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of San Francisco*

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THE PREVENTION OF BONE MINERAL CHANGES INDUCED BY BED REST;
MODIFICATION BY (1) STATIC COMPRESSION SIMULATING WEIGHT
BEARING; (2) COMBINED SUPPLEMENTATION OF ORAL CALCIUM
AND PHOSPHATE; (3) CALCITONIN INJECTIONS; (4) OSCIL-
LATING COMPRESSION; (5) THE ORAL DIOPHOSPHONATE-
DISODIUM ETIDRONATE; (6) LOWER BODY NEGATIVE,
PRESSURE

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(NASA-CR-141453) PREVENTION OF BONE
MINERAL CHANGES INDUCED BY BED REST:
MODIFICATION BY STATIC COMPRESSION
SIMULATING WEIGHT (Public Health Service
Hospital) 55 p HC \$4.25 CSCI 06E

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INTRODUCTION

Bone mineral is lost during bed rest (1-3). This has been reconfirmed in previous studies here in eleven normal males who remained horizontal for up to 36 weeks of continuous bed rest (1,5). Calcium loss from the skeleton as a whole was measured by balance techniques and averaged 0.5% of total body calcium per month. Tenfold greater rates of loss from the central portion of the calcaneus were observed by gamma ray transmission scanning. It is therefore likely that during bed rest, weight bearing bones lose mineral more rapidly than other portions of the skeleton. Remineralization occurs during reambulation at a rate similar to the rate of loss during bed rest (4,5).

Mineral loss during bed rest is probably due to a reduction in the forces which are applied to the skeleton during normal activity (4,5,7-9). These one "G" homeostatic forces are also absent in the hypogravic environment of space flight. Loss of bone mineral during space flight has been expected on theoretical grounds and has been confirmed recently (10-11). This factor will prove hazardous to astronauts on flights of long duration, not only because of the risk of fracture in demineralized bones on return to earth, but also because hypercalciuria might lead to the formation of renal calculi.

The need to reduce these hazards has led to a series of experiments. Bed rest was employed as an analogue of weightlessness. On the basis of available information, initially two regimens were selected to be tested for effectiveness in preventing mineral loss. Eight healthy adult males underwent 24-30 weeks of continuous bed rest. Three were

treated with an exercise regimen designed to resemble normal ambulatory activity (6), and five were fed supplemental potassium phosphate (12). In each subject the results from a 12-week period on therapy were compared with those from one or more control (untreated) bed rest periods.

Treatment with potassium phosphate supplements (1327 mg P/day) entirely prevented the hypercalciuria of bed rest, but fecal calcium tended to increase. Mineral loss from the central calcaneus was similar to that of untreated subjects (14).

The exercise program which made use of the Exer Genic apparatus included a total of 80 minutes of supine exercise daily; 50 minutes involving the legs. The usual negative calcium balance was seen in spite of the exercise regimen. The loss of mineral from the calcaneus as measured by gamma ray transmission scanning was not diminished either.

It is possible that the exercise program did not have any beneficial effect because the forces which could be generated by voluntary muscular effort were not great enough in magnitude or duration to truly resemble those during normal ambulation. (However, even a severe exercise regimen performed in space did not prevent bone mineral loss or negative calcium balance (11).)

Therefore, several additional studies were developed to attempt to modify both the negative calcium balance as well as the calcaneal mineral loss seen with prolonged bed rest.

GENERAL METHODS & MATERIALS

Metabolic Unit Physical Plant:

The Metabolic Unit is located in the southeast wing of the U.S. PHS Hospital in San Francisco. The 10-bed ward is divided into 5 double rooms. Each room is equipped with a sink, television, radio and stereo record player, and 3 rooms have air conditioners and toilets. A centrally located nursing station contains adequate desk space and a medicine closet with a refrigerator. The utility room contains a washer and drier and a refrigerator. The Metabolic Kitchen is completely equipped for preparing measured diets with balances, a freezer, a refrigerator, a stove, a dishwasher, and distilled water. The Metabolic Laboratory consists of 2 rooms with facilities for balance studies including two freezers, a muffle furnace, an atomic absorption spectrophotometer, a Technicon autoanalyzer, distilled water, and miscellaneous equipment. In addition to adequate storage space, there are individual offices for the director, the dietitian, the head nurse, the research fellow or research associate, and the secretary.

Subjects:

Healthy male volunteers aged 19-31 were equilibrated on the metabolic diet for 1-2 weeks and then studied during a 4-6 week baseline ambulatory period without restrictions on their level of activity. Subjects spent the next 3-24 weeks in bed; movements in the horizontal plane were not restricted and they were allowed to raise themselves on one elbow for eating and reading. Defecation and micturition were performed while supine. The subjects were observed frequently by the nursing

staff throughout each 24-hour period and never left the Metabolic Ward without a staff member in attendance. During bed rest, the subjects were required to remain in bed or on a stretcher at all times. They were not allowed to sit up or dangle their legs over the bed.

Balanced Diet:

A palatable food diet was provided. This was usually composed of seven daily menus (3 meals and an evening snack) which were rotated randomly each week and which provided a relatively constant daily intake. All foods, except for some staples, soft drinks and meats were purchased in common lots prior to each study section to assure maximum constancy. Canned whole milk and frozen homogenized eggs were used, and fresh foods were avoided. Standard methods of cooking including steaming, baking and boiling were used. Distilled water was used for food preparation, drinking and rinsing utensils. Individual portions of food were weighed on a Mettler Balance. The subjects were required to eat all of the food served to them. At the end of each meal, the subjects cleaned their plates with a spatula, with bread or by licking, and drank a small quantity of distilled water used to rinse their glassware. All dishes and utensils used in the study were washed in a dishwasher with a distilled water rinse. Tooth paste used by the subjects was free of mineral except silica.

Analyses in duplicate of the diet were obtained at intervals during the study (the number depending on the length of the study). For this purpose an additional serving of each menu was weighed and prepared

throughout the 7 day period. These meals were pooled, homogenized, and aliquots were stored for mineral analysis at the end of each study.

Medication included one hexavitamin tablet daily which was begun on the first day of equilibration. Other medications given include: dioctyl sodium sulfo-succinate (Colace) 0-300 mg/day; occasional aspirin, up to 600 mg, and pseudoephedrine, 60 mg (generally less than once weekly).

Starting in 1971, polyethylene glycol 4000 was used as a stool marker; 500 mg given each 8 hours throughout the course of the study. The drug has been approved for experimental use by the FDA under IND #7781. (Kindly supplied to us by Sandoz Products Limited, 95 The Centre, Feltham, Middlesex, TW13 4EP, England.) Polyethylene glycol has been studied extensively in animal and human subjects without demonstrable toxicity.

Body Measurements:

Daily or weekly weights were obtained using an Acme in-bed scale. During bed rest the weight was taken in the supine position.

Serum, Stool, and Urine Collections:

Balance periods were 7 days in duration. Thirty-five ml of blood were drawn in the fasting state once each week in studies with bed rest lasting 6 weeks or less, and once every 14 days with bed rest studies lasting more than 6 weeks. Twenty-four hour urines were collected daily, acidified with 1 ml of 12 normal HCL/100 ml of urine, and stored at 4° C. Total creatinine content of each daily 24 hour urine was

was determined at the end of each 7 day period. For each subject, the 7 creatinine values were averaged and any value which was greater than 10% from the mean was assumed to represent a collection error and discarded; 6% of all daily specimens were discarded in this fashion. For each subject, the remaining urines were combined into a pool representing the 7 day period and an aliquot was stored at -22° C. Seven day collections of stools were obtained without markers in studies 1-2 and with a continuous marker, polyethylene glycol 4000, for studies 3-5. The stool collections began and ended 16-24 hours after the start and finish of the weekly urine periods to provide a partial correction for intestinal transit time.

Stools were collected in bedpans lined with mylar film and were subsequently transferred with distilled water rinsing into (unused) epoxy-lined 1-gallon (paint) canisters and refrigerated. Upon completion of the 7 day collections the stools were further diluted with distilled water and 300 ml glacial acetic acid to a final weight approximately three times the initial weight. They were homogenized for 30 minutes on a paint shaker, and an aliquot was stored at -22° C for subsequent ashing.

Sweat mineral determinations were not performed during these experiments. A mean value determined from previous sweat collection and analysis (4,12) was used for mineral dermal loss in computing mineral balance.

Ashing of Stool and Diet:

Three methods were employed in stool and diet preparation (4,5). Muffle furnace ashing was used to prepare material for calcium and magnesium analysis. Sulfuric acid digestion was the only method found acceptable for nitrogen analysis; solutions prepared in this manner were also analyzed for phosphorus. Calcium and magnesium analyses using nitric acid digestion were done to confirm muffle furnace data. When the results did not agree within 5 percent, additional determinations were performed.

For the muffle furnace ashing, a weighed aliquot of diet or stool homogenate (approximately 20 gm) was ashed in a covered crucible at 575° C for 72 hours in a muffle furnace. The residual was reconstituted with 2 ml concentrated HCl, 2 ml concentrated NH₄OH, and 1 ml H₂O, followed by three washes of 5 ml of 0.6 normal HCl. Recovery of added calcium was 98.8 ± 3.1 percent (± SD) and that of added phosphorus 98.4 ± 2.7 percent.

Sulfuric acid digestion was performed as follows: an aliquot of the homogenates (approximately 4 gm) was placed in a tared 100 ml volumetric flask and weighed. Twenty ml of concentrated sulfuric acid and 2 selenized granules were added. The solution was boiled for approximately 2 hours, resulting in a fine suspension of brownish material. Recovery of added urea nitrogen was 97.8 ± 2.1 percent and that of phosphorus 100.4 ± 2.4 percent.

Nitric acid digestion was carried out in a similar manner. A quantity of homogenate (approximately 2 gm) was placed in a tared 100 ml volumetric flask and weighed. Ten ml of 90 percent HNO_3 and 4 boiling beads were added. Boiling for approximately 5 minutes at maximal heat on a Kjeldahl burner resulted in a clear solution of about 1 ml volume. The solution was diluted to 100 ml with distilled water and analyzed for calcium and magnesium. Recovery of added calcium was 100.4 ± 2.4 percent.

Laboratory Determinations:

Calcium (4,5) was determined by atomic absorption spectrophotometry on an automated Perkin-Elmer Model 303, using 0.25 percent lanthanum as an electron flux stabilizer and Harleco calcium standard solution C (15,16). Magnesium was also analyzed by atomic absorption spectrophotometry, using magnesium chloride in water as a standard (15,16). Phosphorus was analyzed on a Technicon autoanalyzer by the standard adaptation of the Fiske and SubbaRow technic (17). KH_2PO_4 standard were made up in 0.02 N HCl except for stool and diet solutions which contained sulfuric acid and were compared with standards which had been brought to the same pH with H_2SO_4 .

Ionized calcium concentration was determined with an Orion Model 99-20 serum calcium flow-thru electrode and Model 801 digital pH/mv meter (18). Anaerobic venous serum was obtained by centrifuging filled red top vacutainer tubes which had been fully evacuated before venipuncture. Albumin was determined by autoanalyzer (19).

In several experiments the following laboratory values were determined: alkaline phosphatase by the automated modification of the Bodansky method (20); cholesterol and glucose concentrations were determined by autoanalyzer (21,22); and triglycerides were determined by an automated fluorometric technic (23). Urinary 17-hydroxycorticosteroids were determined in the Metabolic Laboratory at the University of California, San Francisco by the method of Reddy et al (24). Assays of serum concentration of parathyroid hormone and calcitonin were carried out by radioimmunoassay in the laboratory of Dr. Armen Tashjian at the Harvard School of Dental Medicine, Boston, Mass. (25,26).

Total nitrogen was determined on a Technicon autoanalyzer using the standard method (22), except that 0.2 percent perchloric acid was employed in the digestant; urea standards in 0.1 N H₂SO₄ were used. Although recovery of added urea by this method was complete, recovery of creatinine nitrogen was only 62.3 ± 2.1 percent.* Urinary nitrogen excretion was corrected for incomplete recovery of creatinine nitrogen by using this factor and the measured excretion of creatinine (a correction of approximately 2 percent). Urine and serum creatinine concentrations ([Cr]) were determined in a Technicon autoanalyzer by the standard modification of the Folin Wu method (27), employing Technicon standards. Creatinine** (CCr) was calculated from the relationship $CCr = \frac{\text{urine [Cr]} \times \text{urine volume}}{\text{serum [Cr]}}$.

*These findings are similar to those previously reported (4).

**Clearance.

Hydroxyproline was determined by the method of Kivirikko and Prockop (28) using hydroxyproline in distilled water as the standard.

Each of the above methods was found to yield satisfactory results in initial recovery studies. Commercial standard solutions* were included in all subsequent runs for quality control. In addition, all calcium, phosphorus and hydroxyproline determinations were carried out in duplicate, as were urinary nitrogen assays.

Gamma Ray Transmission Scanning:

In the past, bone density has been assessed by visual inspection of radiographs. Using newer technics (29), the changes in bone mineral content can be estimated by the changes in absorption of a monoenergetic gamma ray, specifically the 27.5 KEV emission of ^{127}I . The instrumentation entails using a scanning head consisting of a 3 mm thick NaI crystal scintillation detector moving synchronously with a source holder containing 100 mc of ^{125}I , in the direct alignment with the detector 6 inches away. The tissue to be scanned is surrounded by an appropriate material for tissue equivalency. This is important since the soft tissue around the bone is not always uniform and may add to the bone mass values in an unpredictable way. Scanning is done in parallel lines across the bone studied, and the data so accumulated is stored on magnetic tape and tally punch tape for later processing. Three methods of direct readout are provided: a volumetric display, a 3 dimensional isometric display, and a digital printout. The average count rate

*Commercial standards employed were: Versatol, Versatol A, Hyland Urine, and Brook serum.

through tissue plus tissue equivalent represents 100% transmission. As points along each horizontal row are counted, the count rate for each point is ratioed to 100% transmission and the natural log is then computed for each data point and summed for the entire row. This is expressed as a positive value of absorption. The higher the value, therefore, the denser the bone.

To facilitate immobilization of the leg and reproducibility of position in repeated scans, molds of the heel are constructed. Two baseline scans are done before bed rest begins. Scans are repeated every 3 weeks during bed rest, twice during reambulation, and as possible following completion of the study.

Data Presentation:

Metabolic observations for different activities were carried out for different durations. All studies had concomitant bed rest controls. Accordingly, while all available data is presented on figures and tables depicting individuals, only the portions common to all subjects untreated or similarly treated are shown for those data presentations which employ averages. Complete balance data were obtained on all subjects during the baseline ambulation, the weeks of bed rest, and in several of the studies during the initial 1-3 weeks of reambulation. Complete urinary and serum data were obtained for the last 2-4 weeks of baseline ambulation, the duration of bed rest, and when applicable the weeks of controlled reambulation. When the averages of data presented in these two ways are compared, minor numerical differences are sometimes present.

Study 1

THE EFFECT OF COMPRESSION STRESS SIMULATING WEIGHT BEARING
ON THE CHANGES OF BONE MASS RESULTING FROM BED REST

Specific Aim:

To investigate the ability of compression forces to prevent bone loss during bed rest.

Introduction & Background:

There is ample evidence that bed rest simulates the effects of weightlessness qualitatively, and is a suitable tool for studying the changes to be expected with space flight including bony changes. Skeletal homeostasis is a complex interaction of weight bearing, muscle mass and activity, innervation, skeletal blood flow and humoral influences. The importance of weight bearing and muscular activity may be appreciated from the decreased bone mass and negative mineral balance that result when normal individuals with intact neural, vascular and endocrine systems are put to rest in bed (1,4,5,9). It is important to establish whether the lack of weight bearing or the lack of muscular activity is the major factor responsible for these changes.

Some evidence suggests that exercise without weight bearing is not effective in preventing changes of mineral metabolism and bone mass during bed rest (4,5,30). On the other hand, quiet standing for 2-3 hours per day may be effective in preventing mineral loss (3,30). Time may be an important factor, since weight bearing for less than 2 hours per day does not appear to modify the mineral changes of bed rest

(30,31).

This study is designed to investigate the effect of compressive stress on the longitudinal axis of the human skeleton. There is suggestive evidence that longitudinal compression of the skeleton may modify the bony changes of bed rest in humans (32) and in monkeys (33). Changes in the lower extremity bone mass of normal volunteers undergoing 17 weeks of bed rest will be assessed by gamma ray transmission scanning. Both legs were subjected to constant compressive stresses.

Design of the Study:

A 6 month project was begun in January 1970. The first 2 weeks were for dietary equilibrium, the next 4 weeks were used for baseline ambulatory studies, the next 12 weeks for bed rest, and the final 6 weeks for reambulation (only the first week of reambulation was with metabolic balance).

During bed rest, constant pressures approximately equal to 80% body weight were applied to both legs between the heel and the knee for periods of 200 minutes daily. This was divided between the morning (3 hrs) and the afternoon (1 hr) with 5 minutes of rest every half hour. The longitudinal compression was provided by a Gravitational Acceleration Simulation Suit* (GASS) (Fig 1). Calibrated springs attached to the suit were stretched to provide a compressive force on the longitudinal axis. The force was applied between the soles of the feet and a cinch belt immediately proximal to the iliac crests (two-thirds of the

*This apparatus was designed and maintained by James Gatts, M.D.

force), and between the soles of the feet and straps over the shoulders (one-third of the force). Periodic determinations of bone mass in both legs were conducted throughout the study by gamma ray scans. Diet was controlled, and calcium and phosphorus values in serum and urine were measured at regular intervals.

Study Subjects:

Five healthy volunteers between 21 and 24 years of age were chosen as subjects. TA, MH and AK were selected for constant longitudinal compression and GF and BL as bed rest controls. All signed informed consent.

Procedures:

1. Mineral balance -- calcium, phosphorus and nitrogen.
2. Creatinine clearance; phosphorus clearance.
3. Densitometry -- central os calcis, tibia-1 6 cm proximal to medial malleolus and tibia-2 10 cm proximal to medial malleolus.

Results:

Calcium Metabolism

During bed rest, mean urinary calcium excretion rose from an average baseline value of 180 mg/day to a maximum of 243 mg/day in the 5th week in the two control subjects. The three treated subjects followed the same pattern changing from a baseline of urine calcium of 177 mg/day to 242 mg/day at the 5th week. The value subsequently fell, but did not return to the mean baseline remaining at 220 mg/day by the end of

the control bed rest. Two of the treated subjects' urinary calcium fell during the 10th week of bed rest and returned to baseline values. The third subject's urine calcium also fell but not to the 15th week and was still 40 mg/day above baseline at the end of bed rest.

Fecal calcium remained increased in both controls but in only 2 of the 3 treated subjects for the 17 week bed rest period. The other treated subjects' fecal calcium returned to baseline values in the 8th week of bed rest.

Mean calcium balance for the two control subjects was -188 ± 76 ($X \pm SE$) mg/day, and the balance for the three treated subjects was -138 mg/day ± 4 .

Mean serum calcium concentration show a trend to increase in the controls and to remain the same in the treated subjects but was not significant ($p > .2$). There was no change comparing bed rest with prior ambulatory period.

Phosphorus Metabolism

Mean urinary phosphorus excretion was higher throughout the period of bed rest than during the baseline period in both controls and two treated subjects; the average increment was controls 73 mg/day vs treated 45 mg/day. Negative phosphorus balance was demonstrated in all subjects' control -79 mg/day vs. treated -62 mg/day. There was no appreciable change in mean blood phosphorus concentration during bed rest.

Serum alkaline phosphatase concentration showed no change comparing ambulatory control vs. bed rest, as well as no change comparing

treated subjects with untreated bed rest subjects. There was an increase seen in both groups on reambulating but no difference between the two groups. Reambulation vs. bed rest was significant ($p < 0.05$).

Creatinine clearance was unchanged during bed rest in comparison with the baseline period. There was also no difference between longitudinal compression vs. control group.

Urinary nitrogen excretion increased in comparison with the baseline ambulatory period in both groups equally.

Urinary hydroxyproline increased in both groups; however, the average increment in the treated group was only half of the untreated group, control 6.53 mg/day vs. treated 3.03 mg/day.

Body weights changed only modestly if at all in all subjects throughout the study.

Densitometry data showed equal and significant loss of bone calcium from both groups. Control -19.2% vs. treated -20.5%. Tibia changes were inconsistent but tended toward decrease bone density.

Discussion:

When a normal individual is placed at bed rest for 30-36 weeks, muscle mass and bone mass in the lower extremities decrease (4).

Urine calcium remains increased throughout, and is one index of the bone loss. Exercise in bed for as long as 4 hours per day had no effect in preventing this hypercalciuria, but 3 hours of quiet standing had an effect (3). Lamb showed no effect from two hours per day of walking and back exercises, but relatively equal effects from 3 hours per day of quiet standing or 3 hours per day of vigorous walking and

weight lifting (30).

This study suggests that with constant longitudinal compression that the usual negative calcium balance is not modified. Urinary hydroxyproline which is thought to be a measure of bone breakdown confirms this. Moreover, 20% of the os calcis was lost in both groups as measured by gamma ray transmission scanning.

It is possible that the constant longitudinal compression program did not have any beneficial effect on the os calcis because the normal forces of walking could not be generated with only a constant force on the os calcis.

The next study was designed to in part answer this question.

SUMMARY

Five healthy young men were studied during 17 weeks of continuous bed rest. Three subjects received constant longitudinal compression using the GASS suit applying a force equal to 80% of body weight for 200 minutes/day starting with the first day of bed rest. Two subjects were studied with bed rest only as controls.

There was no modification of negative calcium balance or bone mineral turnover as measured by urinary hydroxyproline excretion. No prevention of the loss of bone mineral from the weight bearing bone, the os calcis was seen.

Study 2

ATTEMPTS TO PREVENT DISUSE OSTEOPOROSIS BY TREATMENT WITH
CALCITONIN, LONGITUDINAL COMPRESSION AND SUPPLEMENTARY
CALCIUM AND PHOSPHATE

Bone mineral is lost when normal human subjects undergo prolonged bed rest (1-9). One hazard of the mineral loss is the increased likelihood of kidney stones which attends hypercalciuria (34). In previous studies, we have shown that this problem can be averted by adding phosphate to the diet (13). However, a more serious disorder, which is not prevented by supplementary phosphate, is the development of disuse osteoporosis.

We have studied the efficacy of three regimens directed at preventing bone mineral loss during bed rest: (1) calcitonin was employed to inhibit the excessive resorption of bone which is thought to be a factor in the development of disuse osteoporosis; (2) intermittent longitudinal compression was applied to the skeleton in order to simulate the forces which are applied by gravity during normal ambulation; and (3) oral calcium and phosphate supplements were used with the hope that the former would increase calcium absorption from the intestine while the latter prevented hypercalciuria.

Six healthy male subjects were studied during 19 weeks of continuous bed rest. The therapeutic regimens were applied singly or in combination for an 8 week period at the beginning or end of bed rest. Conclusions were drawn from a comparison of the data during treatment

periods with those of the intervening 3 weeks of untreated bed rest, and also with the results of previous studies of bed rest without therapy. Changes in skeletal mineral content were assessed directly by gamma ray transmission scanning of the calcaneus, and indirectly by metabolic balance techniques.

Materials & Methods:

The methods and materials employed in this study are those previously reported, except as noted below.

Study Conditions:

Six healthy Caucasian men aged 21-24 years were equilibrated on the metabolic diet for one week and then observed during a 6 week^x baseline ambulatory period without restrictions on their level of activity. The subjects spent the next 19 weeks in bed; movements in the horizontal plane were not restricted and they were allowed to raise themselves on one elbow for eating and reading. Defecation and micturition were performed while supine. The subjects were observed frequently by the nursing staff throughout each 24 hour period, and never left the Metabolic Ward without a staff member in attendance.

Medication:

The subjects were given 200 mg Colace[®] (dioctyl sodium sulfosuccinate) daily throughout the study.

Calcium supplements were given orally as calcium lactate.**

*JF developed infectious mononucleosis during the first baseline week and was only able to participate in the baseline period for the last 4 weeks.

**Manufactured by Bolar Pharmaceutical Company, Copiague, New York; Contractor-American Quinine Company, Plainview, New York.

Sample tablets were randomly selected for mineral analysis during treatment periods. During the first 8 weeks, 7 tablets contained 45.87 ± 0.98 mg Ca/tablet (mean \pm SD). During the last 8 weeks, a new lot of calcium lactate was used; 8 tablets contained 43.37 ± 0.58 mg Ca/tablet. The calcium lactate tablets were disintegrated by standing in 15 ml distilled water for 1 hour and then given between meals at 2 dosage levels: either 17 tablets were given daily (6 at 10:00 a.m., 5 at 2:30 p.m. and 6 at 8:00 p.m.), or 30 tablets were given daily -- 10 at each of the above times.

Supplemental phosphate was administered orally as Hyper-Phos-K.* Each tablet contained K_2HPO_4 and KH_2PO_4 in proportions which provide a pH of 7.4 when dissolved in water. Tablets from the same lot have previously been found to contain 165.9 ± 2.3 mg P/tablet (13). Eight tablets were administered daily -- 3 with breakfast at 8:00 a.m., 2 with lunch at 12:00 noon and 3 with dinner at 5:00 p.m.

Synthetic salmon calcitonin** was administered sc in a dose of 100 Medical Research Council (MRC) U at 9:00 a.m. daily. Prior to the study no hypersensitivity to this preparation of calcitonin was detected by intradermal administration of 0.1 ml of a 1:100 dilution of the drug.

Longitudinal Compression:

Intermittent longitudinal compression was provided by a Gravitational Acceleration Simulation Suit*** (GASS) which was attached to

*Supplied by Davies Rose Hoyt Company, Needham, Mass.

**Supplied by Armour Pharmaceutical Company, Kankakee, Illinois as calcitonin AL-0977 in a gelatin-phenol vehicle.

***This apparatus was designed and maintained by James Gatts, M.D.

a motor (35,36). The compressive forces were alternately stretched and relaxed at a frequency of 45/min. The subjects were required to "stand" on the footboard with one foot at a time. This applied the entire force to one leg at a time, as occurs during normal ambulation. The subjects changed from one side to the other ad libitum -- generally every 1-3 min.

During the first 4 days of the treatment period, the duration and magnitude of force applied to each subject were gradually increased until it could be maintained at a force equal to 80% of his body weight for 200 min/day. This was divided between the morning (3 hr) and the afternoon (1 hr), with 5 min of rest every half-hour. During the final 2 weeks of bed rest, Subjects RB and WR received a compressive force equaling 100% of body weight for 300 min daily.

Collections:

As previously described.

Dermal loss of calcium was not measured in this study, but the balance values include an estimated 19 mg/day (the mean of the determinations in 11 previous study subjects (5,13).

Analytical Methods and Recovery Studies:

Calcium, magnesium, phosphorus, creatinine, alkaline phosphatase and hydroxyproline were determined as previously described.

Immunoreactive parathyroid hormone (PTH) was estimated by the radioimmunoassay technique of Arnaud and co-workers (26a), utilizing a guinea pig antibody to porcine PTH (GPII), ¹³¹I labelled bovine PTH

and dextran-coated charcoal separation of bound and free hormone.

Gamma Ray Transmission Scanning:

The mineral content of the central portion of the calcaneus was assessed by ^{125}I gamma transmission scanning.

Results

Study Design:

The 19 weeks of bed rest were divided into two treatment periods of 8 weeks each, separated by a 3 week period of bed rest without therapy.

Calcium & Phosphorus Metabolism:

The calcium balance became negative and urinary calcium increased during bed rest and treatment with calcitonin. The hypercalciuria subsequently diminished during bed rest without treatment, but the negative balance persisted. The use of calcium and phosphate supplements reduced both the hypercalciuria and the negative calcium balance.

Calcitonin alone did not retard any of the metabolic consequences of bed rest in either of the two subjects so treated. The hypercalciuria was greater than that occurring in untreated subjects.

Intermittent compression alone had no substantial effect on the mineral losses which occur during bed rest.

Calcium and phosphate supplements tended to reduce the hypercalciuria, although the effect was usually not statistically significant; urinary phosphorus excretion was strikingly increased. Calcium balance was significantly less negative in four of the five subjects and

ATTEMPTS TO PREVENT DISUSE OSTEOPOROSIS

TABLE 1. Experimental design and calcium and phosphorus metabolism

	Period	Subject					
		RB	DM	WR	JF	JC	FC
Treatment regimen:							
	Amb	—	—	—	—	—	—
	BR 1-8	Ca P	CT	CT	Compr	Comb	Comb
	BR 9-11	—	—	—	—	—	—
	BR 12-19	Compr	Ca P	Compr	↑Ca P	↑Ca P	Ca P
Urinary calcium (mg/day):							
	Amb	175	214	141	132	218	185
	BR 1-8	188	442	282	208	274	305
	BR 9-11	224	375	195	212	270	262
	BR 12-19	193	227	167	165	214	191
Fecal calcium (mg/day):							
	Amb	985	734	884	818	695	834
	BR 1-8	1705	798	910	884	1422	1462
	BR 9-11	971	936	993	1061	817	892
	BR 12-19	1023	1523	1050	2152	2068	1637
Calcium balance (mg/day):							
	Amb	-150	+62	-15	+60	+97	-10
	BR 1-8	-103	-230	-182	-82	+94	+23
	BR 9-11	-185	-301	-178	-263	-77	-144
	BR 12-19	-206	-7	-207	-13	+22	-85
Urinary phosphorus (mg/day):							
	Amb	1128	1009	938	919	1023	1091
	BR 1-8	1828	1205	1177	1048	1894	1873
	BR 9-11	1208	1195	1114	1072	1204	1108
	BR 12-19	1207	1868	1115	1645	1739	1682
Fecal phosphorus (mg/day):							
	Amb	599	469	550	563	460	556
	BR 1-8	1019	502	504	541	964	1080
	BR 9-11	536	556	583	610	486	601
	BR 12-19	533	1030	611	1245	1183	1284
Phosphorus balance (mg/day):							
	Amb	-57	+192	+182	+188	+187	+23
	BR 1-8	+150	-37	-11	+81	+139	+44
	BR 9-11	-74	-81	-27	-12	-20	-39
	BR 12-19	-70	+99	-56	+107	+75	+31

Amb: Baseline ambulation (6 weeks).

BR 1-8: Bed rest weeks 1-8.

BR 9-11: Bed rest weeks 9-11.

BR 12-19: Bed rest weeks 12-19.

CT: Synthetic salmon calcitonin; 100 MRC U daily. (Treatment with CT was stopped at the beginning of the 7th week of bed rest because of nephrotoxicity (8)).

Compr: Intermittent longitudinal compression; 80% of body wt for 200 min daily (except bed rest weeks 18 and 19; 100% of body wt for 300 min daily).

Ca P: Oral calcium and phosphate supplements; 780 (BR 1-8) or 733 (BR 12-19) mg Ca and 1327 mg P daily.

↑Ca P: Oral calcium and phosphate supplements; 1294 mg Ca and 1327 mg P daily.

Comb: A combination of CT, Compr and Ca P.

The numbers shown are the means of the weekly determinations.

phosphorus balance became positive.

Combined therapy with all three regimens appeared to attenuate the negative calcium and phosphorus balances in both subjects so treated. Urinary phosphorus excretion was increased in both subjects and urinary calcium was not affected.

Calcaneus Mineral:

The changes in the mineral content of the central calcaneus were assessed by gamma ray transmission scanning.

Calcaneus mineral loss was within the 95% confidence limits in all of the current subjects. The absence of bone loss in subjects (RB and WR) might be ascribed to the intermittent compression during the last 8 weeks of bed rest; however, substantial losses were seen in JF and FC during the first 8 weeks at a time when they, too, were receiving compression therapy.

Parathyroid Hormone:

Parathyroid hormone levels showed no consistent change during the study. In particular, the levels were not higher during treatment with calcitonin.

The parathyroid hormone values for WR were abnormally high on one occasion during baseline as well as once during bed rest. The explanation for this finding is unknown. Eight ambulatory serum calcium values over a period of one year have fallen in the range 9.6-10.3 except for one value of 10.7 mg/dl. There is no history of kidney stones, ulcer disease or pancreatitis.

Hydroxyproline:

A rise in urinary hydroxyproline excretion was seen in all subjects except JC. The rise exceeded the 95% confidence limits in DM while he was receiving calcitonin. Treatment with mineral supplements alone was associated with reduced levels of hydroxyproline excretion in three of five subjects receiving this regimen.

Other Metabolic Data:

Urinary excretion of nitrogen was higher during bed rest than during baseline ambulation. The treatment programs did not appear to influence this phenomenon. Fasting serum calcium concentrations remained relatively constant throughout the study. Fasting serum phosphorus concentrations hardly changed during bed rest, although the levels tended to be decreased during treatment with calcium and phosphorus supplements.

No consistent changes in serum alkaline phosphatase activity were noted. There was no apparent trend towards a change of creatinine clearance. Body weight fell in those whose initial weight exceeded 75 kg and rose in the subject who weighed less than 58 kg in the beginning. Subjects with intermediate weights showed little change. These data perhaps reflect the constant 2500 calorie diet which all subjects received, regardless of their size.

Clinical Observations:

There were no untoward medical or psychological events during bed rest. The intermittent compression apparatus was uncomfortable to wear,

frequently leading to minor backaches and shoulder discomfort. Tedium was also a problem because it was difficult to carry on other activities, such as reading, while being joggled in the suit. For these reasons, this regimen was generally disliked by the subjects.

Reambulation was undertaken slowly; during the first week the subjects gradually increased the amount of time spent on their feet, until normal activity was achieved. There were no signs of orthostatism but tenderness of the joints of the feet was noted for 1-3 weeks and easy fatigability for somewhat longer. All subjects had returned to their pre-study health status by the end of the second month.

Summary:

Six healthy men were studied during 19 weeks of continuous bed rest and 3 treatment regimens were tested for their effectiveness in preventing bone mineral loss:

1. Synthetic salmon calcitonin (100 MRC U daily) did not prevent the negative calcium and phosphorus balances which are observed during untreated bed rest. The increase in urinary calcium and hydroxyproline excretion was unusually large in one of the two subjects.

2. Intermittent compression in the longitudinal axis was applied by springs attached to a special suit; a force equal to 80% of body wt was applied 45 times per min for 4 hr daily. The negative mineral balances were not substantially affected by this regimen.

3. Calcium and phosphate supplements were administered, increasing daily intake of calcium from 1.0 to 1.8 or 2.3 gm, and that of

phosphorus from 1.7 to 3.0 gm. Calcium balances were significantly less negative than those of control subjects in four of five cases; phosphorus balances showed similar patterns.

4. Combined administration of these 3 regimens to 2 subjects also produced a beneficial response.

These conclusions which are based on mineral balance data were only partially confirmed by gamma ray transmission scanning of the central calcaneus, and some discrepancies were noted. We conclude that the 2 month course of calcium and phosphate supplements retarded the development of disuse osteoporosis, but that the intermittent compression and calcitonin were ineffective.

Study 3

PREVENTION OF BONE MINERAL LOSS DURING PROLONGED BED REST
WITH CALCIUM AND PHOSPHATE SUPPLEMENTS

Specific Aim:

To evaluate the ability of calcium and phosphate supplements to prevent disuse osteoporosis during immobilization.

Introduction:

In an ongoing series of experiments, we have investigated several treatment programs in an attempt to prevent mineral loss:

- a) horizontal exercise employing the Exer Genie apparatus;
- b) static longitudinal compression which simulated weight bearing stresses;
- c) oscillating longitudinal compression to simulate dynamic stresses of walking;
- d) daily subcutaneous injections of synthetic salmon calcitonin;
- e) oral phosphate supplements; and
- f) oral supplementation of calcium and phosphate.

In the completed study, #2 (37), it was found that calcium and phosphate supplements reduce calcium and phosphorus losses during bed rest, and may modify the bone mineral loss. This regimen will be further evaluated in these studies.

Methods -- Study A Subjects:

Six subjects were investigated during a baseline period of 5 weeks followed by 17 weeks of continuous bed rest, and finally 2 weeks of reambulation all under balance conditions. Four subjects received oral calcium and phosphate supplements during the bed rest period, and two subjects were untreated controls.

Diet & Medication:

The diet consisted of whole food prepared in 7 daily menus which provided a constant weekly intake of minerals and hydroxyproline. Mean daily calcium intake was 1027 ± 24 mg (SD) and that of phosphorus was 1656 ± 23 mg. Other constituents included: 1 hexavitamin tablet daily, 200 mg Colace^R (dioctyl sodium sulfosuccinate) daily, and 500 mg of polyethylene glycol 4000 given orally three times a day with meals as a continuous stool marker.

Calcium lactate supplements were given between meals to provide an additional calcium intake of 1315 ± 57 mg. The calcium lactate tablets were disintegrated by standing in 15 ml distilled water for 1 hour. Thirty tablets were given daily -- 10 at 10:00 a.m., 10 at 2:30 p.m., and 10 at 8:00 p.m.

Additional phosphorus was also given -- 1419 ± 20 mg P, orally. The tablets contained K_2HPO_4 in proportions providing a pH 7.4 when dissolved in water. Eight tablets were given daily -- 3 with breakfast at 8:00 a.m., 2 with lunch at noon and 3 with dinner at 5:00 p.m.

The calcium and phosphorus supplements were started with the bed rest phase.

Collections were as previously described. Dermal loss of calcium was not measured in this study, but the balance values include an estimated 19 mg/day.

Analytical Methods & Recovery Studies:

Calcium (total ionized), phosphorus, creatinine, alkaline phosphatase, serum protein and urine hydroxyproline were measured as described. Gamma ray transmission scanning was performed on the os calcis weekly.

Study B Subjects:

Four volunteers were studied; 3 for 6 weeks and 1 for 4 weeks of ambulatory control. All subjects underwent continuous bed rest; 2 for 24 weeks (NL, SW); 1 for 5 weeks (JM) and 1 for 8 weeks (PM). SW received additional calcium and phosphate supplements for 18 weeks and then remained at bed rest without the supplements for 6 weeks. PM received the additional calcium supplements for the entire 24 weeks; however, he reambulated after week 8. NL was untreated during the first 18 weeks and then received the supplements between weeks 19 and 24. JM was untreated through 5 weeks of bed rest and then withdrew from the study.

Diet & Medication:

Similar diet and medications were used as described above. Calcium lactate supplements provided 1219 ± 49 mg Ca/day. Twenty-eight tablets were given daily -- 10 at 10:00 a.m., 9 at 2:30 p.m., and 9 at 8:00 p.m. Additional phosphorus was given as KH_2PO_4 , 12 tablets daily -- 4 with

breakfast, 4 with lunch and 4 with dinner, totaling 1440 ± 36 mg P/day.

Dermal loss of calcium was again estimated.

Analytical Methods & Recovery Studies:

Calcium (total and ionized) phosphorus, creatinine, serum protein, alkaline phosphatase and urinary hydroxyproline were measured as described. Gamma ray transmission scanning was performed on the os calcis bi-weekly.

Calcium⁴⁷ kinetic studies were carried out on three occasions: during weeks 3 and 4 of baseline, during weeks 5 and 6 of bed rest and during weeks 17 and 18 of bed rest. For each study approximately 10 microcuries of sterile ⁴⁷CaCl₂ were injected intravenously. Serum was obtained at 0.5, 1, 1.5, 2.5, 4.8, 12, 16, 20 and 24 hours; urine was collected in 8 hour portions the first day, 12 hour portions the second day and then daily through day 9; and stool was collected from the day of injection through day 10 following administration of the isotope. Urine and stool specimens were treated with oxalate to concentrate the calcium and enhance counting efficiency. All specimens were counted in 4 cc aliquots on an Autogamma II (Nuclear Chicago). A rewrite of the Los Alamos least squares program for Fortran IV was used to calculate slopes and intercepts of the specific activity curves. The modified closed three compartmental model of Hansen et al (38) was used in the calculation of pool sizes and exchange rates. Bone accretion and resorption rates were determined from formulae described by Heaney and Whedon (39).

In interpreting data, two subjects from the previous study will be included in this section; JF and JC who received calcium supplements of 1294 mg Ca/day and phosphorus of 1327 mg P/day. The calcium and phosphate supplements were begun after 11 weeks of bed rest.

Results:

Calcium Metabolism

During bed rest, mean urinary calcium excretion rose from an average baseline value of 178 ± 20 mg/day ($\bar{X} \pm \text{SEM}$) to a maximum of 220 ± 27 mg/day in the 5th week. The value then fell slightly and plateaued (200 mg/day). The average increment in urinary calcium excretion during bed rest was 27 mg/day.

Calcium balance data is reported. Mean calcium balances for the four subjects of Part A, three subjects of part B, and the two subjects of study #2 is 38 ± 88 mg/day. Positive calcium balance occurred during the first 13 weeks of the study, averaging +73 mg/day. Calcium balance was negative from the 14th to the 18th week, averaging -109 mg/day.

Mean serum calcium and ionized calcium during treated bed rest was unchanged from ambulatory non calcium supplemented control periods.

Phosphorus Metabolism

All subjects were in positive phosphorus balance throughout the bed rest period when calcium and phosphate supplements were administered. Urine phosphorus values increased proportional to the increase in dietary phosphorus. There was no increasing loss in urine phosphorus

excretion during bed rest as is usually seen with bed rest. Serum phosphate concentrations remained normal and unchanged throughout the study.

Other Metabolic Data

Urinary hydroxyproline excretion was consistently higher throughout the bed rest period than during the baseline ambulatory period.

Urinary nitrogen excretion was increased as has been previously shown in untreated bed rest.

Creatinine clearance was unchanged during bed rest in comparison with the baseline period.

There was no change in serum alkaline phosphatase in phase A subjects.

Calcium Kinetics

Calcium kinetics were performed on NL, JM, FM, and SW. Prior to bed rest, the mean bone formation rate was 534 mg/day, turnover rate 806 mg/day, bone resorption rate 509 mg/day, gastrointestinal (GI) absorption was 297 mg/day and percent GI absorption was 31%. After 5 weeks of bed rest, the untreated subjects demonstrated an increase rate in bone formation rate, turnover and resorption rate. After 17 weeks of bed rest, bone formation rate decreased while turnover continued to increase. In the calcium-phosphate treated group at 5 weeks of bed rest, bone turnover was increased; however, bone formation and resorption rate were inconclusive. At 17 weeks, bone formation rate showed no change from ambulatory control, both turnover and bone resorption rate was significantly increased.

Densitometry:

At the end of bed rest, percent mineral loss from the calcaneus was: TA -- 18% (17 weeks), PM -- 1% (8 weeks), SW -- 5% (17 weeks), RB -- +3% (17 weeks), PH -- +3.5% (17 weeks).

Summary:

Four of the five subjects did not develop the usual negative calcium balance during the first 12 weeks of bed rest. Negative calcium balance was seen in all after the 13th week. Urine calcium did not change from baseline throughout the study. Four subjects showed no appreciable calcaneal mineral loss.

Study 4

EFFECT OF THE DIPHOSPHONATE EIDP ON MINERAL METABOLISM
DURING PROLONGED BED REST

Introduction:

The diphosphonates have received attention because of their potential therapeutic usefulness in a wide variety of skeletal disorders for which no satisfactory treatment is yet known (40-45). As stable analogs of naturally occurring pyrophosphate, they have been shown to retard both the formation and dissolution of hydroxyapatite crystals in vitro (46-48). Inhibition of bone formation and resorption has also been demonstrated in tissue culture (48,49) and in several species of intact animals (50-56). Preliminary studies have indicated that relatively small amounts of diphosphonate may effectively retard bone resorption while bone formation proceeds undiminished (57); in fact, the evidence suggests that a paradoxical enhancement of formation may occur at this optimal dosage level.

Disuse osteoporosis consequent to prolonged bed rest is a useful model for determining the efficacy of diphosphonates in human demineralization disorders for several reasons: (1) the metabolic balance changes in subjects undergoing bed rest of several months duration are consistent and well-defined, so that significant variations from the usual pattern can be recognized easily (3,13,58,59); (2) gamma ray transmission scanning of the calcaneus gives a precise estimate of its mineral content and detects mineral loss in virtually all subjects during prolonged

bed rest (5,13,58,59,60); and (3) the difficult task of reversing established disease is avoided by instituting therapy when bed rest begins and evaluating its usefulness in the prevention of the mineral loss.

Four healthy young men were studied under conditions of strict metabolic balance for 26 weeks including 20 weeks of continuous bed rest. For the bed rest period they were randomly assigned to one of two drug dosage schedules of Disodium etidronate (R)-- either 5 or 20 mg/kg/day. The effects of these regimens were assessed by three largely independent techniques: (1) metabolic balance; (2) ^{125}I gamma ray transmission scanning of the calcaneus; and (3) ^{47}Ca kinetic studies.

General Study Design:

The methods were the same as those previously described. Four healthy Caucasian males aged 22-29 years participated in the current study. Metabolic balance collections were begun after a 10-day period of dietary equilibration. The subjects were ambulatory but confined to the Metabolic Unit for 4 weeks of baseline evaluation. Disodium etidronate (R)* was started three days before beginning bed rest. RA and RS were randomly assigned to receive 5 mg/kg/day, and TM and TO to receive 20 mg/kg/day. Bed rest was continuous for the next 20 weeks; movements in the horizontal plane were not restricted and subjects were

*Disodium etidronate (disodium-ethane-ethane-1-hydroxy-1, 1-diphosphonate, or EHDP) was supplied by the Procter & Gamble Company, Miami Valley Laboratories, Cincinnati, Ohio 45239

allowed to raise their heads as high as 30° to eat, read and use the bed pan. Calcaneal mineral content and appropriate serum chemistries were determined bi-weekly throughout bed rest. ⁴⁷Ca kinetic studies were carried out on three occasions: during weeks 3 and 4 of baseline, during weeks 5 and 6 of bed rest and during weeks 18 and 19 of bed rest. After the 20th week the EHDP was discontinued and reambulation begun.

Metabolic Balance:

The whole food metabolic diet consisted of seven different daily menus which recurred each week of the study. On six different occasions an entire week's diet was analyzed for mineral content with the following mean daily values \pm 2 SD: calcium 1039 \pm 32 mg and phosphorus 1701 \pm 30 mg. Subjects were allowed to supplement their caloric intake with kool-aid and sour ball candies when they wished to curb their appetites. These supplements were recorded and small (0-18 mg/day for calcium; 0-47 mg/day for phosphorus) adjustments made in the calculated dietary intake. Each subject received one hexavitamin daily. Dioctyl sodium sulfosuccinate (Colace[®]) was given during bed rest (100 mg p.o. b.i.d.) to prevent constipation.

Disodium etidronate[®] was given in tablet form one hour prior to breakfast with fruit juice. The EHDP was analyzed for phosphorus and the amount included in each subject's balance calculations as supplemental phosphorus intake (the increments were: RA, 97 mg/day; RS, 72 mg/day; TM and TO, 367 mg/day). EHDP phosphorus contributed to the

total fecal excretion of phosphorus but was not detected by our method for analyzing urinary phosphorus.

Polyethylene glycol* was administered as two capsules three times daily with meals, and balance was calculated using the ratio of administered to recovered polyethylene glycol (61). Analyses of 26 capsules revealed the content of polyethylene glycol to be 257 ± 5 mg (SD).

Results

Metabolic Balance:

Low Dose EHDP (5 mg/kg/day)

Neither subject differed significantly from previous untreated subjects in the magnitude of their hypercalciuria, hyperphosphaturia, or negative calcium and phosphorus balances. During the first week of bed rest, one subject (RS) showed an unusually sharp rise in urinary and fecal calcium. Serum alkaline phosphatase declined in subject RA, and in both subjects urinary hydroxyproline excretion rose to levels at or above the upper limits seen in the previous untreated control subjects. No substantial changes were observed in the serum concentrations of phosphorus or ionized calcium. No abnormalities were noted in urinalysis, complete blood count, creatinine clearance, partial thromboplastin time or the serum levels of glutamic oxalacetic transaminase, bilirubin and glucose.

*The polyethylene glycol 4000 was supplied by Sandoz Products Ltd. of P.O. Box Horsforth No. 4, Calverley Lane, Horsforth, Leeds, England.

High Dose EHDP (20 mg/kg/day)

During the first week of bed rest, both subjects (TM and TO) showed a sharp rise in urinary and fecal calcium excretion and a moderate decrease in urinary and fecal phosphorus excretion. In weeks 2 through 12, the usual patterns of hypercalciuria, hyperphosphaturia and negative calcium and phosphorus balance were seen. During the last 8 weeks of bed rest, a major shift toward positive calcium and phosphorus balance occurred in both subjects.

Urinary hydroxyproline excretion decreased during bed rest in distinct contrast to the rise which is usually seen. The serum phosphorus level rose promptly, persisted at levels about 3 mg/dl above baseline during EHDP therapy and fell to normal after discontinuation of the drug. Serum alkaline phosphatase values tended to decline throughout the treatment period. Parathyroid hormone levels were slightly lower during bed rest than during baseline, but the differences were not statistically significant. Serum total and ionized calcium concentrations were unchanged and the routine laboratory tests listed for the low dose group remained within normal limits.

Calcaneus Scans:

All four subjects lost significant amounts of mineral from the calcaneus. These losses were well within the range of observations made in previous untreated subjects undergoing prolonged bed rest.

Calcium Kinetic Studies:

Low Dose EHDP (5 mg/kg/day)

Bone accretion rate remained constant or fell moderately during bed rest. Bone resorption rate increased markedly at weeks 5-6 and

declined toward the formation rate by weeks 18-19. Endogenous fecal calcium excretion increased during bed rest, and there were decreases in gastrointestinal absorption of calcium and in the miscible calcium pool size.

High Dose EHDP (20 mg/kg/day)

Bone accretion and resorption rates fell progressively and in parallel fashion to levels 50% below baseline by the end of bed rest. There was a decrease in the miscible calcium pool and an increase in gastrointestinal calcium absorption at the fifth week of the study; subsequent changes in gastrointestinal calcium absorption were inconsistent.

Summary:

The effect of the diphosphonate EHDP on bone mineral metabolism was tested in four healthy young men during 20 weeks of continuous bed rest. Two subjects received 20 mg/kg/day and the other two 5 mg/kg/day throughout the period of study. Administration of the drug in low dosage had relatively little effect -- it appeared that the usual increase in bone accretion rate during bed rest was prevented and there was a paradoxical accentuation of the bed rest induced increase in hydroxyproline excretion. Skeletal mineral loss, assessed by calcium measurements and gamma ray absorptiometry of the calcaneus, occurred at the same rate previously noted in untreated control subjects.

Two types of drug effect were apparent at the higher dose: one was immediate and sustained -- a rise in serum phosphorus concentra-

tion and a fall in serum alkaline phosphatase activity. The other was delayed and progressive -- a decline in urinary hydroxyproline excretion and in the rates of bone accretion and resorption. The usual negative mineral balance developed during the first half of the study, then disappeared during the last few weeks. However, calcaneal mineral losses, assessed by gamma ray absorptiometry, were not prevented.

Study 5

THE ROLE OF ORTHOSTATIC FACTORS IN THE LOSS OF
BONE MINERAL DURING PROLONGED BED REST

Specific Aims:

To determine

1. whether orthostatic factors influence bone mineral loss during prolonged bed rest; and
2. whether these factors can be manipulated to prevent demineralization.

Significance of This Research:

Since various therapeutic attempts to prevent the negative calcium balance and loss of calcaneal density during prolonged bed rest have met with only limited success, the role of hydrostatic forces were examined. These forces can be simulated by an apparatus which provides lower body negative pressure (LBNP), and which is suitable for use during either bed rest or space flight. Amounts of lower body negative pressure as high as 50 mm Hg (alternating with 25 mm Hg every 2-4 minutes) for 8 hours per day appear to be feasible (62-64).

Plan of Research Study A:

Eleven subjects between the ages of 22-31 were studied. After a 10-day equilibration period, four subjects had 4 weeks of ambulatory control and seven had 2 weeks of ambulatory control. Then the subjects were put to bed for 4 or 6 weeks during which an orthostatic modification was attempted.

	SUBJECTS				
	LO	KF	NL	SK	BN
Amb Control (wks)	2	2	2	2	4
Length of BR (wks)	6	6	6	6	6
Intervention	BR only	BR + static LBNP ¹	BR + quiet standing ²	BR + quiet sitting ³	quiet sitting; wks 5-6 LBNP ⁴
Reambulation Control	0	0	0	0	0

1. Static lower body negative pressure (LBNP) was for 4 hours daily of -30 mm Hg in the morning and early afternoon
2. Quiet standing was for 3 hours/day at 30 minute intervals: 7:30 a.m., 10:00 a.m., noon, 3:00 p.m., 5:00 p.m., 9:00 p.m. Shoes were taped to floor.
3. Quiet sitting with legs dangling for 8 hours/day in four 2-hour shifts from 7:30 a.m. through 10:00 p.m.
4. During bed rest weeks 5 and 6, quiet sitting was replaced by 4 hours of cyclic LBNP/day at -33 Hg; 2 minutes on and 1 minute off.

	SUBJECTS					
	GT	TO'B	RT	GJ	RB	AM
Amb Control (wks)	4	4	4	2	2	2
Length of BR (wks)	6+4 ⁵	6+6 ⁶	4	4	4	4
Intervention	BR + C-LBNP	BR + quiet standing	BR + C-LBNP during wk ¹	BR +	BR +	BR + C-LBNP during wk ⁴
Reambulation Control	0	0	2	2	2	2

5. GT returned to 4 weeks of bed rest only after a reambulation period of 4 weeks.
6. TO'B returned to 6 weeks of bed rest plus C-LBNP after a reambulation period of 6 weeks.

Plan of Research Study B:

Five subjects (OC, DS, CS, EL, CJ) were studied before, during and after 6 weeks of bed rest as follows:

Equilibrium period -- 1 week.

Ambulatory control phase -- 3 weeks.

Bed rest phase -- 6 weeks.

Recovery phase - 1 week.

Total re-establishment of normal activity -- 11 weeks.

At the end of the 11 weeks of normal activity, this study was repeated on the same five subjects. During bed rest, the five subjects were allocated to receive the following regimen:

Subjects 1 and 2 of the first group (A) received no treatment during the first 12 weeks of the study, and (B) received 4 hours per day of cyclic LBNP (2 minutes on; 1 minute off at -50 Hg) during the bed rest of the third 12 week period.

Subjects 3, 4 and 5 (A) received cyclic LBNP 4 hours per day as described during the bed rest of the first 12 weeks of study, and (B) received no treatment during the bed rest of the third 12 weeks of study.

Medications:

All subjects received a hexavitamin tablet once daily; dioctyl sodium sulfosuccinate (Colace^R) orally in a dose of 100 mg twice daily to prevent constipation; polyethylene glycol 4000, a fecal marker, in an oral dose of 500 mg three times daily.

LBNP Treatment:

A specially constructed box with a vacuum cleaner attached was used to expose the subjects to negative pressure from the iliac crests to the toes.

In study A subjects were supported in the LBNP box by a saddle. The LBNP was given at a constant -30 mm Hg over a 4 hour period. In study B each subject was supported by his feet resting comfortably on a foot board at the end of the box. The LBNP was given cyclicly over a 4 hour period; 2 minutes on and 1 minute off at -50 mm Hg. The treatment was given during mid morning in either two 2-hour periods (study A) or one 4-hour period (study B). A trained attendant and a physician was with the subjects at all times during the LBNP. Pulse and blood pressure were monitored at 0, 5, 15, 30 minutes and every additional 30 minutes of LBNP

Balance Diet:

The diet was prepared in the same fashion as in previous studies. The whole food diet was composed of seven daily menus, each consisting of three meals and an evening snack. On the average, the diet contained 1 gm of calcium and 1.5 gm of phosphorus.

Results Study A:

The usual rise in urine calcium and negative calcium balance was seen in the subjects treated with quiet sitting, quiet standing and constant LBNP. By the end of the 4th week of bed rest, the mean urinary calcium for the two subjects (SK, BN) sitting 8 hours daily was 431 ± 29 mg/day ($\bar{X} \pm \text{SEM}$). Urine calcium at 6 weeks of bed rest of KF was 370 mg/day. The two subjects', NL and TO'B who stood 3 hours/day, mean urine calcium was 270 ± 25 mg/day but had increased only 60 mg/day over the 6 weeks.

Calcium balance (delta change) was -125 mg/day after 4 weeks for those sitting; -215 mg for NL and -77 mg for TO'B after 6 weeks of quiet standing; and -220 mg for KF after 6 weeks of constant LBNP.

AM, RB, GJ, RI who underwent only 4 weeks of bed rest and LO, 6 weeks of bed rest, all showed increased urine calcium by week 3 of bed rest (314 ± 56 mg/day). AM and RT received constant LBNP during the final week of bed rest. Both had slightly more urine calcium than the previous week, 319 vs 315 mg/day.

Calcium balance (delta change) was -158 ± 50 mg/day after 3 weeks in all subjects. The two subjects who received constant LBNP during the 4th week of bed rest had a negative calcium balance of -143 ± 39 mg/day which was greater than the preceding bed rest untreated week.

Significant change in urine calcium and increased negative calcium balance was seen in BN who during bed rest was treated by 8 hours/day chair sitting for 4 weeks and then 2 weeks of cyclic LBNP (4th week

calcium balance -183 mg/day; 6th week calcium balance -170 mg/day).

The two subjects who had cyclic LBNP both showed relatively no change in urine calcium or calcium balance when compared with ambulatory control (urine calcium, ambulatory: C-LBNP TO'B, 218 mg/day vs 217 mg/day; GT 122 mg/day vs 151 mg/day; calcium balance TO'B, +42 mg/day vs +17 mg/day; GT +85 mg/day vs 1 mg/day).

Phosphorus Metabolism:

Phosphorus balance, change from baseline, became negative and urinary phosphorus increased in all. The subjects untreated or treated with constant LBNP during the last week of bed rest had a negative phosphorus balance of -39 ± 23 mg/day; those treated with LBNP for 6 weeks, -94 ± 16 mg/day; 8 hours of quiet sitting, 28 ± 67 mg/day, and 3 hours of quiet standing, -68 ± 28 mg/day.

Other Metabolic Data:

Urine hydroxyproline excretion increased in all subjects comparable to that seen in bed rest alone

Urine nitrogen excretion also increased.

Creatinine clearance did not change throughout the study.

Phase B:

Calcium Metabolism -- urinary calcium excretion rose from an average baseline value of 218 ± 10 mg/day to a mean of 276 ± 4 mg/day during untreated bed rest and 268 ± 3 mg during the 6 weeks of C-LBNP.

Calcium balance became negative similarly in both treated and untreated groups (-90 mg/day vs -82 mg/day). Mean serum calcium was not

changed.

Phosphorus Metabolism -- both the urine excretion and phosphorus balance were similar in the treated vs the untreated groups.

Other Metabolic Determinations -- urinary hydroxyproline excretion increased over baseline by 10 mg/day in the untreated group and 12 mg/day in the C-LBNP group.

Urine nitrogen excretion increased similarly in both groups over ambulatory control period.

Creatinine clearance remained the same throughout the study.

General -- the LBNP caused only minor problems to the subjects, e.g. passing of flatus and the feeling of cold feet. Several subjects complained of intermittent low back pain which was probably positional. Two subjects complained of scrotal pain which was relieved after a scrotal support garment was worn. Four subjects intermittently slept in the LBNP box.

Partial protection from the orthostatism of bed rest was seen in Phase B study (65).

Summary:

1. Cyclic LBNP did not prevent the negative calcium balance of bed rest.
2. Hydrostatic forces do not play a major role in the maintenance of bone homeostasis.
3. LBNP continuous at -33 mm Hg or cyclic at -50 mm Hg does not cause any major adverse effects.

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