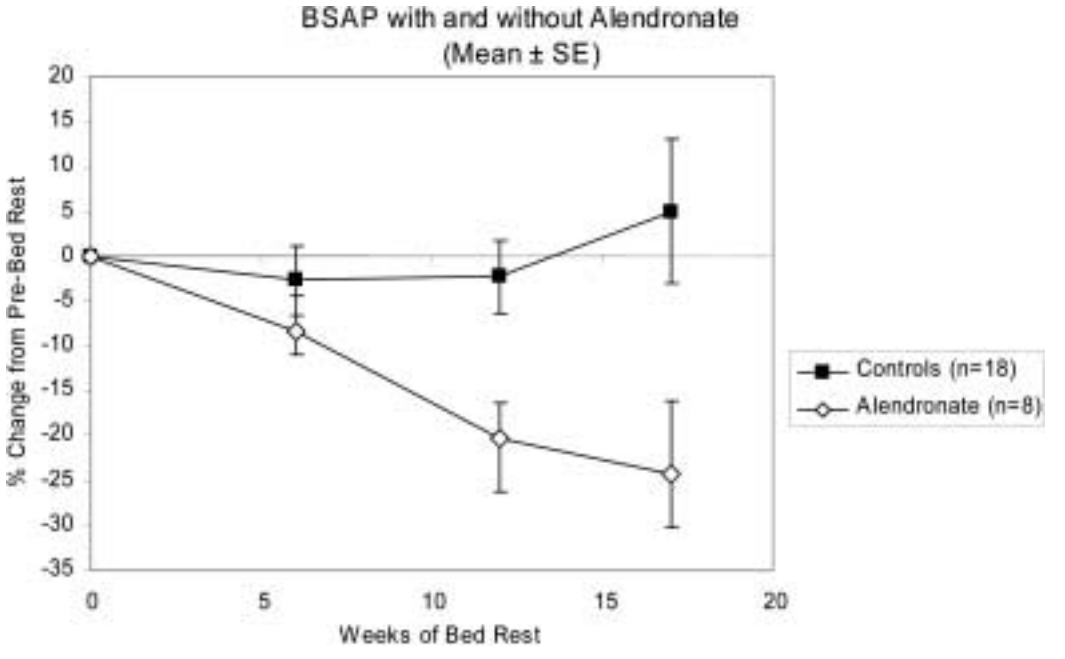

Figure 10. Change in BSAP during bed rest, with and without alendronate. Adapted from LeBlanc et al.[12].

did not prevent bone loss. IV pamidronate administered 14 days before bed rest prevented bone loss. It is noted that pamidronate is several times less potent than zoledronic acid and requires administration at 3-month intervals compared to once every 12 months for IV zoledronic acid.

NASA has approved a study "Bisphosphonates as a Countermeasure to Space Flight Induced Bone Loss" to test whether either IV administered zoledronic acid (4 mg once before flight) or oral alendronate (70 mg/wk before and during flight) are effective in preventing space flight-induced bone loss. From the results of the ground based studies described above, it is expected that both agents will be effective.

Percentage Change from Pre-Bed Rest BMD			
	Controls (n=13)	**Alendronate (n=8)**	**Control vs. Alendronate *p* value**
Lumbar Spine	-1.6*	1.8	<0.05
Femoral Neck	-2.0*	2.1*	<0.01
Trochanter	-3.9**	0.0	<0.01
Pelvis	-3.6**	1.6	<0.01
Calcaneus	-10.3**	-4.9*	NS
Distal Radius	-0.5	0.7	NS

Table 8. Change in BMD during bed rest, with and without alendronate. *$p<0.05$; **$p<0.01$, significantly different from baseline. Adapted from LeBlanc et al.[12].

Anabolic Agents

Anabolic agents such as PTH have not been tested in space flight or bed rest studies. The only anabolic agent approved by the FDA for the treatment of osteoporosis is the recombinant teriparatide, or rhPTH. Teriparatide is a PTH fragment that has shown efficacy in increasing vertebral BMD and reducing both vertebral and non-vertebral fracture risk in osteoporotic women[40]. Some safety questions have been raised based on the results of rat, but not monkey or human, studies. Teriparatide also requires daily injection and has not been tested in bed rest. A PTH-related peptide, PTHrP, also has an anabolic effect on bone formation[41] and clinical trials are underway to study the effects of this molecule. While these anabolic agents are too early in their development cycle to warrant their use as space flight countermeasures, such compounds may hold promise for future application.

OPG and RANK-L Compounds

Osteoprotegerin (OPG) was identified several years ago as a possible candidate for osteoporosis therapy and tested in clinical trials for osteoporosis[42] and metastatic cancer[43]. OPG acts as a decoy receptor to which the receptor activator of nuclear factor-KB ligand (RANK-L) has binding affinity. RANK-L normally binds to osteoclastic precursor cells, and the interruption of this event should prevent bone resorption by osteoclasts. OPG has fallen out of favor recently due to issues raised regarding its efficacy and safety, but has been replaced by the development of a human monoclonal antibody for RANK-L. Amgen is currently testing

such a monoclonal antibody, AMG 162, for the treatment of osteoporosis[44,45]. Like the PTH compounds described above, compounds such as AMG 162 are in clinical testing and will require more ground-based testing (e.g., bed rest) and safety verification before they can be considered for use as countermeasures to space flight induced bone loss.

Summary

1. Astronauts and cosmonauts who participated in long-duration flights aboard Mir and ISS have shown consistent loss of regional bone mineral, with 92% experiencing a minimum 5% loss in at least one skeletal site and over 40% experiencing a 10% or greater loss in at least one skeletal site. These losses occurred in spite of the crewmembers' participation in exercise regimens aboard the Mir and ISS space stations.
2. All data to date support the idea that the lack of mechanical forces on the skeleton in microgravity leads to increased bone remodeling. A now commonly accepted idea is that increased remodeling by itself is associated with a weaker skeleton and an increased fracture risk over and above that caused by actual bone loss.
3. In addition to increased remodeling, space flight remodeling is uncoupled, i.e., resorption is increased while formation is little changed.
4. This uncoupling of bone resorption and formation leads to increased urinary and fecal calcium excretion and negative Ca balance.
5. Negative Ca balance is manifested in bone loss. This loss is greatest in the lower limbs, i.e., pelvis, hips, and legs, with little loss in the upper skeleton. Trabecular loss in the hip is 50% greater than the integral (trabecular + cortical) loss. Although the trabecular bone loss on a percentage basis is greater than cortical bone loss in certain skeletal sites, on a mass basis the greatest loss is from cortical bone.
6. A consistent finding is the large variation in bone loss between bones sites and between individuals. These observations are seen in space flight and in bed rest studies. In the case of bed rest, physical activity, body weight and diet are strictly controlled, suggesting that genetic variation may play an important role. Recovery of bone after bed rest or space flight does occur, but the rate of recovery is variable and ultimate recovery may take several years. There is evidence of architectural changes, i.e., geometric expansion at the femur neck.
7. Most efforts to create an effective countermeasure to space-induced deconditioning during flight have involved exercise. A practical prescription has not as yet been demonstrated. Anti-resorptive agents such as bisphosphonates have been shown to be effective in ground-based analogue studies but have not yet been tested in flight. It is likely that some combination of exercise with drug therapy will prove to be effective in preventing space flight induced bone loss.

References

1. Mack PB, LaChance PL. Effects of recumbency and space flight on bone density. Am J Clin Nutr 1967; 20:1194-1205.
2. Vose GP. Review of roentgenographic bone demineralization studies of the Gemini space flights. Am J Roentgenol Radium Ther Nucl Med 1974; 121:1-4.
3. Lutwak L, Whedon GD, Lachance PA, Reid JM, Lipscomb HS. Mineral, electrolyte and nitrogen balance studies of the Gemini-VII fourteen-day orbital space flight. J Clin Endocrinol Metab 1969; 29:1140-1156.
4. Brodzinski RL, Rancitelli LA, Haller WA, Dewey LS. Calcium, potassium, and iron loss by Apollo VII, 8, IX, X and XI astronauts. Aerosp Med 1971; 42:621-626.
5. Vogel JM, Anderson JT. Rectilinear transmission scanning of irregular bones for quantification of mineral content. J Nucl Med 1972; 13:13-18.
6. Rambaut PC, Leach CS, Johnson PC. Calcium and phosphorus change of the Apollo 17 crew members. Nutr Metab 1975; 18:62-69.
7. Rambaut PC, Leach CS, Whedon GD. A study of metabolic balance in crewmembers of Skylab IV. Acta Astronaut 1979; 6:1313-1322.
8, Rambaut PC, Johnston RS. Prolonged weightlessness and calcium loss in man. Acta Astronaut 1979; 6:1113-1122.
9. Whedon GD, Lutwak L, Rambaut PC, Whittle MW, Smith MC, Reid J, Leach CS, Stadler CR, Sanford DD. Mineral and nitrogen metabolic studies, experiment M071. In: Johnson RS, Dietlein LE (eds) Biomedical Results from Skylab NASA SP-377. NASA, Washington, DC; 1977:164-174.
10. Smith SM, Nillen JL, LeBlanc A, Lipton A, Demers LM, Lane HW, Leach CS. Collagen cross-link excretion during space flight and bed rest. J Clin Endocrinol Metab 1998; 83:3584-3591.
11. LeBlanc A, Schneider V, Spector E, Evans H, Rowe R, Lane H, Demers L, Lipton A. Calcium absorption, endogenous excretion, and endocrine changes during and after long-term bed rest. Bone 1995; 16(Suppl.4):301S-304S.
12. LeBlanc AD, Driscol TB, Shackelford LC, Evans HJ, Rianon NJ, Smith SM, Feeback DL, Lai D. Alendronate as an effective countermeasure to disuse induced bone loss. J Musculoskelet Neuronal Interact 2002; 2:335-343.
13. Shackelford LC, LeBlanc AD, Driscoll TB, Evans HG, Rianon NG, Smith SM, Spector E, Feeback DL, Lai D. Resistance exercise as a countermeasure to disuse induced bone loss. J Appl Physiol 2004; 97:119-129.
14. Zerwekh JE, Ruml LA, Gottschalk F, Pak CY. The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. J Bone Miner Res 1998; 13:1594-1601.

15. Watanabe Y, Ohshima H, Mizuno K, Sekiguchi C, Fukunaga M, Kohri K, Rittweger J, Felsenberg D, Matsumoto T, Nakamura T. Intravenous pamidronate prevents femoral bone loss and renal stone formation during 90-day bed rest. J Bone Miner Res 2004; 19:1771-1778.
16. Biriukov EN, Kraasnykh IG. Changes in the optical density of bone tissue and in the calcium metabolism of the astronauts. In: Nikivaev AG, Sevastianov VI (eds) Kosmicheskaia Biologiia I Meditsina, Moscow; 1970:42-45.
17. Gazenko OG, Genin AM, Egorov AD. Summary of medical investigations in the U.S.S.R. manned space missions. Acta Astronaut 1981; 8:907-917.
18. Stupakov GP, Kazeikin VS, Kozlovskii AP, Korolev VV. Evaluation of the changes in the bone structures of the human axial skeleton in prolonged space flight. Kosm Biol Aviakosm Med 1984; 18:33-37.
19. Oganov VS, Cann C, Rakhmanov AS, Ternovoi SK. Study of the musculoskeletal system of the spine in humans after long-term space flights by the method of computerized tomography. Kosmicheskaia Biologiia I Aviakosmicheskaia Meditsina 1990; 24:20-21.
20. Schneider VS, LeBlanc AD, Taggart LC. Bone and mineral metabolism. In: Nicogossian AE, Huntoon CL, Pool SL (eds) Space Physiology and Medicine. Lea & Febiger, Philadelphia, PA; 1994:327-333.
21. Oganov VS, Grigoriev AI, Voronin LI, Rakhmanov AS, Bakulin AV, Schneider VS, LeBlanc AD. Bone mineral density in cosmonauts after flights lasting 4.5-6 months on the Mir orbital station. Aviakosm Ekolog Med 1992; 26:20-24.
22. LeBlanc A, Schneider V, Shackelford L, West S, Oganov V, Bakulin A, Voronin L. Bone mineral and lean tissue loss after long duration space flight. J Musculoskelet Neuronal Interact 2000; 1:157-160.
23. LeBlanc AD, Schneider VS, Evans HG, Engelbretson DA, Krebs JM. Bone mineral loss and recovery after 17 weeks of bed rest. J Bone Miner Res 1990; 5:843-850.
24. LeBlanc AD, (unpublished data).
25. Smith SM, Wastney ME, Morukov BV, Larina IM, Nyquist LE, Abrams SA, Taran EN, Shih CY, Nillen JL, Davis-Street JE, Rice BL, Lane HW. Calcium metabolism before, during, and after a 3-mo spaceflight: kinetic and biochemical changes. Am J Physiol 1999; 277(1 Pt 2):R1-10.
26. Smith SM, Wastney ME, O'Brien KO, Morukov BV, Larina IM, Abrams SA, Davis-Street JE, Oganov V, Shackelford L. Bone markers, calcium metabolism, and calcium kinetics during extended-duration space flight on the Mir space station. J Bone Miner Res 2005; 20:208-218.
27. Lang T, LeBlanc A, Evans H, Lu Y, Genant H, Yu A. Cortical and trabecular bone mineral loss from the spine and hip in long-duration spaceflight. J Bone Miner Res 2004; 19:1006-1012.
28. Lane HW, Gretebeck RJ, Smith SM. Nutrition, endocrinology, and body composition during space flight. Nutr Res 1998; 18:1923-1934.
29. LeBlanc A, Lin C, Shackelford L, Sinitsyn V, Evans H, Belichenko O, Schenkman B, Kozlovskaya I, Oganov V, Bakulin A, Hedrick T, Feeback D. Muscle volume, MRI relaxation times (T2), and body composition after spaceflight. J Appl Physiol 2000; 89:2158-2164.
30. Vico L, Chappard D, Alexandre C, Palle S, Minaire P, Riffat G, Morukov B, Rakhmanov S. Effects of a 120 day period of bed-rest on bone mass and bone cell activities in man: attempts at countermeasure. Bone Miner 1987; 2:383-394.
31. Van der Wiel HE, Lips P, Nauta J, Kwakkel G, Hazenberg G, Netelenbos JC, Van der Vijgh WJ. Intranasal calcitonin suppresses increased bone resorption during short-term immobilization: a double-blind study of the effects of intranasal calcitonin on biochemical parameters of bone turnover. J Bone Miner Res 1993; 8:1459-1465.
32. Inoue M, Tanaka H, Moriwake T, Oka M, Sekiguchi C, Seino Y. Altered biochemical markers of bone turnover in humans during 120 days of bed rest. Bone 2000; 26:281-286.
33. Smith SM, Davis-Street JE, Fesperman JV, Calkins DS, Bawa M, Macias BR, Meyer RS, Hargens AR. Evaluation of treadmill exercise in a lower body negative pressure chamber as a countermeasure for weightlessness-induced bone loss: a bed rest study with identical twins. J Bone Miner Res 2003; 18:2223-2230.
34. Arnaud SB, Sherrard DJ, Maloney N, Whalen RT, Fung P. Effect of 1-week head-down tilt bed rest on bone formation and the calcium endocrine system. Aviat Space Environ Med 1992; 63:14-20.
35. Lueken SA, Arnaud SB, Taylor AK, Baylink DJ. Changes in markers of bone formation and resorption in a bed rest model of weightlessness. J Bone Miner Res 1993; 8:1433-1438.
36. Nicogossian AE, Sawin CF, Grigoriev AI. Countermeasures to space deconditioning. In: Nocogossian AE, Huntoon CL, Pool SL (eds) Space Physiology and Medicine. Lea & Febiger, Philadelphia; 1994:447-467.
37. Rubin C, Recker R, Cullen D, Ryaby J, McCabe J, McLeod K. Prevention of postmenopausal bone loss by a low-magnitude, high-frequency mechanical stimuli: a clinical trial assessing compliance, efficacy, and safety. J Bone Miner Res 2004; 19:343-351.
38. Shapiro J, Beck TJ, Mustapha B, Ruff CB, Ballard P, Brintzenhofeszoc K, Caminis J. Zoledronic acid counteracts bone loss in the spinal cord injury model of microgravity. J Bone Miner Res 2004; 19:S445.
39. Shapiro J. (unpublished data).
40. *Forteo* prescribing information, Eli Lilly and Company; 2006.
41. Miao D, He B, Jiang Y, Kobayashi T, Soroceanu MA, Zhao J, Su H, Tong X, Amizuka N, Gupta A, Genant HK, Kronenberg HM, Goltzman D, Karaplis AC. Osteoblast-

derived PTHrP is a potent endogenous bone anabolic agent that modifies the therapeutic efficacy of administered PTH 1–34. J Clin Invest 2005; 115:2402-2411.
42. Bekker PJ, Holloway D, Nakanishi A, Arrighi M, Leese PT, Dunstan CR. The effect of a single dose of osteoprotegerin in postmenopausal women. J Bone Miner Res 2001; 16:348-260.
43. Body JJ, Greipp P, Coleman RE, Facon T, Geurs F, Fermand JP, Harousseau JL, Lipton A, Mariette X, Williams CD, Nakanishi A, Holloway D, Martin SW, Dunstan CR, Bekker PJ. A phase I study of AMGN-0007, a recombinant osteoprotegerin construct, in patients with multiple myeloma or breast carcinoma related bone metastases. Cancer 2003; 97(Suppl.3):887-892.
44. Bekker PG, Holloway DL, Rasmussen AS, Murphy R, Martin SW, Leese PT, Holmes GB, Dunstan CR, DePaoli AM. A single-dose placebo-controlled study of AMG 162, a fully human monoclonal antibody to RANKL, in postmenopausal women. J Bone Miner Res 2004; 19:1059-1066.
45. McClung MR, Lewiecki EM, Bolognese MA, Woodson G, Moffell A, Peacock M, Miller PD, Lederman S, Chesnut CH, Murphy R, Holloway DL, Bekker PG. AMG 162 increases bone mineral density (BMD) within 1 month in postmenopausal women with low BMD. J Bone Miner Res 2004; 19(Suppl.1):S20.