Alendronate as an Effective Countermeasure to Disuse Induced Bone loss

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Prevention of Bone Loss in Microgravity

Abstract

Microgravity, similar to diuse immobilization on earth, causes rapid bone loss. This loss is believed to be an adaptive response to the reduced musculoskelatal forces in space and occurs gradually enough that changes occurring during short duration space flight are not a concern. Bone loss, however, will be a major impediment for long duration missions if effective countermeasures are not developed and implemented. Bed rest is used to simulate the reduced mechanical forces in humans and was used to test the hypothesis that oral alendronate would reduce the effects of long duration (17 weeks) inactivity on bone. Eight male subjects were given daily oral doses of alendronate during 17 weeks of horizontal bed rest and compared with 13 male control subjects not given the drug. Efficacy was evaluated based on measurements of bone markers, calcium balance and bone density performed before, during and after the bed rest. The results show that oral alendronate attenuates most of the characteristic changes associated with long duration bed rest and presumably space flight.

Keywords: microgravity, bone, bed rest, alendronate

INTRODUCTION

The body's normal adaptive response to reduced gravitational forces causes untoward effects in many organ systems, e.g., neurovestibular disturbances, cardiovascular deconditioning, loss of red blood cells and plasma volume, loss of muscle mass and strength and decreased bone density (1). The potential for significant and possibly irreversible loss of bone is one of the most important medical concerns for long-duration manned space flight. Despite the implementation of a comprehensive exercise program aboard the Mir space station, regional decreases in bone density of 1-1.6% per month occur (2-4). This bone loss is not uniform, displaying significant heterogeneity between individuals and between bone sites within a given individual. While the data is currently being analyzed and not yet published, it appears that recovery does occur, although slowly and sometimes incompletely. Similar to the variable response seen during the loss of bone, recovery of bone appears to show variability in the rate and degree of bone restitution following flight (5).

NASA's ability to assure the health of the astronauts during long-duration space flight is compromised without an effective countermeasure to disuse bone loss. Countermeasures for disuse bone loss could employ exercise, biochemical prescriptions or some combination of both. Exercise is the most desirable countermeasure for maintaining muscle volume and strength since it has positive effects on the cardiovascular as well as the musculoskelatal system. The results from Mir, however, indicate that exercise alone may prove difficult as the only countermeasure for bone loss prevention. As opposed to exercise, an effective biochemical intervention would require minimal crew time, minimal stowage space and no power. No biochemical countermeasure has been attempted during weightlessness. However, several attempts have been made using ground-based bed rest studies. Bed rest is commonly used to simulate some of the effects of space flight on the musculoskelatal system. The skeletal changes, i.e., decreases in bone density, increased bone resorption markers, increased urinary and fecal calcium (Ca)

excretion and negative Ca balance were found to be qualitatively similar, although smaller in magnitude, during bed rest than in space flight (2-8).

Two of the early bisphosphonates, etidronate and clodronate, were tested as potential countermeasures to disuse bone loss. During the last eight weeks of a 20-week bed rest study, Ca balance shifted toward positive with high dose etidronate (20 mg/kg/day). Significant calcaneal mineral losses however were observed (9-10). This drug was not pursued since etidronate is associated with an accumulation of osteoid tissue in both animals and man when it was given at the anti-resorptive dose for extended periods of time (11-12). Clodronate was tested in an unpublished 17 week bed rest study, the results of which indicated that Ca balance was maintained. CT-densitometry of the spine in the nine male treated subjects approached statistical significance in preventing lumbar spine bone loss compared to five controls. Single photon absorptiometry of the calcaneus, however, showed no statistically significant difference between the two groups and one treated test subject showed severe calcaneal density loss while maintaining normal Ca balance (13). Clodronate was withdrawn from clinical investigation in the United States due to a potential serious adverse reaction. In 1995, the FDA approved alendronate for the treatment of post-menopausal osteoporosis and Paget's disease of bone. One published short duration bed rest study indicated that alendronate might be safe and effective in preventing bed rest induced bone loss (14). This study compared two groups of men, one treated with alendronate (n=8) and the other with placebo (n=8) and consisted of 2 weeks of pre bed rest and 3 weeks of bed rest. The treated subjects were given 20 mg/day for all 5 weeks. During the bed rest phase subjects sat up briefly to injest the pill and were then returned to the lying down position. During the pre bed rest ambulatory control period, alendronate-treated subjects exhibited reduced urinary calcium compared to the placebo group. Both the placebo and alendronate groups showed significant increases in urinary calcium excretion during bed rest. The excretion during bed rest in the alendronate group, however, was below the baseline (pre bed rest) level of the placebo group, suggesting that this dose level

might be effective against bone loss. Two of the treated subjects had asymptomatic decreased serum Ca during the ambulatory phase of the study that reversed during bed rest. No adverse reactions to alendronate were noted in this study.

Although the above studies suggest that bisphosphonates might be effective, it was important to NASA physicians to verify safety and efficacy using ground based models prior to routine use on long duration space missions. The purpose of this study was to test the hypothesis that oral alendronate (10mg/day) would reduce the effects of long duration (17 weeks) bed rest on bone as evidenced by the effects on bone markers, calcium balance and bone density. Because the drug was not approved for use in young women, this study was limited by NASA to normal males in an age group similar to the astronaut corps. The alendronate treated subjects (n=8) were compared to untreated bed rest male subjects (n=13). Since the drug was not expected to have an effect on muscle, changes in lean body mass (LBM) were used to determine comparability of the treated and control groups.

METHODS

The Baylor College of Medicine and the NASA, Johnson Space Center Human Use Committees reviewed and approved this research protocol. Informed consent was obtained from all subjects. The design of this study was to evaluate drug efficacy using each individual as their own control (pre vs. bed rest) and by comparison with untreated male controls. The latter consisted of 8 men from previous 17-week studies by the same investigative team using the same research protocol (7-8) and five men from a concurrent bed rest study investigating another countermeasure.

Research subjects were recruited using local newspaper advertisements. All potential subjects were required to: pass a Class III physical, have a bone mineral density (BMD) result within two standard deviations above or below the age/sex-matched mean and normal drug and psychological (MMPI)

screens. Individuals with a history of upper G.I. problems such as ulcers, dysphagia, symptomatic esophageal disease, gastritis, or duodenitis were excluded from the study. In addition, the treated subjects were tested for pill tolerance. The tolerance test consisted of ingesting a 10 mg dose of alendronate (FOSAMAX, Merk & Co) with 8 ounces of water and then immediately lying down for 30 minutes. No tested subject reported any discomfort from this procedure.

A total of nine men were recruited to participate in the drug treatment portion of the study. One individual passed all the pre-study physicals and screening procedures, but was subsequently found to have a hiatal hernia. This individual was removed from the study resulting in a total of eight males in the treatment group. One other subject, early in the bed rest period, complained of heartburn in the evening and revealed having similar problems prior to entering the study. The symptoms and their timing, occurring in the evening, did not seem related to the ingestion of alendronate, taken in the morning. Therefore, the subject was put on 50 mg/day Prilosec and continued in the study without further problems. The eight treated subjects were 22-56 years of age (mean=35), 160-185 cm in height (mean=173) and 65-98 kg in weight (mean=77). The controls consisted of 13 men, 22-56 years of age (mean=32), 156-191 cm in height (mean=171) and 65-99 kg in weight (mean=76). The protocol for all subjects was the same except for some differences in measured variables between previous and current controls as indicated in the results.

The studies were divided into three periods; a pre-bed rest period, 17 weeks of bed rest and a post bed rest recovery period. During the pre-bed rest period, the subjects were ambulatory while housed in the testing facility. During this time, all baseline measurements were obtained. In bed rest, the subjects were not permitted to sit up, but were allowed to raise themselves on one elbow while maintaining continuous contact with the bed, for eating, using a portable computer or reading. For defecation, the subjects were allowed to move to a portable commode, located next to the bed. Generally this occurred about 3-7 times per week and required prior permission from ward personnel. The subjects

were closely monitored at all times to prevent unauthorized activity. The treated subjects were given a 10mg tablet of alendronate each day during the bed rest period. The fasting subjects were asked to briefly sit up each morning to take the pill with 8 ounces of water. After the pill was swallowed, subjects immediately returned to lying down. Subjects consumed no food, medicine or drink (other than plain water) for 12 hours before and 90 minutes after ingestion of the alendronate dose. During the post-bed rest period, the subjects were allowed to resume ambulatory activities *ad lib*, but were cautioned to avoid excessive walking during the first several days.

Subjects were maintained on a metabolic diet during the entire study. The diet was prepared in a similar fashion as previously described (7). Briefly, the diet was composed of 7 daily menus, each consisting of three meals and an evening snack. All food portions were separately weighed. The subjects were required to eat all food. On the average, the diet contained about 1 g of calcium, 1.7 g of phosphorus, and 15 g of nitrogen per day. In addition, all subjects received a hexavitamin tablet once daily which provided approximately 14,000 I.U. of vitamin A, 630 I.U. of vitamin D, 3 mg thiamin, 5 mg riboflavin, 42 mg niacin, and 192 mg ascorbic acid. The mineral content of the diet was confirmed on 20 different weeks during the study. During these weeks additional meals were prepared that were subsequently homogenized, ashed and analyzed. A description of the mineral analysis has been published previously (15). The subjects were weighed weekly using an in-bed scale. The caloric intake was adjusted to maintain approximate constant body weight during the study. In these instances, the caloric content was adjusted while maintaining a constant mineral content using a diet analysis software package (Nutritionist V, First Databank).

Bone Densitometry

Bone mineral of the whole body, spine (L1-L4), femur neck, trochanter, proximal and distal radius and calcaneus were measured 3 times during baseline ambulation and 3 times at the end of bed

rest. The mean pre and post bed rest values were used for analysis. All current bone densitometry measurements were obtained using a Hologic QDR 2000+ whole body densitometer. The older published BMD measurements were obtained from various DEXA instruments, but all pre and post measurements on a given individual were performed on the same instrument and obtained and analyzed by the same operator. Routine acquisition and analysis software were used to obtain BMD and soft tissue scans except for the calcaneus. These scans were obtained using a special jig and the Hologic spine acquisition software. Phantom scans were performed regularly in order to assess the short-term and long-term performance of the densitometer. Approximately 20 times per month (and on the morning of each day of testing), a Hologic spine phantom was scanned. This phantom is an anatomical model of the lumbar spine, composed of hydroxyapatite and encased in plastic. The precision error for phantom bone mineral density is approximately 0.5%. The precision of bone and soft tissue DEXA measurements in our laboratory has been published (16-18).

Urine and Fecal Ca Excretion and Ca Balance

Twenty-four hour urine samples were collected daily throughout the study. During collection, the 24 hr voidings were stored at 4° C. At the end of each 24 hour period, the total volume was measured and duplicate aliquots (2% of total volume) were obtained. One aliquot was acidified with HCl and both aliquots were then frozen. The aliquots from each day were combined to from a 7 day pool and stored frozen at -22° C for subsequent analysis. Individually collected fecal samples were combined into weekly, 7 day pools and frozen for Ca analysis. Urinary Ca was analyzed using plasma-mass spectrometry weekly, while fecal Ca was analyzed on 4 occasions; during 3 weeks of baseline, and at 4-6 weeks, 10–12 weeks and 15-17 weeks of bed rest. Ca balance was determined using fecal and urinary Ca data during these same intervals. Calculations of metabolic balance were based on the following: Ca intake in mg/day minus output mg/day (urinary + fecal Ca). Net balance was obtained as the difference

between baseline and bed rest.

Markers of Bone Metabolism and regulatory Hormones

In order to determine changes in the markers of bone metabolism and regulatory hormones, blood and urine samples were obtained during the ambulatory period and the 3 bed rest time periods described above. The blood samples were obtained in the early morning, after a 12 hour fast, during the 3rd day of the last week of each 3-week interval. Measures obtained from serum were: total calcium (ion-sensitive electrode, Beckman Coulter Instruments, Brea, CA); ionized Ca (ion- sensitive electrode, i-STAT Corporation, Princeton, NJ); total alkaline phosphatase (spectrophotometry); bone-specific alkaline phosphatase (enzyme-linked immunosorbent assay, Metra Biosystems, Palo Alto, CA); 1,25 Dihydroxyvitamin D and 25 Hydroxyvitamin D by radioimmunoassay (DiaSorin, Inc., Stillwater, MN); osteocalcin (radioimmunoassay, Biomedical Technologies, Stoughton, MA); and mid-molecule and intact molecule PTH (radioimmunoassay, Nichols Institute Diagnostics, San Juan Capistrano, CA). Measures obtained from urine were: total calcium (ion- sensitive electrode, Beckman Coulter Instruments, Brea, CA); n-telopeptide (enzyme-linked immunosorbent assay, Ostex Int, Seattle, WA); and pyridinium and deoxypyridinoline crosslinks (enzyme-linked immunosorbent assay, Metra Biosystems, Palo Alto, Ca). The bone specific alkaline phosphatase, intact molecule (iPTH) and 25(OH)D were obtained on the concurrent controls and drug treated subjects only.

Lean Body Mass Measurements

Changes in whole body and regional lean body mass were determined from the whole body DEXA scans. Reproducibility of these measurements in our laboratory has been determined in a published precision study (18). Coefficients of variation are <1% for total body lean tissue mass and <2% for regional lean tissue mass.

STATISTICS

Except for the Ca excretion which are given in mg/day, the data are expressed as percent change from pre bed rest values. The percentage changes are reported as mean ± SE for the control and treatment groups. A paired t test was used to determine if the changes during bed rest for the individual variables were statistically significant. Differences between the control and treatment group means were tested using unpaired t test. Within and between group differences for groups of variables were tested using Wilk's Lambda statistic (19) by SAS proc GLM (SAS Institute, NC). The groups tested using the later were: BMD, urine and fecal Ca excretion, resorption markers, formation markers and lean body mass. A p value of less than 0.05 was used for determining significance.

RESULTS

Table 1 gives the BMD changes as a percent of baseline (mean ±SE) for the control and treated subjects. During bed rest, the BMD of the control group decreased in all regions except the arms and radius. The arms and radius were unchanged as reportedly previously (7). Only the 5 concurrent control data are presented for the radius since the instrument (Osteon, Inc.) used previously, scanned somewhat *different* regions and therefore could not be combined with the current DEXA data. The results, however, were the same. The treated group showed no loss in BMD except the calcaneus which decreased about 5% compared to 10% in the controls. As opposed to a loss in the control group, the femur neck of the treated group showed an increase in BMD. Comparing the treated subjects to controls, significant differences were found in the spine, femur neck, trochanter, and pelvis. Compared to controls, the changes in the whole body, legs and arms although smaller in the treated group, did not reach significance. All BMD regions, from table 1, that decreased in the control group (lumbar spine, femoral neck, trochanter, calcaneus, total body, pelvis, legs) were combined for testing for a treatment effect using the Wilk's Lambda statistic. For the controls, the within group differences were significant

($p=0.006$) while the treatment group were not ($p=0.68$), i.e., the controls lost BMD while the treated subjects did not. The between group testing was significant ($p=0.019$), demonstrating a significant effect from the treatment.

Table 2 gives the hormonal and bone marker changes as a percent of baseline for the control and treated subjects. In the controls, the three resorption markers all increased and the iPTH (intact molecule) decreased during bed rest. In the current study both intact and mid-molecule PTH assays were performed while previously, only the latter was performed. In the treated group, the NTx resorption marker decreased while the other two resorption markers increased, but significantly less than in the controls. The formation markers all decreased relative to baseline in the treated group while unchanged in the controls. Within group changes in the control and treatment groups of the combined resorption markers using the Wilk's Lambda statistic were significant ($p<0.001$). Comparing the resorption markers between the two groups, the Wilk's Lambda was significant ($p<0.001$), confirming a significant drug effect. Similar to the paired t test results, the Wilk's Lambda indicated no significant within group change in the formation markers in the controls, but a significant decrease in the treated group ($p<0.001$). Unlike the unpaired t test, the Wilk's Lambda did not show a significant difference in formation markers between the control and treated groups. Both serum Ca and ionized Ca decreased slightly in the treatment group.

Table 3 gives the fecal and urine Ca excretion and balance in the treated and control groups. Compared to baseline, urinary Ca excretion increased on average 72 mg/day in controls while the treated group demonstrated a decrease of 24 mg/day. The difference in urinary Ca excretion between control and treated groups was significant. On the other hand, fecal Ca excretion showed a somewhat different response. While the controls showed a significant increase of 147 mg/day, the treated group also showed an increase of 86 mg/day although it did not reach significance. The Wilk's Lambda statistic showed that the between group changes of the combined urine and fecal excretion were significant ($p=0.0145$).

The overall mean Ca balance was -213 mg/day in the controls and a nonsignificant -62 mg/day in the treatment group.

Table 4 gives the lean body mass measurements obtaine from the DEXA measurements. Lean body mass was only available in 6 of the 8 previous subjects resulting in 11 controls. The lean tissue in the legs and total body showed similar decreases, although the whole body change in the alendronate group was not significant. The arms show a nonsignificant increase in both groups. This table demonstrates that the LBM changes in the control and treated groups were similar.

DISCUSSION

This bed rest study demonstrated that alendronate ingestion is an effective countermeasure during bed rest, and likely in weightlessness as well. Thirteen untreated bed rest subjects show loss of bone mass in all regions with the exception of the wrist and arms. Compared to baseline, the alendronate treated subjects show no loss in BMD except the calcaneus which appears to be half that seen in the controls. All 3 bone resorption markers were elevated in the controls while decreased or elevated significantly less in the treated group. Formation markers, unchanged in the controls are decreased in the treated group as expected, if alendronate is suppressing remodeling. Net Ca balance was positively effected by alendronate treatment. The data indicate, however, that alendronate may not completely prevent bone loss. For example, in the treated group, there was loss in the calcaneus, the pyridinoline markers were elevated, fecal Ca appears elevated and net Ca balance is negative-the latter two did not reach significance. Also this study lasted only 4 months, while missions to the planets may last many years. Extrapolating effectiveness much beyond the study duration is not warranted at this time. Therefore , research designed to elicit the basic mechanism/s is reccomended.

Other than the one subject with a hiatal hernia, described in the methods, the daily ingestion of

the alendronate pill was well tolerated over the 4 months in bed rest. All bisphosphonates have a theoretical potential of producing hypocalcemia by inhibiting bone resorption. In one study, bed rest subjects were given 20mg/day of alendronate for two weeks prior to and during three weeks of bed rest. While ambulatory, two of eight subjects developed mild, asymptomatic hypocalcemia that reversed when the subjects went into bed rest (14). In the current study, where the drug was given during bed rest, only a small decrease in total serum and ionized Ca., 4.2% and 2% respectively, were observed.

The DPD and PYD were elevated compared to baseline in the treated group, but significantly less compared to the control subjects. The difference in NTx response between the two groups is much more pronounced. This probably reflects the fact that NTx is a more specific index of bone resorption, while DPD and PYD (as measured by ELISA) are confounded, especially in cases where there is coincident muscle loss (non-bone collagen breakdown). There was also discordance between the intact and mid-molecule PTH assay results. It would appear that the intact assay better reflects the changes in total and ionized serum Ca.

From ground-based human bed rest studies it appears that decreased muscle forces results in an uncoupling of bone resorption and formation which leads to rapid bone loss (6,8). In these ground based studies, resorption markers increase very early after the onset of bed rest and remained elevated for the duration of bed rest (120 days). Formation markers on the other hand, indicate bone formation increasing slowly or not at all. During space flight, crew members exhibit a rapid and sustained increase in resorption markers that is accompanied by continuous Ca loss, similar to bed rest (6, 20). While formation markers during bed rest are unchanged, less published flight data is available concerning formation markers, but it clearly does not increase. In any case, there does appear to be an uncoupling similar to bed rest, the exact mechanism of which is not completely understood. Therefore, a strategy that targets the increased osteoclastic activity would seem to be a promising approach. The results of this study confirm that the uncoupling of bone remodeling is a critical part of the underlining mechanism for

the loss of bone in disuse. Further, these results indicate that reducing remodeling using alendronate can prevent this loss by uncoupling the usual relationship between muscle and bone atrophy.

For short duration missions on the order of a few weeks to a month, the amount of bone that can be lost is of little concern especially if lost bone is eventually recovered. With increasingly lengthy flights such as tours aboard the space station and trips to nearby planets the amount and distribution of lost bone becomes important. The Russians, having operated the space station Mir for many years, recognized this problem and implemented a comprehensive countermeasure program. This program consisted of several hours of exercise a day (21). Despite the comprehensive nature of this program and its success in ameliorating many untoward effects of weightlessness, its effect on bone was obviously not complete, since crew members lost substantial bone during flight (2-3). However, it is unknown what the loss would have been without this countermeasure program since there wasn't a nonexercising control group. Also, there were individual differences in crew compliance performing the exercise regime as a result of personal preference or mission constraints (22). For example, exercise is generally not practical during first days of flight because of scheduling constraints early in the mission while many crew members increase their exercise intensity in the weeks just prior to return to Earth. Reports from returning crew also indicate significant variability among crew members in the amount, scheduling and type of exercise performed during flight. Without disregarding the possibility that the exercise program may have prevented some bone loss, it does appear to require modification. The current exercise program carried out on the International Space Station (ISS) has modified the Russian program to include greater amounts of resistive exercise using a device that is intended to provide mechanical forces on the bones and muscles that mimic weightlifting in one G. The effectiveness of this program is currently being evaluated with ISS crew members; this regime will require many flights to determine efficacy. Even if the current exercise program is successful, which it may not, having a safe pharmacological countermeasure available will be important. The current exercise requires considerable amount of time

and in some situations, such as what we saw aboard MIR in the late 1990's , there may not be adequate time for exercise. A combination of alendronate with exercise might reduce the time needed to protect the skeleton, but needs investigation. There may be other situations when the crew members cannot exercise, e.g., injury, equipment failure or sickness. Other forms of alendronate, e.g., the 8 day pill marketed by Merk would simplify the use of this drug in space. Newer bisphosphonates being investigated in various laboratories, may prove even more effective, e.g., injectable forms that are effective for several months are particularly appealing.

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TABLE 1. PERCENTAGE CHANGE FROM BASELINE OF BONE MINERAL DENSITY AFTER 17 WEEKS OF BED REST (MEAN ±SE)

	Control (n = 13)	*Alendronate* (n = 8)
Lumbar spine	-1.6 ± 0.8[a]	1.8 ± 0.9[c]
Femoral neck	-2.0 ± 0.9[a]	2.1 ± 0.6[b,d]
Trochanter	-3.9 ± 0.6[b]	0.0 ± 0.7[d]
Calcaneus	-10.3 ± 2.3[b]	-4.9 ± 1.6[a]
Distal radius	-0.5 ± 0.6[*]	0.7 ± 1.0
Proximal radius	-0.6 ± 0.3[*]	-0.8 ± 0.5
Total Body	-0.8 ± 0.3[a]	-0.3 ± 0.7
Pelvis	-3.6 ± 0.8[b]	1.6 ± 1.0[d]
Legs	-1.9 ± 0.7[a]	-0.7 ± 0.8
Arms	-0.7 ± 0.9	0.2 ± 0.6

[a] baseline vs bed rest $p < 0.05$, [b] baseline vs bed rest $p < 0.01$, [c] control vs alendronate $p < 0.05$
[d] control vs alendronate $p < 0.01$, [*] 5 control subjects only

TABLE 2. AVERAGE PERCENT CHANGE FROM BASELINE OF BONE METABOLISM MARKERS AND REGULATORY HORMONES DURING 17 WEEKS OF BED REST (MEAN ±SE).

	Control (n = 13)	*Alendronate* (n = 8)
NTx (nmol/mmol)	40 ± 9[b]	-25 ± 5[b,d]
PYD (nmol/mmol)	44 ± 4[b]	27 ± 8[a,c]
DPD (nmol/mmol)	35 ± 4[b]	16 ± 6[a,c]
Alkaline Phosphatase (U/L)	8 ± 5	-5 ± 1[a,c]
Bone Specific Alk Phos (U/L)	-6 ± 5[*]	-18 ± 3[b,c]
Osteocalcin (ng/ml)	18 ± 11	-13 ± 6[a,c]
1,25 (OH)2 Vit D (pg/ml)	-15 ± 6	3 ± 13
25 Vit D (pg/ml)	8.4 ± 14[*]	-0.3 ± 7
PTH (MM) (pg/ml)	-6 ± 2	4 ± 7
PTH (IM) (pg/ml)	-23 ± 6[*,a]	31 ± 16[c]
Serum Calcium (mg/dl)	1.8 ± 1.0	-4.3 ± 1.1[b,d]
Ionized Ca (mmol/L)	0.5 ± 0.4	-2.0 ± 0.6[a,d]

[a] baseline vs bed rest $p < 0.05$, [b] baseline vs bed rest $p < 0.01$, [c] control vs alendronate $p < 0.05$, [d] control vs alendronate $p < 0.01$, [*] 5 control subjects only

TABLE 3. CALCIUM EXCRETION AND BALANCE (MEAN ±SE).

	Control (n = 13)		*Alendronate (n = 8)*	
	Pre-bed rest	Bed rest	Pre-bed rest	Bed rest
Ca intake (mg/day)	1002 ± 12	1007 ± 13	1026 ± 9	1027 ± 12
Urine Ca (mg/day)	176 ± 20	248 ±27[b]	187 ± 35	163 ± 29
Fecal Ca (mg/day)	724 ± 42	871 ± 30[b]	707 ± 43	793 ± 24
Ca balance (mg/day)	102 ± 49	-109 ± 38[b]	121 ± 29	69 ± 29
Net Ca balance (mg/day)		-213 ± 36[b]		-62 ± 38[c]
Delta Urine Ca (mg/day)		72 ± 8[b]		-24 ± 131[d]
Delta Fecal Ca (mg/day)		147 ± 36[b]		86 ± 37

[a] baseline vs bed rest $p<0.05$, [b] baseline vs bed rest $p<0.01$, [c] control vs alendronate $p<0.05$,
[d] control vs alendronate $p<0.01$

TABLE 4. PERCENTAGE CHANGE FROM BASELINE IN LEAN BODY MASS AFTER 17 WEEKS OF BED REST (MEAN ±SE).

	Control (n = 11)	*Alendronate* (n = 8)
Whole Body	-3.0 ± 1.1[a]	-1.6 ± 0.9
Legs	-8.5 ± 1.2[b]	-8.4 ± 1.0[b]
Trunk	-1.6 ± 1.5[*]	0.6 ± 1.1
Arms	2.5 ± 1.4	3.0 ± 1.7

[a] baseline vs bed rest $p < 0.05$, [b] baseline vs bed rest $p < 0.01$, [*] 9 control subjects only