The Effect of Supplemental Oral Phosphate on the Bone Mineral Changes during Prolonged Bed Rest

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ABSTRACT Five healthy young men were studied during 24–30 wk of continuous bed rest. During the first 12 wk of bed rest, untreated subjects increased calcium excretion in the urine by 109 mg/day and in the feces by 147 mg/day. The rate of total body calcium loss was 0.5–0.7% per month. Losses of central calcaneus mineral, assessed by gamma ray transmission scanning, occurred at a tenfold higher rate, whereas the mineral content of the radius did not change. Changes in phosphorus balance resembled the calcium pattern, and increased excretion of nitrogen and hydroxyproline also occurred during bed rest. Upon reambulation, the subjects' calcium balance became positive in 1 month and recovery of their calcaneus mineral was complete within 10–20 wk.

Treatment with potassium phosphate supplements (1327 mg P/day) entirely prevented the hypercalciuria of bed rest, but fecal calcium tended to increase. During the first 12 wk, calcium balance was slightly less negative (mean − 193 mg/day) than during bed rest without added phosphate (mean − 267 mg/day). This effect was not seen during the second 12 wk of bed rest. The patterns of magnesium excretion were similar to those of calcium. Fecal and urinary phosphorus excretions were doubled, and phosphorus balance became positive (+ 113 mg/day). Mineral loss from the central calcaneus was similar to that of untreated subjects. It is concluded that this form of phosphate supplementation reduces urinary calcium excretion but does not prevent bone loss during bed rest.

INTRODUCTION

Normal individuals who undergo prolonged periods of bed rest develop disuse osteoporosis (1). Both urinary and fecal excretion of calcium are increased during bed rest, the over-all rate of mineral loss being about 0.5% of the body calcium store per month (1–4). A tenfold greater rate of mineral loss from the central calcaneus has been demonstrated by gamma ray transmission scanning (1).

The administration of oral phosphate supplements reduces urinary calcium excretion in normal individuals (5, 6), in patients with various disorders of calcium metabolism (6–12), and in patients at bed rest (3, 13). The effect of phosphate supplementation on fecal calcium excretion is less certain. It has been thought that it is unchanged or only slightly increased, and that calcium balance, reflecting chiefly the urinary effect, changes in a positive direction (3, 6–8). If this balance effect were confirmed, it might represent an increase in skeletal mineral content. Thus, phosphate could have a useful role in the prevention or treatment of demineralization disorders. One such disorder, disuse osteoporosis, is a suitable model to test this hypothesis; it is a rapidly progressing condition whose course has been well characterized in the past (1–4).

The current study was designed to determine whether administration of oral potassium phosphate prevents the development of disuse osteoporosis. Five healthy male subjects underwent 24–30 wk of continuous bed rest. In each subject, the results from a 12 wk period of phosphate supplementation were compared with those from one or more untreated bed rest periods. Changes in skeletal mineral content were assessed indirectly by calcium balance techniques, and directly by gamma ray transmission scanning of the calcaneus and radius.

METHODS

The methods and materials employed in this study are similar to those previously reported (1).

Study subjects. Five healthy male volunteers aged 19–27 yr underwent continuous bed rest for either 24 wk (R. W., R. G., J. G.) or 30 wk (F. K., R. W. R.). The subjects were required to remain horizontal throughout bed rest, although movements in this plane were not restricted. Subjects were allowed to raise themselves on one elbow for eating and reading. Defecation and micturition were performed while supine. Data obtained during bed rest were compared with information before (baseline ambulatory period) and after (reambulatory period) bed rest. At least 8 wk of baseline ambulatory data were collected, although only the last 5 wk of this period are reported to ensure that adequate equilibration had occurred. During this period, the subjects engaged in a relatively normal level of activity supplemented by 30 min twice daily of walking at 3 mph up a 6° treadmill. The subjects were never allowed to leave the Metabolic Ward unless a staff member was in attendance to assure rigid adherence to the metabolic balance.

Diet and medications. As in the previous study (1), the whole food diet was composed of seven daily menus which provided a constant mineral intake (Table I). Other dietary constituents (including the contents of one hexavitamin tablet daily) were calculated to be: 2484 calories, 106 g protein, 94 g fat, 308 g carbohydrate, 729 mg cholesterol, 3.87 g sodium, 3.17 g potassium, 15 mg iron, 112 μg iodine, 14,200 IU vitamin A, 3.1 mg thiamine, 5.0 mg riboflavin, 42 mg niacin, 192 mg ascorbic acid, 629 IU vitamin D, 74 μg folate, 1.9 mg vitamin B_6, and 7 μg vitamin B_{12}.

Supplemental phosphate was administered orally as Hyper-Phos-K tablets.[1] Each tablet contained a mixture of K_2HPO_4 and KH_2PO_4 in proportions which provide a pH of 7.4 when dissolved in water. 14 tablets were randomly selected for mineral analysis and found to contain 165.9 ±2.3 mg P/tablet (SD). Eight tablets were given daily (two with each meal and two at the evening snack) to provide 1327 mg P/day. All subjects were given 200 mg Colace (dioctyl sodium sulfosuccinate)[2] daily throughout the study.

Serum, stool, urine and sweat collections. The balance periods were 7 days in duration. 35 ml of blood were drawn in the fasting state weekly (J. G., R. G.) or biweekly (R. W., F. K., R. W. R.). 24 hr urines were collected daily, acidified with 1 ml of 12 N HCl/100 ml urine, and stored at 4°C. At the end of each 7 day period, the creatinine content of the daily urines was determined. For each subject, the seven creatinine values were averaged and any 24 hour urine whose creatinine content deviated more than 10% from the mean was assumed to represent a collection error and discarded; 5.6% of all daily specimens were discarded in this fashion. For each subject, the re-

[1] Supplied by Davies Rose Hoyt, Division of the Kendall Company, Needham Heights, Mass.

[2] Mead Johnson Laboratories, Evansville, Ind.

TABLE I
Dietary Mineral Content

	Date*	Calcium	Phosphorus	Nitrogen	Magnesium
		mg/day	*mg/day*	*g/day*	*mg/day*
Predicted Values		1002	1553	16.3	292
Measured Values	11/25/68	998	1614	17.8	301
	12/18/68	969	1602	16.9	296
	1/17/69	996	1563	17.2	296
	2/20/69	982	1624	17.6	302
	3/19/69	999	1614	18.1	294
	4/18/69	975	1567	17.6	282
	5/23/69	960	1584	17.5	293
	6/27/69	954	1599	17.1	298
	7/25/69	988	1602	16.9	302
	8/29/69	965	1625	16.7	299
	9/26/69	984	1512	17.1	300
	10/24/69	981	1593	17.5	296
	11/14/69	979	1573	17.3	300
	12/12/69	994	1573	17.2	302
	1/9/70	980	1623	17.3	290
Mean for subjects F. K. and R. W. R.	(11/25/68–9/26/69)*	979	1591	17.3	297
Mean for subjects R. W., J. G., and R. G.	(5/23/69–1/9/70)*	976	1593	17.2	298

Predicted values were calculated from standard tables. Measured values were determined monthly as follows: An additional serving of each menu was prepared for a week. The food was pooled, homogenized, and analyzed for mineral content. The results were divided by seven to yield daily values.

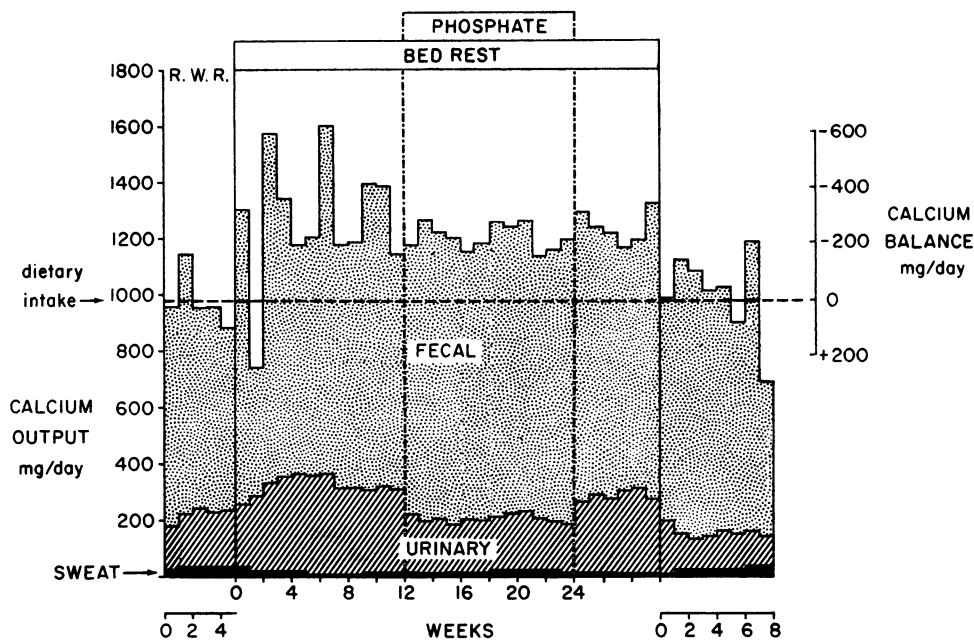* Bed rest was started on 1/27/69 (F. K. and R. W. R.), 6/16/69 (R. W.), and 8/25/69 (J. G. and R. G.).

FIGURE 1 The effect of phosphate supplements (1327 mg P/day) on calcium balance during bed rest in a representative subject (R. W. R.). Calcium output in the sweat, urine, and stool is plotted cumulatively on the ordinate against time on the abscissa.

maining urines were combined into a pool representing the 7 day period and a sample was stored at −22°C.

7-day collections of stools were obtained without markers beginning and ending 16 hr after starting the urine collection (timed to provide a partial correction for intestinal transit time). The methods for homogenization of the stools, and for determination of cutaneous mineral loss have been described previously (1).

Ashing procedures and recovery studies. Specimens were digested in nitric acid for calcium and magnesium determinations, and in sulfuric acid for phosphorus and nitrogen determinations, as previously described (1). Recovery studies were carried out by adding a known amount of mineral to a stool homogenate after removal of the initial sample. The mixture was then homogenized a second time, and a second sample removed. Recovery of added calcium was 101.3 and 98.3% at the beginning of the study, 99.4 and 97.7% during the study, and 100.0 and 101.3% after completion of the study. Recovery of added phosphorus was 101.4, 96.9, 101.0, and 102.2% at the beginning and 104.5 and 106.5% after completion of the study. Recovery of added urea nitrogen was 94.8, 99.3, 97.9, and 99.4% at the beginning and 91.3 and 98.6% after completion of the study. Recovery of added magnesium was 101.9 and 100.7% after completion of the study.

Laboratory determinations. Calcium, magnesium, phosphorus, creatinine, and hydroxyproline were determined as previously described (1). Total nitrogen was analyzed with a Technicon AutoAnalyzer [8] (14) using 0.2% perchloric acid in the digestant and urea standards in 0.1 N H_2SO_4. Alkaline phosphatase was determined by the automated modification of the Bodansky method (15). The mineral content of the central portion of the calcaneus, and of the distal 8 cm of the radius, was assessed by gamma ray transmission scanning, as previously described (16, 17).

[8] Technicon Instruments Corporation, Tarrytown, N. Y.

RESULTS

Calcium balance. Two subjects were not treated during the first 12 wk of bed rest; urinary calcium excretion rose to a maximum during the 6th wk which was almost twice baseline and then gradually declined (Figs. 1 and 2). The values fell sharply with the administration of phosphate during the second 12 wk, and rose again to higher levels during the final 6 wk of bed rest after phosphate had been discontinued. In the three subjects who received phosphate during the first 12 wk of bed rest, hypercalciuria did not develop until the second 12 wk, after phosphate was withdrawn. Fecal calcium excretion was increased during bed rest in all subjects (Fig. 2, Table II). Each subject had a higher mean value during phosphate supplementation than during untreated bed rest; the average increment was 64 mg/day.

During the first 12 wk of bed rest, the average calcium balance for the untreated subjects was − 267 mg/day (Fig. 2, Table II). The mean value for the phosphate-treated group was less negative (− 193 mg/day). During the second 12 wk when regimens were crossed over, the mean calcium balances for the two groups were similar (− 217 and − 214 mg/day). Return to consistently positive values did not occur until the 5th wk of reambulation.

Phosphorus balance. The urinary phosphorus excretion pattern of untreated subjects resembled the pattern observed for urinary calcium (Fig. 3). During

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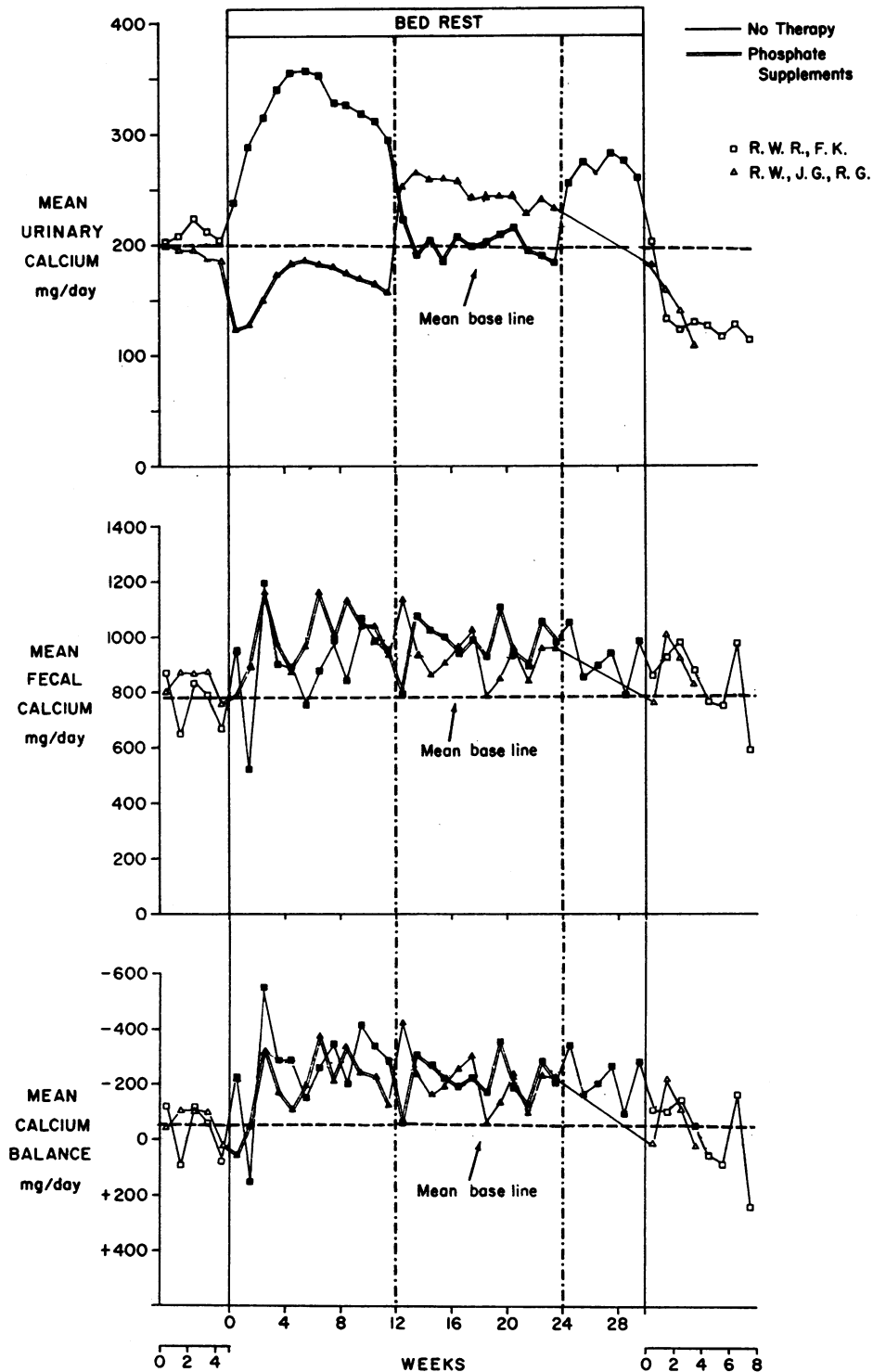
FIGURE 2 The effect of phosphate supplements on calcium metabolism during prolonged bed rest. Mean weekly values during periods of phosphate supplementation (double line) are compared to those during periods without therapy (single line). R. W. R. and F. K. (squares) completed 30 wk of bed rest, whereas R. W., J. G., and R. G. (triangles) reambulated after 24 wk. The mean of the five base line observations for all five subjects is shown as a horizontal dashed line.

TABLE II
Summary of Calcium Balance Data

	No. weeks	Therapy	Subject		Mean	Therapy	Subject			Mean
			R. W. R.	F. K.			R. W.	J. G.	R. G.	
				mg/day				*mg/day*		
Urinary calcium										
Amb	5		187	233	210		154	201	223	193
Bed A	12	None	306	332	319	Phos	168	159	166	164
Bed B	12	Phos	187	214	201	None	199	235	309	248
Bed C	6	None	276	262	269		↓	↓	↓	
Reamb	8*		127	142	135		71	169	161	134
Fecal calcium										
Amb	5		756	770	763		896	779	824	833
Bed A	12	None	945	875	910	Phos	1050	946	1000	999
Bed B	12	Phos	999	957	978	None	1007	856	940	934
Bed C	6	None	950	891	921		↓	↓	↓	
Reamb	8*		846	837	841		878	798	855	844
Dermal calcium										
Amb	5		36	25	31		11	22	16	16
Bed A	12	None	16	18	17	Phos	4	6	6	5
Bed B	12	Phos	19	17	18	None	7	8	10	8
Bed C	6	None	11	22	17		↓	↓	↓	
Reamb	8*		28	27	28		28	14	26	23
Calcium balance										
Amb	5		0	−49	−24		−86	−26	−87	−66
Bed A	12	None	−288	−246	−267	Phos	−246	−135	−197	−193
Bed B	12	Phos	−226	−209	−217	None	−237	−123	−283	−214
Bed C	6	None	−258	−196	−227		↓	↓	↓	
Reamb	8*		−21	−27	−24		−1	−5	−66	−24

Calcium intake was 979 (R. W. R., F. K.) or 976 mg/day (R. W., J. G., R. G.).
Amb, base line ambulatory period; bed A, bed B, and bed C are three consecutive bed rest periods;
Reamb, the reambulatory period; Phos = supplemental phosphate therapy, 1327 mg P/day.
↓, period omitted (subjects R. W., J. G., and R. G., underwent only 24 wk of bed rest).
* Reambulatory period was of 8 wk duration except in the cases of J. G. (4 wk) and R. G. (6 wk).

phosphate treatment a twofold elevation in both urinary and fecal phosphorus excretion occurred. Phosphorus balance during bed rest without treatment averaged − 106 mg/day (first 12 wk), and − 34 mg/day (second 12 wk); the values during phosphate supplementation were + 112 and + 125 mg/day, respectively.

Calcaneus and radius mineral. Serial assessments of the mineral content of the central calcaneus were carried out by gamma ray transmission scanning (Fig. 4). All subjects lost mineral steadily during bed rest. The rate of loss was not reduced by phosphate supplementation; mean total loss of mineral during 12 wk periods with supplementation was actually slightly greater than during 12 wk control periods (16.8% vs. 14.3% of the base line value). Total mineral loss during 24–30 wk of bed rest ranged from 26 to 39% of the baseline values. Mineral content of the calcaneus began to in-

crease promptly upon reambulation, and stabilized near the base line value after 10–20 wk.

Serial assessments of the mineral content of the radius revealed no major changes during the study. The mean changes during the two 12 wk periods of phosphate supplementation were + 0.1 and + 0.8%, and those during the 12 wk control periods were − 0.5 and + 0.5% of the base line value.

Miscellaneous data. Nitrogen balance was more negative during bed rest than during ambulatory periods; the effect of phosphate on this parameter was variable (Fig. 5). During phosphate treatment, mean urinary magnesium excretion was 23 mg/day lower than untreated bed rest values (Fig. 6). Mean fecal excretion of magnesium during treatment was 24 mg/day higher than untreated bed rest values. Magnesium balance was not consistently altered by either bed rest or phosphate.

Dermal calcium loss, which was assessed monthly, was always less than 3% of the total calcium loss (Table II). Mean dermal loss of nitrogen ranged from 3.2 to 3.9% of total output during the study, and mean dermal loss of magnesium ranged from 0.7 to 2.6% of total output. Dermal loss of phosphorus was unmeasurable (less than 1 mg/day).
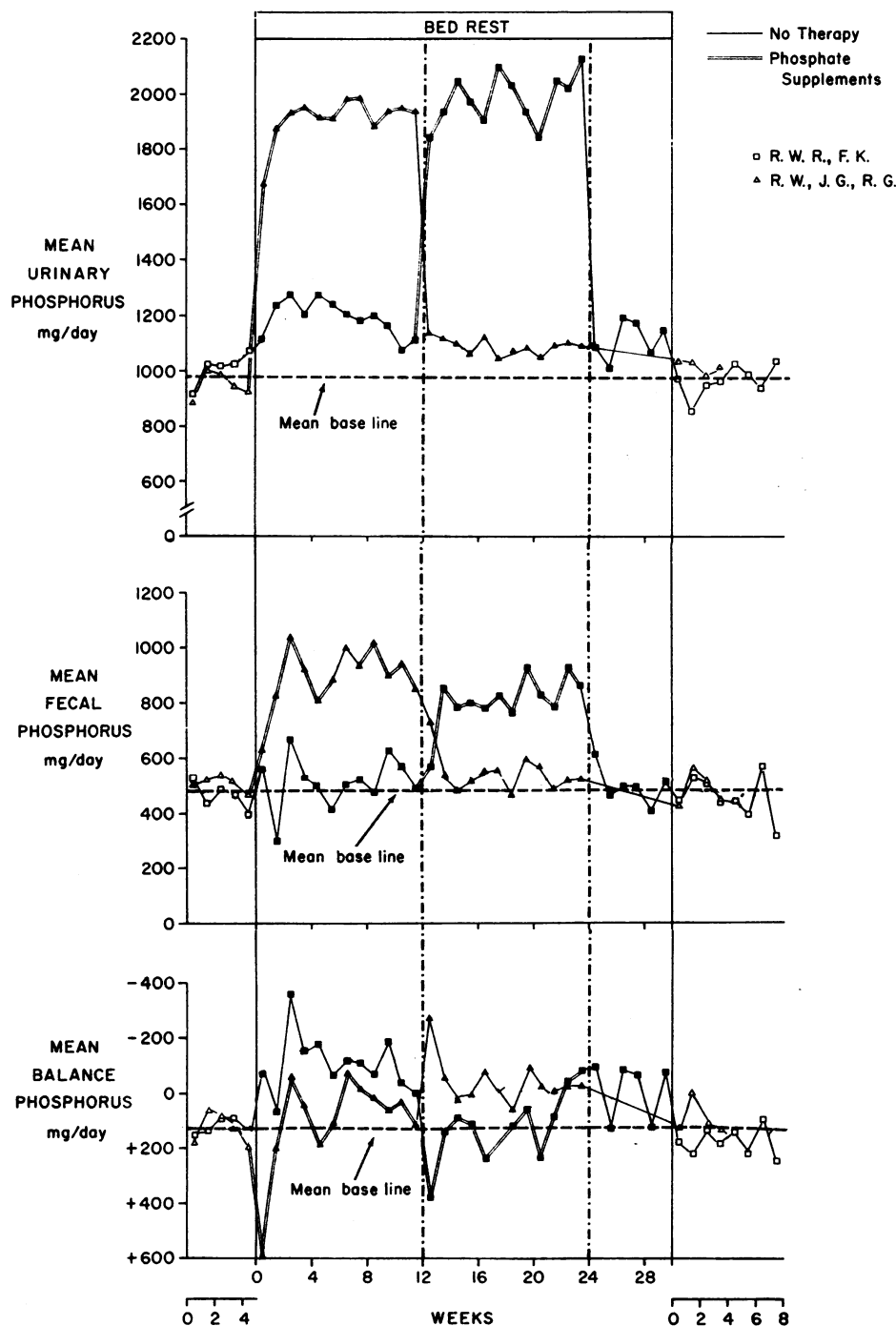
Slight increases in the mean fasting serum calcium

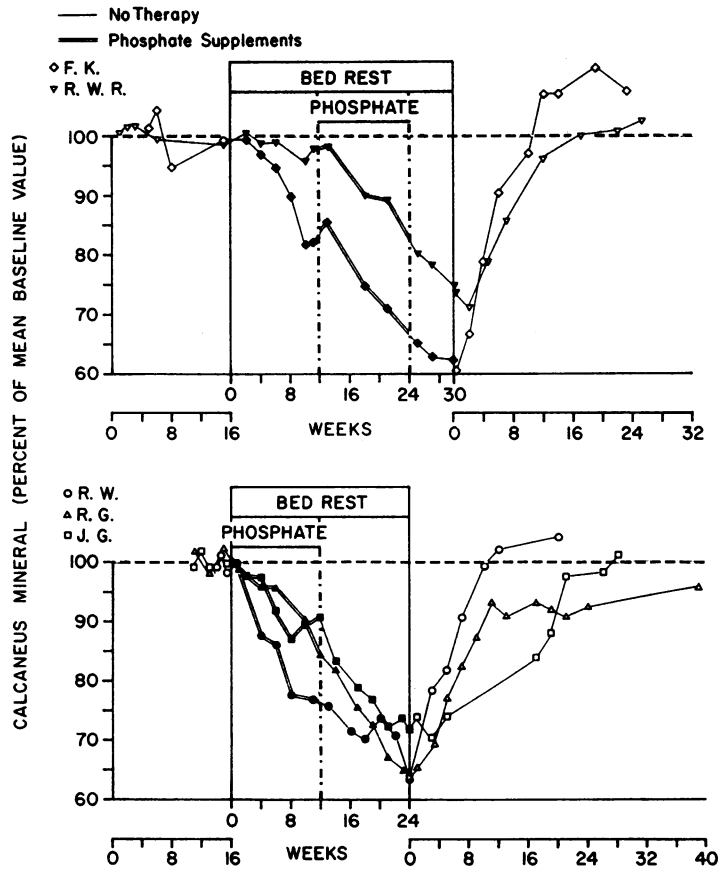FIGURE 3 The effect of phosphate supplements on phosphorus metabolism during prolonged bed rest.

FIGURE 4 The lack of effect of phosphate supplements on loss of calcaneus mineral during prolonged bed rest. Individual value for each of the five subjects are shown as solid symbols during bed rest and as open symbols during ambulatory periods. For each subject, the values are expressed as per cent of the mean base line.

concentrations were usually noted during bed rest; the average increment of 0.2 mg/100 ml was not affected by phosphate administration (Fig. 7). Serum alkaline phosphatase activity did not change consistently during bed rest, but four of the five subjects had increased levels during reambulation. Urinary hydroxyproline excretion reached a maximum during the 2nd month of bed rest, and fell to near base line during the final months in both treated and untreated subjects (Fig. 8).

Creatinine clearance was relatively constant during the study (Fig. 8). Body weight was stable except for R. W. R. and F. K., who gained 2 and 6 kg, respectively.

There were no significant medical or psychiatric complications. Weekly examinations of urinary sediment revealed no crystalluria. Upon reambulation, plantar tenderness during the 1st wk was minimized by limiting the daily duration of ambulation. Mild fatigue was noted for several months and then disappeared.

DISCUSSION

Periods of bed rest without phosphate supplements were associated with increased excretion of calcium and phosphorus as expected (1). During the first 12 wk of bed rest, the mean calcium balance was − 267 mg/day, a loss of total body calcium of approximately 0.7% per month (assuming the total body calcium to be 1250 g [18]). The rate of loss decreased during the subsequent months, averaging 0.5% per month in untreated subjects. Phosphorus balance changes were similar to the calcium balance patterns.[4]

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[4] During the first 12 wk of bed rest without treatment, mean phosphorus balance was − 106 mg/day. If the change in bone mass were the only factor contributing to deviations in phosphorus balance, the expected value would be − 117 mg/day (assuming the P : Ca ratio of bone mineral to be 0.44 [19]). However, changes in nonskeletal protoplasm also affect phosphorus balance (20). The increased
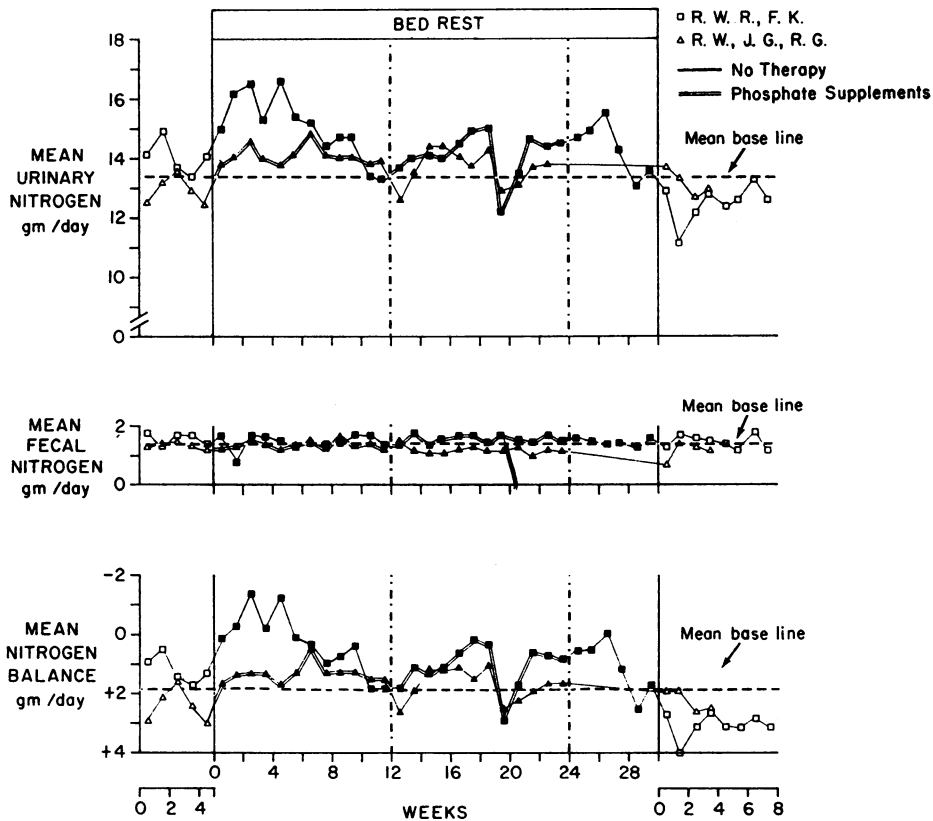
FIGURE 5 Nitrogen metabolism during prolonged bed rest with and without phosphate supplementation. The data are plotted in the same fashion as those in Fig. 2.

In contrast to these changes in the whole body calcium store, tenfold greater rates of mineral loss were observed from the central calcaneus. It should be noted that these large changes in the calcaneus contributed relatively little to the over-all mineral loss; assuming that this bone is 1/500th of the skeletal mass (21), its 26–39% mineral loss during the study represents only 5 mg of calcium per day. The fact that changes occurred in the calcaneus but not in the radius supports the hypothesis that during bed rest mineral loss occurs only from bones which are normally weight bearing.

The mechanism of bone loss during bed rest is probably increased bone resorption, which has been documented in other studies of immobilization by isotopic (22, 23) microradiographic (24), and tetracycline labeling (25) techniques. Accelerated resorption is probably manifested in the current study by the increase in

hydroxyproline excretion. The local increase in bone resorption might result from a reduction of the mechanical forces on the legs (26–28), perhaps mediated by alteration of piezoelectric potentials (29).

Increased bone resorption would lead to the observed increase in serum calcium concentration, which in turn could increase urinary calcium excretion by two mechanisms: directly by increasing filtered load, and indirectly by suppressing parathyroid hormone secretion. The mechanism for the fecal calcium increase is more obscure. If reduction in parathyroid hormone concentration does occur, a reduction in gastrointestinal absorption of calcium might result (30). It is also possible that increased circulating glucocorticoids, reduced vitamin D activity, or adaptation by unknown mechanisms may play a role (31). Alternatively, the data may reflect increased secretion of calcium into the gastrointestinal tract, rather than reduced absorption.

Reambulation was attended by a prompt fall in serum concentration and urinary excretion of calcium, perhaps reflecting a sudden return of bone resorption to normal or subnormal levels. In support of this, hydroxyproline levels tended to fall. A rapid increase in bone formation

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nitrogen loss during bed rest represents loss of lean body mass, and greater losses of phosphorus would be anticipated. This prediction is fulfilled if all balance values are expressed as change from base line; the theoretical phosphorus balance (20) during the first 12 wk of bed rest in the untreated subjects was − 163 mg/day, and the mean observed change from baseline was − 227 mg/day.

is also possible; mean serum alkaline phosphatase activity increased in four of the five subjects.

Reduction in fecal calcium excretion was more gradual, and the negative balance persisted for the first month of reambulation, in accord with previous observations (1, 2, 32). Calcium balance did become positive at about the 5th wk of reambulation; this event has not been documented in previous studies because observations of recovery have been less extensive.

In contrast with the balance observations, recovery of calcaneus mineral usually began soon after reambulation. Recovery proceeded at a more rapid rate than loss during bed rest, and was virtually complete after 10–20 wk. The discrepancy between the balance and densitometric data during the first month of reambulation might be explained by redistribution of mineral within the body during this period. The data confirm the reversibility of mineral loss due to immobilization, a process which has been the subject of controversy in the past (1, 32–35).
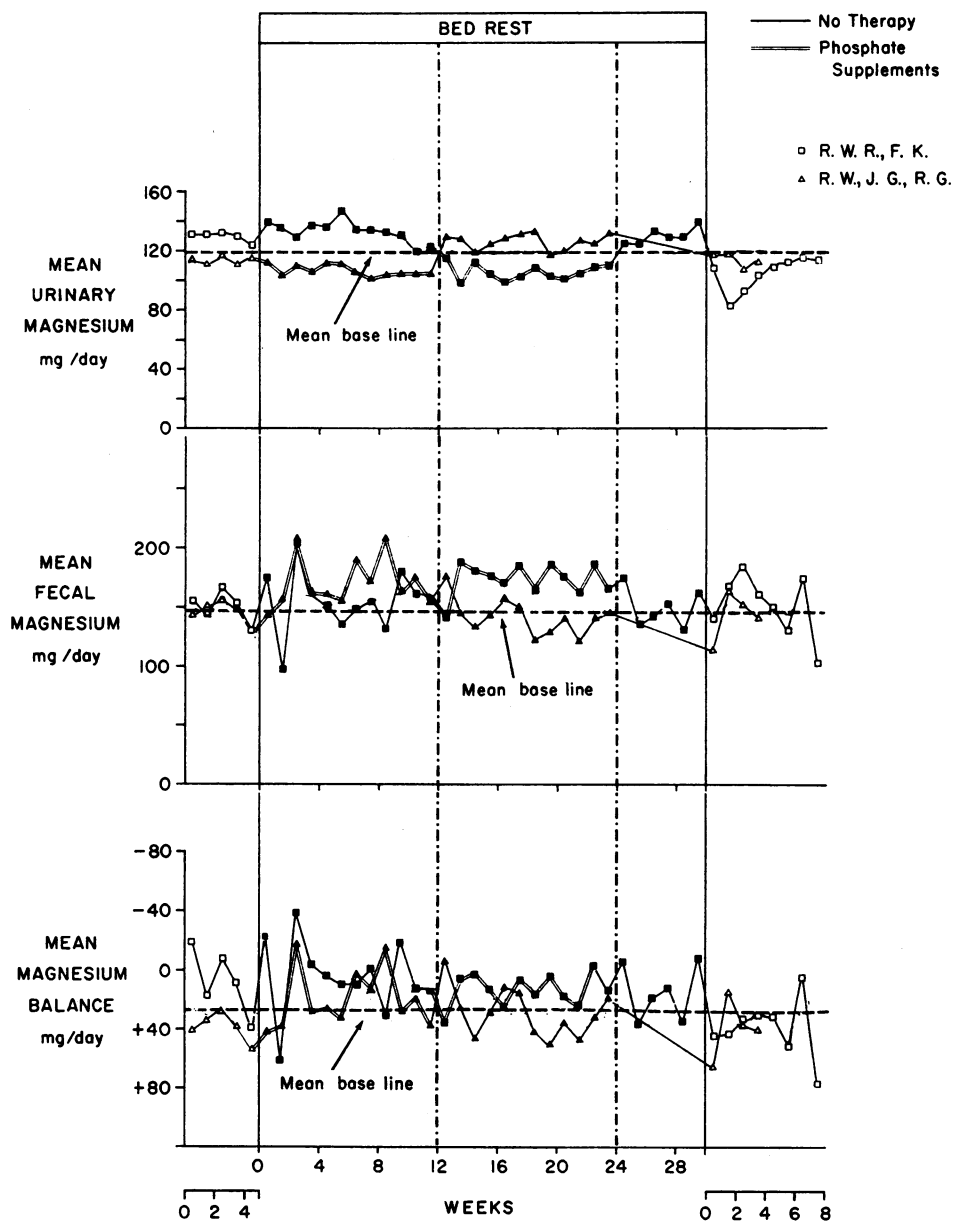

FIGURE 6 Magnesium metabolism during prolonged bed rest with and without phosphate supplementation.
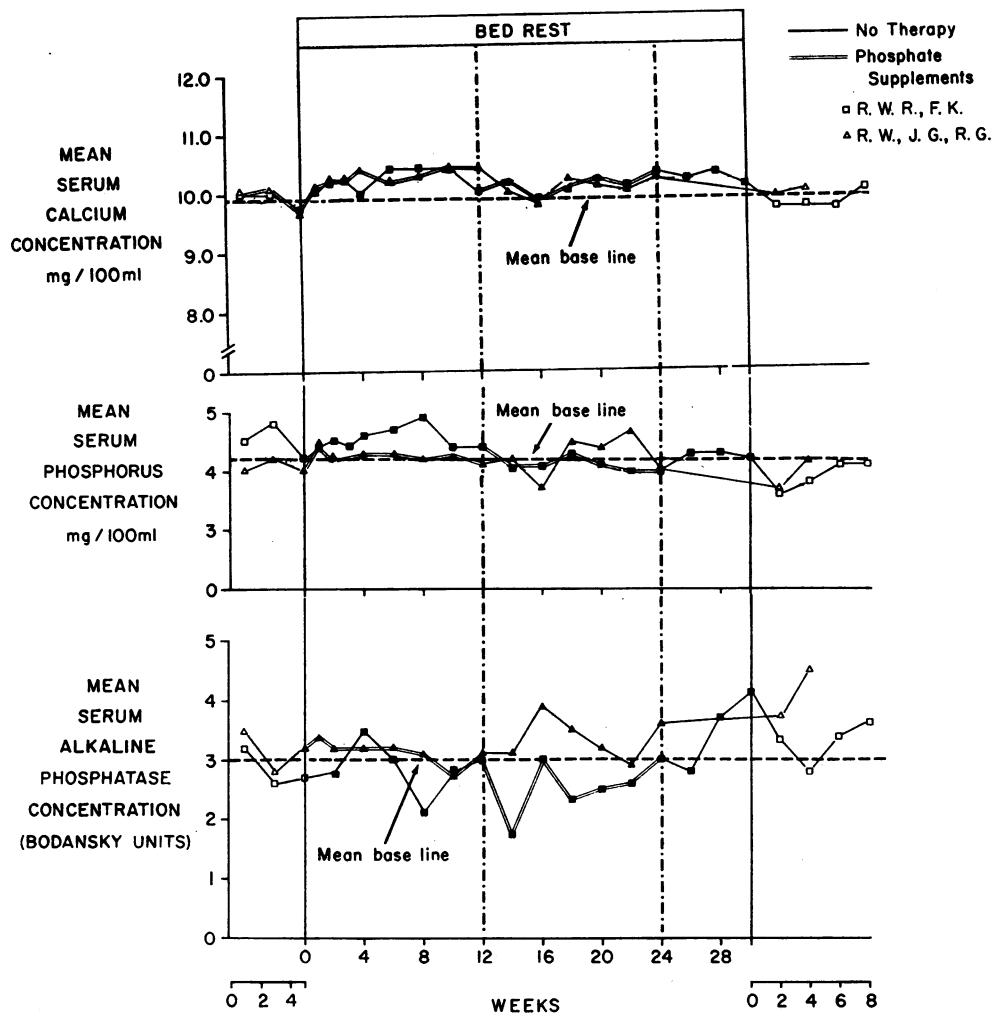
FIGURE 7 Mean serum calcium and phosphorus concentrations, and alkaline phosphatase activity during prolonged bed rest with and without phosphate supplementation.

Use of phosphate supplements consistently prevented the hypercalciuria which occurs during bed rest without treatment. Reduction of urinary calcium by oral phosphate loading has been shown in many previous studies (3, 5–13). The mechanism for this effect is uncertain. In two previous studies of normocalcemic individuals (5, 36), reductions in serum calcium concentration of about 0.2 mg/100 ml have followed oral phosphate supplementation; this may occur as a physiochemical response to the slight increase in serum phosphorus concentration. These serum changes were not observed in the current study, perhaps because only fasting sera were analyzed. If small reductions in serum calcium concentration during the day do occur, they could decrease urinary calcium excretion by reducing the filtered calcium load or increasing parathyroid hormone secretion (37). It is also possible that

the effects of phosphate supplementation on urinary calcium simply reflect the response of bone to an alkaline load. Bicarbonate feeding reduces urinary calcium excretion (38), probably by protecting the skeleton from resorption for the purpose of providing phosphate buffer.

Previous investigators have reported little change or a slight increase in fecal calcium during periods of phosphate supplementation (3, 6–8). In the current study, fecal calcium was increased by an average of 64 mg/day when periods of phosphate supplementation were compared with untreated bed rest periods. This might be a result of complexing of calcium and phosphate in the gut.

A modest change of calcium balance in a positive direction has been noted previously in studies of phosphate supplementation (3, 6–8). The most extensive
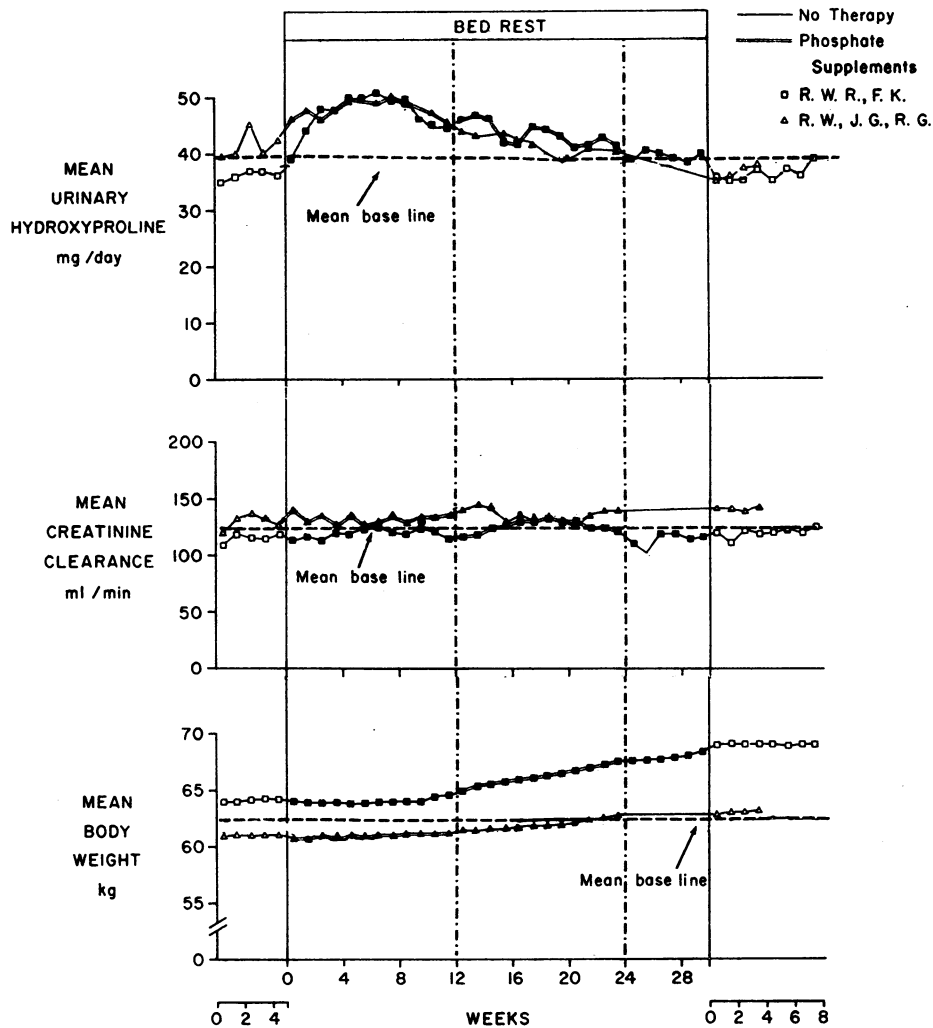
FIGURE 8 Mean urinary hydroxyproline, endogenous creatinine clearance, and body weight
during prolonged bed rest with and without phosphate supplementation.

and pertinent of these was carried out by Goldsmith
et al. (3); 1–2 g phosphorus per day were administered
for 10–30 days to six volunteers at bed rest. Mean cal-
cium balance during bed rest without treatment was
— 152 mg/day, and that during phosphate supplementa-
tion — 78 mg/day. This effect of phosphate would be in
accord with in vitro and animal studies in which phos-
phate has been judged to inhibit bone resorption (39–
41) or stimulate bone formation (40, 42–44).

In the current investigation, the data during the first
4 wk of bed rest resemble those of the Goldsmith study
(3); mean calcium balance was less negative during
phosphate supplementation than during untreated bed
rest (— 125 vs. — 228 mg/day). However, this effect
was diminished by the end of 12 wk (— 193 vs. — 267
mg/day), and was not seen during the second 12 wk
of bed rest (— 217 vs. — 214 mg/day). Moreover, loss

of calcaneus mineral was not reduced by phosphate
administration, and bone resorption and formation
were not altered enough to change urinary hydroxy-
proline excretion or serum alkaline phosphatase levels.
It is possible that substantially different conclusions
would result from use of a different dose of phosphate.
However, selection of the supplementary 1327 mg P/
day was based on a careful review of previous ex-
perience (3, 5–12). The data are most consistent with
Eisenberg's conclusion (based on intravenous infusion
data), that phosphate does not affect bone formation
or resorption in man (45).

Phosphate supplementation was associated with a
positive phosphorus balance of about 100 mg/day. This
is unexpected, since depletion of both the skeleton and
the total lean body mass is inferred from the calcium
and nitrogen balance measurements. The most plausible

explanation for these findings is that the phosphate composition of one or several components of the lean body mass increases during phosphate supplementation. In support of this hypothesis, the relative mineral constitution of bone salt depends on numerous factors including dietary phosphorus (46).

Renal calculus formation is a common complication of immobilization (47) which is probably prevented by phosphate supplementation. This protection has been established in habitual stone formers (9, 11), but is neither confirmed nor refuted in the current study.

The early improvement in calcium balance during phosphate supplementation leaves open the possibility that this agent may have a role in the prevention or treatment of demineralization disorders. However, the failure to alter substantially the metabolic changes which occur during prolonged bed rest, and the lack of any attenuation of the calcaneal mineral loss, lead to the conclusion that this form of therapy alone does not prevent disuse osteoporosis.

ACKNOWLEDGMENTS

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