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Abnormalities in Circadian Patterns of Bone Resorption and Renal Calcium Conservation in Type I Osteoporosis*

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ABSTRACT. We compared changes over 24 h in 15 postmenopausal normal women (mean [±SD] age, 64 ± 7 yr) with those in 15 postmenopausal women with type I osteoporosis and vertebral fractures (mean age, 64 ± 5 yr). The serum osteocalcin concentration, a sensitive index of bone formation, increased by about 5% at night in both groups. Urinary deoxypyridinoline excretion, a sensitive index of bone resorption, increased by 48% at night ($P < 0.01$) in the normal women, whereas in the osteoporotic women it was 62% higher overall ($P < 0.05$), and the increase persisted into the morning. At night, urinary fractional excretion of calcium decreased by 20% ($P < 0.001$) in the normal women, but was unchanged in the osteoporotic women; this circadian pattern differed between groups ($P < 0.05$). The serum ionized calcium concentration did not change at night in either group. There was a trend ($P = 0.07$) for blunting of the nocturnal increase in the serum intact PTH level in osteoporotic women. Thus, the nocturnal serum ionized calcium level is maintained by decreased urinary calcium excretion and increased bone resorption in postmenopausal normal women, but almost entirely by increased resorption in postmenopausal osteoporotic women. This greater dependence on bone resorption during the nocturnal fast may account in part for the greater bone loss in osteoporotic women. (*J Clin Endocrinol Metab* **74:** 487–494, 1992)

TYPE I (postmenopausal) osteoporosis results from loss of trabecular bone during the first 20 yr after menopause and is characterized by fractures of the distal forearm and vertebrae (1). Bone turnover in this form of osteoporosis has been extensively studied by calcium kinetics and bone histomorphometry over the last 2 decades. In general, these studies have found bone turnover to be heterogeneous: increased in most osteoporotic women, but decreased in some (2, 3).

Although these methods provide good assessments of overall bone turnover, the findings represent the integral values for a 2- to 3-week interval and, thus, are incapable of detecting short term changes in calcium homeostasis and bone turnover that are produced by such daily events as meals and the immobility of sleep. However, the use of recently developed assays for sensitive biochemical markers of bone turnover that are specific to bone makes circadian studies feasible in humans. Pyridinoline and deoxypyridinoline (Dpyr), the nonreducible cross-links of collagen, are found in bone collagen. They are present in the diet, but are not absorbed in the gut (4). Pyridinoline is found in a number of tissues, including cartilage, tendon, ligament, and aorta, but Dpyr is specific to bone (5, 6).

Urinary Dpyr excretion reflects bone collagen breakdown. It correlates well with radioisotopic measurements of bone resorption (7) and is a sensitive measure of bone resorption in a number of metabolic bone diseases (8), including osteoporosis (9). Osteocalcin (also called bone Gla protein) is a 49-amino acid peptide secreted by osteoblasts. Its concentration in serum reflects bone formation, as assessed independently by bone histomorphometry and radiocalcium kinetics in normal subjects (10) and by bone histomorphometry in osteoporotic subjects (11).

This study had two aims. The first was to relate previously documented changes in circadian patterns of calcium homeostasis to possible changes in circadian patterns of bone turnover, as assessed by using the new biochemical markers. The second was to determine whether circadian patterns of bone turnover and calcium homeostasis are abnormal in osteoporosis. The finding of such abnormalities could provide better insight into the mechanism underlying bone loss and could lead to new strategies to prevent it from occurring.

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Materials and Methods

Experimental subjects

We studied 15 healthy postmenopausal women (Table 1), aged 51–75 yr, who were 4–24 yr postmenopausal. None had entered menopause before the age of 40 yr, and none had any disease or was taking any medication known to affect bone density. We excluded nightshift workers and subjects who recently had traveled across several time zones. No subject in this group had a vertebral fracture evident on anteroposterior or lateral radiographs of the lumbar and thoracic spine. Data for 8 of these subjects for circadian patterns in mineral metabolism, but not for biochemical markers of bone turnover, have been previously reported (12).

We also studied 15 women (Table 1), aged 57–72 yr, who had vertebral fractures and were 8–35 yr postmenopausal. All had 3 or more mild wedge fractures (anterior vertebral height, 75–85% of posterior height), 1 or more severe wedge fractures (anterior height, <75% of posterior height), or both. All fractures had occurred after minimal or no trauma.

No subject had ever taken sodium fluoride, estrogens within the preceding 6 months, or calcium within the preceding 3 months. The studies were approved by the Mayo Institutional Review Board, and signed informed consent forms were obtained. All studies were carried out at the Mayo Clinical Research Center.

Study protocol

The experimental subjects consumed a low gelatin diet for the 3 days immediately before the study. Dietary intake of calcium was estimated from a 7-day diet record and an interview with a trained dietician. The study diet was low in gelatin and matched the habitual intake of calcium. Meals were served at 0800, 1200, and 1800 h and were consumed within 30 min (Fig. 1).

The subjects were admitted to the Clinical Research Center at 0615 h, and an iv cannula was inserted. Blood was drawn (11 mL) every 2 h from 0700 h until 0700 h the next day. The cannula was flushed with 2 mL sterile saline (154 mmol/L) after each blood sample was drawn. For Ca^{2+} measurement, 600 μL blood were placed in each of two serum separator tubes (SST Microtainer tubes, Becton Dickinson Vacutainer Systems, Rutherford, NJ), allowed to clot for 30 min, centrifuged at 3000 rpm for 10 min, refrigerated, and stored unopened at 4 C until analyzed (within 24 h). Serum was separated from the remainder of the blood (~10 mL) and stored at −70 C until analyzed.

Urine was collected for three 8-h periods, starting at 0700 h. Glacial acetic acid was used as a preservative. Aliquots of the collection were stored at −20 C.

All subjects were ambulant (sitting or walking) from 0800–2230 h and recumbent from 2230–0700 h.

Biochemical measurements

Measurements of serum ionized calcium, phosphorus, osteocalcin, and PTH and urinary pyridinium cross-links from an individual were all performed in single assays. Ca^{2+} in samples collected anaerobically was measured with a Radiometer ICA 1 analyzer (Radiometer Copenhagen, Copenhagen, Denmark). The Ca^{2+} values reported are those at the pH of the specimen (not corrected to pH 7.40). The inter- and intraassay coefficients of variation for serum ionized calcium with aqueous controls and a normal pool (adjusted to pH 7.4) were less than 1.6% and less than 0.8%, respectively. Serum and urinary total calcium levels were measured in duplicate by atomic absorption spectroscopy (IL 751 atomic absorption spectrophotometer, Instrumentation Laboratories, Lexington, MA), serum and urinary phosphorus levels were measured by a phosphomolybdic acid method, and serum albumin was measured by a bromcresol green method using a Rotachem centrifugal analyzer (Travenol Laboratories, Inc., Round Lake, IL). Serum and urinary creatinine levels were measured by an automated chemistry analyzer (Beckman Astra 8 analyzer, Beckman Instruments, Inc., Brea, CA).

Serum osteocalcin was measured by RIA (13) with an antiserum raised in rabbits to bovine osteocalcin. Homogeneous bovine osteocalcin was used for tracer and standard. Antibody-bound and free ^{125}I-labeled osteocalcins were separated by the double antibody method. The intraassay variation was less than 7%; the interassay variation was less than 10%. All measurements were made in duplicate. Serum osteocalcin levels are greatly reduced by hemolysis. As a result, there were only complete data on eight subjects in each group.

Serum intact PTH was measured in duplicate by IRMA (Allegro intact PTH immunoassay system, Nichols Institute Diagnostics, San Juan Capistrano, CA). The intraassay variation was less than 8%; the interassay variation was less than 11%.

The fractional excretion of calcium (FECa) was calculated by: FECa = (urinary calcium/serum ionized calcium) × (urinary creatinine/serum creatinine). The fractional excretion of phosphorus (FEPhos) was calculated by: FEPhos = (urinary phos-

TABLE 1. Baseline characteristics (mean ± SD) of 15 normal and 15 osteoporotic women studied

Variable	Normal	Osteoporotic	*P*	Difference mean (95% CI)[a]
Age, yr	64.3 ± 6.9	64.2 ± 4.8	0.95	0.1 (−4.3–4.6)
Years postmenopause	14.7 ± 6.7	17.9 ± 8.0	0.24	−3.2 (−8.7–2.3)
Height, cm	161.5 ± 7.9	157.7 ± 5.6	0.14	3.8 (−1.3–8.9)
Weight, kg	69.5 ± 8.7	65.7 ± 10.1	0.28	3.8 (−3.2–10.8)
Dietary Ca, mg/day[b]	864 ± 304	791 ± 239	0.47	73 (−131–277)

[a] 95% confidence interval for difference (normal − osteoporotics) in means.

[b] Obtained from 7-day diet record.

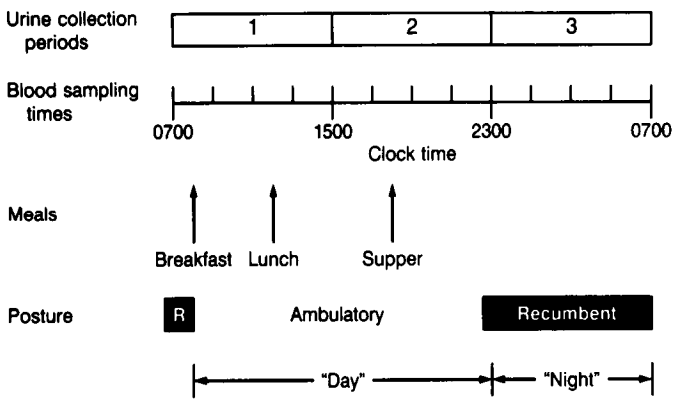

FIG. 1. Study protocol, showing timing of urine collections, blood sampling, meals, and posture.

phorus/serum phosphorus) × (urinary creatinine/serum creatinine.

Urinary Dpyr was measured by fluorometric detection after reverse phase high pressure liquid chromatography (5). The urine sample was prepared for HPLC by acid hydrolysis, followed by separation on a cellulose CF1 column (6). The interassay variation was less than 11%, and all measurements were made in duplicate.

All studies and measurements were made at the Mayo Clinic, except for measurements of urinary Dpyr, which were made at the Department of Human Metabolism and Clinical Biochemistry, University of Sheffield.

Statistical analyses

Circadian patterns in the 13 serum and 3 urine measurements were examined by standardizing each woman's data. The mean and SD of all plasma or urine measurements for each woman were calculated, and then individual values were expressed as an R (relative deviate) score: R score = (observed value − mean over all time points)/SD over all time points. The R score at a given time point indicates how far, in SD units, above (positive values) or below (negative values) the individual's mean value the reading is. The mean of each woman's R scores is 0, and the SD is 1. Urine values were analyzed in both standardized and original (untransformed) scales.

Trends throughout the day for the serum data were assessed in two ways. First, for each woman we applied the white noise test with $\alpha = 0.05$ (14) to the 13 2-h R scores to test the null hypothesis that there is no pattern over time and that these scores merely represent random fluctuations (15). Under the null hypothesis of no circadian pattern, we might expect at most 5% of the subjects to exhibit significant fluctuations.

Second, we fit polynomial equations for R score on time to each woman's data. If there are no important time trends, then the mean of the polynomial coefficients will be near 0 over all women.

Hence, time trends also were tested by using a one-sample Hotelling T^2 test of the hypothesis that the mean vector of polynomial coefficients was 0. Our goal in fitting polynomials was to represent the 13 2-h values accurately by the fewest possible number of coefficients. The terms included in the polynomial and the reported R^2 values are based on the fit of the model to the time series of mean values.

There were only three time periods for the urine data, which made the analysis somewhat simpler. Time trends were assessed by multivariate analysis of variance to test the hypothesis that the mean of the three time periods did not differ from each other.

To test whether the values from the normal women were different from the values from the osteoporotic women overall, we used multivariate analysis of variance (Hotelling T^2 test) on the mean vector of polynomial coefficients for serum data and on the mean vector of the three readings for urine data.

To compare day and nighttime values, we calculated the mean of the daytime values (for urine the collection period was 0700–2300 h; for serum, this included 2-h samples obtained from 0700–2100 h) and the nighttime values (urine, 2300–0700 h; serum, 2300–0700 h) for each woman from the raw data. We then compared daytime and nighttime values within groups by using the paired test or signed rank test, as appropriate. Comparisons between normal and osteoporotic women used the two-sample test or rank sum test.

All data were analyzed with the Statistical Analysis System (15). All statistical tests were two-sided, with significance declared if $P \leq 0.05$. $P \geq 0.10$ or larger is reported as not significant.

Results

Calcium homeostasis

The standardized serum Ca^{2+} levels followed a circadian pattern in normal women ($R^2 = 0.43$; $P < 0.01$; by fitting first, second, fourth, sixth, eighth, and ninth order polynomial terms; 4 of 15 significant by the white noise test) and in osteoporotic women (3 of 15 significant by the white noise test; P = NS by fitting the above polynomials; Fig. 2, *upper panel*, and Table 2). Mean nighttime and daytime serum Ca^{2+} levels were not different (Table 3).

The standardized serum total calcium and serum albumin levels exhibited significant circadian patterns in both groups (Table 2). The mean nighttime serum total calcium value was lower than the mean daytime value by 1.2% for normal women ($P < 0.01$) and osteoporotic women ($P < 0.05$); the mean nighttime serum albumin value was lower than the mean daytime value by 5.9% for normal women ($P < 0.001$) and by 6.3% for osteoporotic women ($P < 0.001$; Table 3).

The standardized urinary calcium excretion did not show a significant pattern in either group (Table 4). Urinary calcium excretion showed a 17.9% decrease at night in normal subjects ($P < 0.05$), but only a 1.1% increase at night in osteoporotic women (P = NS); this difference between groups was of borderline significance ($P = 0.09$; Table 3).

The standardized FECa followed a circadian pattern in normal women ($P < 0.001$) and, to a lesser extent, in osteoporotic women ($P = 0.08$; Table 4 and Fig. 3, *lower*

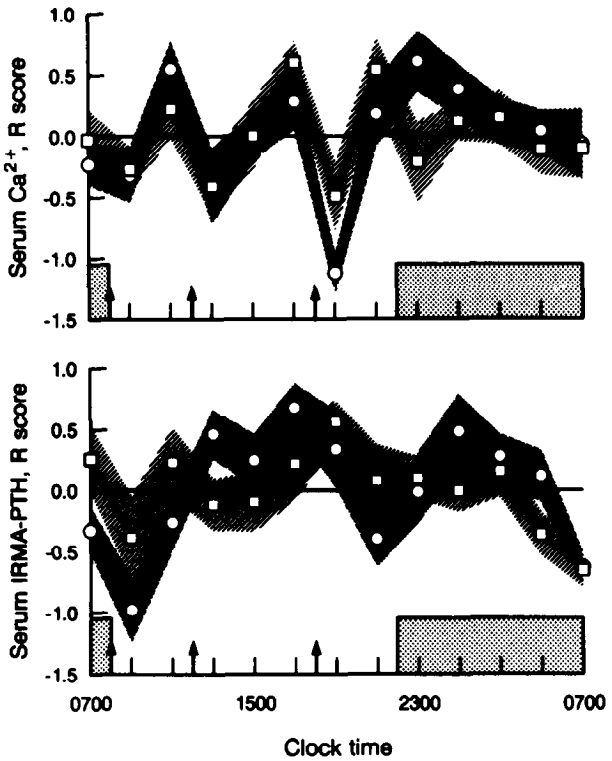

FIG. 2. *Upper panel,* Serum Ca^{2+}. Circadian variations (mean ± SE) in 15 normal women (○) and 15 osteoporotic women (□) women. *Arrows* indicate meal times; *horizontal bars* represent the period of recumbancy. At night (■), serum Ca^{2+} did not decrease. *Lower panel,* Serum intact PTH. Patterns differed ($P = 0.07$) between groups.

panel). The difference between these patterns was significant ($P < 0.05$). The mean FECa showed a 20.0% decrease at night in normal women ($P < 0.001$) and only a 2.3% decrease in osteoporotic women (P = NS); this difference between groups was significant ($P < 0.05$; Table 3).

PTH

The standardized serum intact PTH level followed a circadian pattern in normal ($R^2 = 0.78$; $P < 0.01$; by fitting first, second, third, fourth, and fifth order polynomial terms; 3 of 15 significant by the white noise test) and osteoporotic women ($R^2 = 0.65$; $P < 0.01$; by fitting polynomials; 4 of 15 significant by the white noise test). This difference between the groups reached borderline significance ($P = 0.07$). The peaks for PTH occurred at 1700 and 0100 h in the normal women, but only at 2300 h in the osteoporotic women (Fig. 2, *lower panel*).

Bone turnover

The standardized serum osteocalcin level followed a circadian pattern in normal and osteoporotic women, based on analysis by the white noise test (four of eight and three of eight had significant results, respectively; Fig. 4). The pattern was also present when tested by polynomial fitting (using first, second, third, sixth, and ninth order terms in the polynomials) for both the normal women ($R^2 = 0.84$; $P < 0.03$) and the osteoporotic women ($R^2 = 0.91$; $P < 0.01$; Table 2). The patterns did not differ significantly between the groups. There was a 4.5% increase at night in normal women ($P < 0.05$) and a 5.0% increase at night in osteoporotic women ($P < 0.01$); these increases did not differ significantly between groups (Table 3).

The standardized serum Ca^{2+} × phosphorus product followed a circadian pattern in normal women and osteoporotic women (12 of 15 and 11 of 15 had significant white noise tests, respectively). The pattern was also present when tested by polynomial fitting (using first, second, third, fifth, and sixth order terms in the polynomials) for both normal ($R^2 = 0.80$; $P < 0.001$) and osteoporotic women ($R^2 = 0.97$; $P < 0.01$; Table 2). The pattern did not differ significantly between the groups. There was a 9.8% increase at night in normal women ($P < 0.001$) and a 5.8% increase at night in osteoporotic women ($P < 0.01$); this difference between groups was not significant (Table 3).

The standardized urinary Dpyr excretion showed a significant circadian pattern in both groups ($P < 0.01$). There was a 48% increase in mean Dpyr excretion in normal women ($P < 0.01$), and this nighttime increase persisted into the morning in the osteoporotic group (Fig. 3, *upper panel,* and Table 4). The difference in circadian pattern between groups was significant ($P < 0.05$).

Other measurements

The standardized serum phosphorus level followed a circadian pattern in normal women ($R^2 = 0.82$; $P < 0.001$; by fitting a polynomial with first, second, third, fifth, and sixth order terms; 11 of 15 significant by the white noise test) and osteoporotic women ($R^2 = 0.99$; $P < 0.001$; by fitting polynomials; 12 of 15 significant by the white noise test); the patterns did not differ significantly between the two groups (Table 3). The mean serum phosphorus value showed a 9.3% increase at night in normal women ($P < 0.001$) and a 5.8% increase at night in osteoporotic women ($P < 0.01$). The serum phosphorus concentration had two peaks, at 1500 and 0100 h (the latter was the greater of the two).

The FEPhos followed a circadian pattern in normal women ($P < 0.02$) and osteoporotic women ($P < 0.03$), with a peak between 1500–2300 h.

Creatinine clearance did not follow a circadian pattern in either group, nor was there any overall change at night.

TABLE 2. *P* values for circadian changes in standardized serum variables fitted by polynomial equations

Variable	Group	WNT[a]	R^{2b}	*P*	
				No circadian pattern[c]	Osteoporotics *vs.* normals[d]
Osteocalcin	Normal	4/8	0.84	<0.03	NS
	Osteoporotic	3/8	0.91	<0.01	
$Ca^{2+} \times P$	Normal	12/15	0.80	<0.001	NS
	Osteoporotic	11/15	0.97	<0.01	
Ca^{2+}	Normal	4/15	0.43	<0.01	NS
	Osteoporotic	3/15	0.16	NS	
Total Ca	Normal	6/15	0.89	<0.001	NS
	Osteoporotic	8/15	0.75	<0.02	
Albumin	Normal	12/15	0.92	<0.001	0.08
	Osteoporotic	13/15	0.85	<0.001	
PTH	Normal	3/13	0.78	<0.01	0.07
	Osteoporotic	4/15	0.65	<0.01	
Phos	Normal	11/15	0.82	<0.001	NS
	Osteoporotic	12/15	0.99	<0.001	

[a] Summary of the white noise test (WNT) performed on each individual. Tabled values are the number of individuals with a significant ($P < 0.05$) nonrandom pattern/the total number of individuals with complete data. Under the null hypothesis of no pattern, one would expect to see about 1 significant result in 15 individuals ($= 0.05 \times 15$).

[b] Squared multiple correlation coefficient obtained by fitting the mean values over subjects against collection time.

[c] Two-tail *P* from Hotelling t^2 test of the null hypothesis of no circadian pattern. The mean vector of polynomial regression coefficients is tested against the 0 vector; where regression coefficients were obtained separately for each subject and then averaged over subjects.

[d] Two-tail *P* from Hotelling t^2 test comparing the vectors of mean polynomial coefficients over subjects in osteoporotics and normals.

TABLE 3. Day/night differences in variables related to bone turnover and calcium homeostasis

Variable	Means of raw data				P	
	Group[a]	Day[b]	Night[b]	Δ at night, %[c]	Within groups[d]	O *vs.* N[e]
Serum						
Osteocalcin, ng/mL	N	8.91	9.31	4.5	<0.05	NS
	O	9.70	10.19	5.0	<0.01	
Ca^{2+}, mmol/L	N	1.248	1.253	0.4	NS	NS
	O	1.263	1.263	0	NS	
Total Ca, mmol/L	N	2.35	2.32	−1.2	<0.01	NS
	O	2.39	2.36	−1.2	<0.05	
Albumin, g/L	N	40.2	37.8	−5.9	<0.001	NS
	O	39.7	37.2	−6.3	<0.001	
PTH-IRMA, ng/L	N	41	42	2.5	NS	NS
	O	39	38	−2.1	NS	
P, mmol/L	N	1.21	1.33	9.3	<0.001	NS
	O	1.31	1.39	5.8	<0.01	
Urine						
Ca, mmol/8 h	N	1.47	1.21	−17.9	<0.05	0.09
	O	1.59	1.61	1.1	NS	
FECa	N	0.031	0.025	−20.0	<0.001	0.05
	O	0.036	0.036	−2.3	NS	
FEPhos	N	0.168	0.149	−11.3	0.05	NS
	O	0.145	0.138	−4.4	NS	

[a] N, normals; O, osteoporotics.

[b] Day includes urine collection periods 1 and 2 (0700 to 2300) or blood samples taken from 0700 to 2100. Night includes urine collection period 3 (2300 to 0700) or blood samples taken from 2300 to 0700 the following day.

[c] $100 \times$ [(mean night − mean day)/mean day].

[d] Two-tail *P* from paired *t* test or signed-rank test of the null hypothesis of no "day-night" difference.

[e] Two-tail *P* from two-sample *t* test or rank-sum test of the null hypothesis that the "day-night" difference is the same for both.

TABLE 4. Urinary excretion during three periods

Variable	Group[a]	Period means[b]			P	
		1	2	3	All periods equal[c]	Osteoporotics *vs.* normals[d]
Dpyr, nmol/8 h	N, raw	28.4	29.0	39.3	NS	NS
	O, raw	55.4	40.9	62.8	0.08	
	N, std	−0.66	−0.29	0.95	<0.001	<0.01
	O, std	0.15	−0.70	0.55	<0.01	
Ca, mmol/8 h	N, raw	1.46	1.49	1.21	0.05	NS
	O, raw	1.53	1.65	1.61	NS	
	N, std	0.17	0.17	−0.34	NS	NS
	O, std	−0.20	0.04	0.16	NS	
FECa	N, raw	0.032	0.030	0.025	<0.001	<0.05
	O, raw	0.033	0.039	0.036	NS	
	N, std	0.56	0.26	−0.82	<0.001	<0.02
	O, std	−0.24	0.46	−0.22	0.08	
FEPhos	N, raw	0.16	0.18	0.15	<0.02	NS
	O, raw	0.13	0.16	0.14	<0.03	
	N, std	0.05	0.50	−0.55	<0.02	NS
	O, std	−0.15	0.50	−0.35	<0.01	
Cl_{cr}, mL/s	N, raw	1.23	1.34	1.33	NS	NS
	O, raw	1.23	1.28	1.35	NS	

[a] Shown as raw and standardized (std) data for normals (N) and osteoporotics (O).
[b] Periods were 0700–1500, 1500–2300, and 2300–0700.
[c] Two-tail P from Hotelling t^2 test of the null hypothesis that all periods are equal.
[d] Two-tail P from Hotelling t^2 test of the null hypothesis that normals and osteoporotics have the same mean period vectors.

Discussion

In the normal postmenopausal women, circadian changes in both calcium homeostasis and bone turnover were clearly evident. Although the serum Ca^{2+} concentration was altered by meals, it remained constant during the 14-h overnight fast. This conforms to the findings in most previous studies of the circadian pattern of Ca^{2+} in normal subjects (16–20), although some earlier smaller studies found that it decreased minimally at night (21, 22). In a previous study we found a small decrease in serum Ca^{2+} in women between the age of 20–70 yr that occurred between 0600–0800 h (12). The apparent nocturnal decrease in total serum calcium probably was the result of hemodilution caused by the recumbent posture, because serum albumin also decreased (23). The lack of change in serum Ca^{2+} level despite the overnight fast requires a homeostatic adjustment. In the normal women this was achieved by both decreased FECa and increased bone resorption. This nocturnal decrease in calcium excretion has been reported in younger subjects (24–26) and may represent an intrinsic endogenous rhythm, because it is not abolished by maintenance of erect posture or by feeding at night (27).

The main hormone that maintains the serum Ca^{2+} concentration within narrow limits is PTH. We measured intact PTH because it has a short half-life (28) and, thus, should be a good indicator of short term changes in PTH secretion. Two peaks were identified:

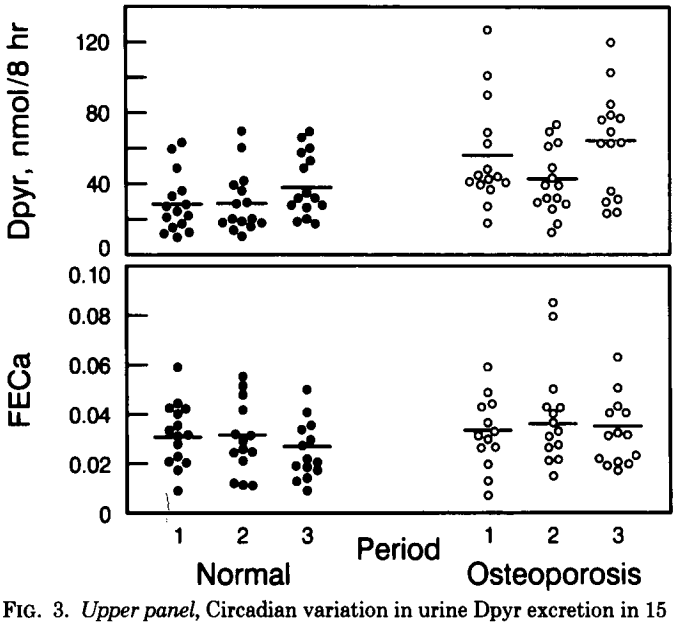

FIG. 3. *Upper panel*, Circadian variation in urine Dpyr excretion in 15 normal women (●) and 15 osteoporotic women (○). The day is divided into three 8-h periods: period 1, 0700–1500 h; period 2, 1500–2300 h; and period 3, 2300–0700 h. *Horizontal lines* represent the means. There was a circadian variation in both groups ($P < 0.01$), and the patterns differed between groups ($P < 0.05$). *Lower panel*, Circadian variation in FECa in normal women (●; $P < 0.001$) and osteoporotic women (○; $P = 0.008$); the patterns differed significantly ($P < 0.05$).

one in the afternoon and one at night. This pattern is consistent with two recent studies using midregion PTH assays (20, 29). Earlier studies showed only a nocturnal

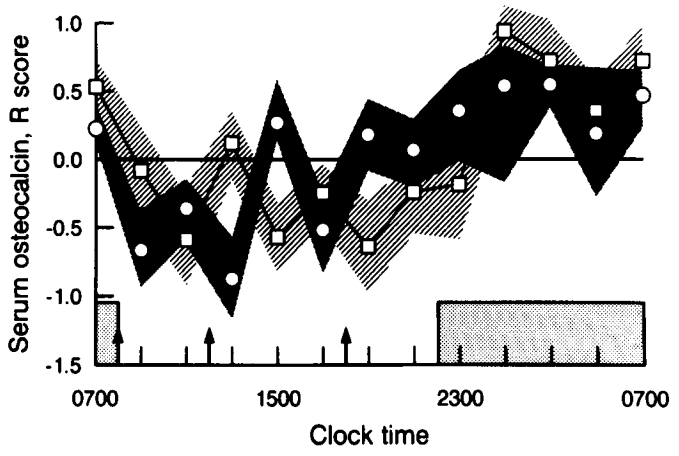

FIG. 4. Circadian variation in serum osteocalcin (BGP) in 15 normal women (O) and 15 osteoporotic women (□), standardized to the R score. *Shaded areas* represent the mean ± SE. Both groups showed a significant circadian pattern. *Arrows* indicate meal times; *stippled bars* represent recumbency.

peak in PTH secretion, but this may have been due to changes in the serum protein concentration at night (21, 23). PTH increases both renal tubular reabsorption of calcium and bone resorption (30), and a nocturnal increase in serum PTH may maintain the serum Ca^{2+} level constant through both mechanisms.

As assessed by the biochemical markers, both bone formation and bone resorption increased during sleep, suggesting that physiological repair of the skeleton may occur then. Similar findings during sleep/inactivity periods have been noted in experimental animals. In rats there was a peak in bone collagen synthesis (31, 32) during the inactive (light) period and a peak in the metaphyseal mineralization rate (33) during the active (dark) period. Bone resorption activity *in vitro* was increased in bone removed during the inactive (light) period in rats (31), and radiocalcium kinetic studies in dogs have shown increased ^{45}Ca release from bone during the inactive (fasting) period (34). Thus, in these animals osteoblastic activity and bone resorption may increase during inactive periods, whereas bone mineralization may increase during active periods.

We found that the serum concentration of osteocalcin, a specific osteoblast product, increased at night to a similar extent in both normal and osteoporotic women. However, the magnitude of the increase was not as great as that reported for younger healthy subjects (35–37). Pietschmann *et al.* (38) found an 18% increase at night in osteoporotic women; we found a 5% increase. The nocturnal increase was probably not due to decreased clearance, because creatinine clearance was unchanged. The product of serum Ca^{2+} × phosphorus concentration is probably important in promoting bone mineralization, and this also peaked at night in both groups. These findings are consistent with but not proof of, a small increase in bone formation at night.

In contrast to the normal postmenopausal women, the women with type I osteoporosis were unable to conserve calcium by decreasing urinary excretion; they maintain their serum Ca^{2+} level at night almost exclusively by increasing bone resorption. This failure of renal adaptation and the greater than normal increase in bone resorption during the long nocturnal fast may account in part for their greater bone loss. The role, if any, of immobilization during sleep in the pathogenesis of this abnormality is unclear.

The results in the osteoporotic women are consistent with an increased sensitivity of bone-resorbing cells to PTH action. To prevent serum Ca^{2+} from decreasing during the nocturnal fast, PTH secretion increased in both the normal and osteoporotic postmenopausal women. In the normal women, the higher serum PTH concentration increases bone resorption, but also promotes renal conservation. In the osteoporotic women, however, the putative increase in bone sensitivity to PTH would lead to increased release of calcium from bone. This would blunt the increase in the serum PTH concentration, resulting in a lower circulating concentration, which would impair renal adaptation.

Increased sensitivity of bone to PTH in osteoporosis was suggested by Heaney (39) years ago on the basis of theoretical considerations. Recently, Kotowicz *et al.* (40) documented this experimentally by the significant positive correlation of serum PTH with rate of bone resorption, as assessed by a new histomorphometric method. They found that, for each unit of increase in serum PTH concentration, there was an almost 2-fold greater increase in the bone resorption rate in osteoporotic postmenopausal women than in age-matched normal postmenopausal women.

Whatever the mechanism of this abnormality, its recognition has practical implications for treatment. Because the highest rates of bone resorption occur nocturnally in osteoporotic women, antiresorptive therapeutic agents may be more effective if administered at night. Horowitz *et al.* (41) reported that calcium supplements administered at bedtime decreased fasting urinary hydroxyproline excretion, and the same may be true for administration of other short-acting antiresorptive drugs, such as calcitonin.

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References

1. Riggs BL, Melton LJ. Medical progress series: involutional osteoporosis. N Engl J Med. 1986;314:1676–86.
2. Parfitt AM. Bone remodeling: relationship to the amount and structure of bone and the pathogenesis and prevention of fractures. In: Riggs BL, Melton III LJ, eds. Osteoporosis: etiology diagnosis and management. New York: Raven Press; 1988;45–93.
3. Eriksen EF, Hodgson SF, Eastell R, Cedel SL, O'Fallon WM, Riggs BL. Cancellous bone remodeling in type I (postmenopausal osteoporosis): quantitative assessment of rates of formation, resorption and bone loss at tissue and cellular levels. J Bone Mineral Res. 1990;5:311–9.
4. Colwell A, Eastell R, Assiri AMA, Russell RGG. Effects of diet on deoxypyridinoline excretion [Abstract 225]. Proc of the 3rd Int Symp on Osteoporosis. 1990;64.
5. Eyre DR, Koob TJ, Van Ness KP. Quantitation of hydroxypyridinium crosslinks in collagen by high performance liquid chromatography. Anal Biochem. 1984;137:380–8.
6. Black D, Duncan A, Robins SP. Quantitative analysis of the pyridinium crosslinks of collagen in urine using ion-paired reversed-phase high-performance liquid chromatography. Anal Biochem. 1988;169:197–203.
7. Eastell R, Hampton L, Colwell A, et al. Urinary collagen crosslinks are highly correlated with radioisotopic measurements of bone resorption [Abstract 173]. Proc of the 3rd Int Symp on Osteoporosis. 1990;51.
8. Uebelhart D, Gineyts E, Chapuy MC, Delmas PD. Urinary excretion of pyridinium crosslinks: a new marker of bone resorption in metabolic bone disease. Bone Mineral. 1990;8:87–96.
9. Robins SP, Duncan A, Riggs BL. Direct measurement of free hydroxypyridinium crosslinks of collagen in urine as new markers of bone resorption in osteoporosis [Abstract 172]. Proc of the 3rd Int Symp on Osteoporosis. 1990;51.
10. Eastell R, Delmas PD, Hodgson SF, Eriksen EP, Mann KG. Bone formation rate in older normal women: concurrent assessment with bone histomorphometry, calcium kinetics, and biochemical markers. J Clin Endocrinol Metab. 1988;67:741–8.
11. Brown JP, Delmas PD, Malaval L, Edouard C, Chapuy MC, Meunier PJ. Serum bone Gla-protein: a specific marker for bone formation in postmenopausal osteoporosis. Lancet. 1984;1:1091–3.
12. Calvo MS, Eastell R, Offord KP, Bergstralh EL, Burritt MP. Circadian variation in ionized calcium and intact parathyroid hormone: evidence for sex differences in calcium homeostasis. J Clin Endocrinol Metab. 1991;72:69–76.
13. Delmas PD, Stenner D, Wahner H, Mann KG, Riggs BL. Increase in serum bone γ-carboxyglutamic acid protein with aging in women. J Clin Invest. 1983;71:1316–21.
14. Fuller WA. Introduction to statistical time series. New York: Wiley and Sons; 1976.
15. Statistical analysis system. User's guide. Version S Cary, NC: SAS Institute; 1985.
16. Markowitz M, Rotkin L, Rosen JF. Circadian rhythms of bone minerals in humans. Science. 1981;213:672–4.
17. Markowitz ME, Rosen JF, Mizruchi M. Circadian variations in serum zinc (Zn) concentrations: correlation with blood ionized calcium, serum total calcium, and phosphate in humans. Am J Clin Nutr. 1985;41:689–96.
18. Perry III HM, Province MA, Droke DM, Kim GS, Shaheb S, Avioli LV. Diurnal variation of serum calcium and phosphorus in postmenopausal women. Calcif Tissue Int. 1986;38:115–8.
19. Portale AA, Halloran BP, Morris Jr RC. Dietary intake of phosphorus modulates the circadian rhythm in serum concentration of phosphorus. Implications for the renal production of 1,25-dihydroxyvitamin D. J Clin Invest. 1987;80:1147–54.
20. Calvo MS, Kumar R, Heath III H. Elevated secretion and action of serum parathyroid hormone in young adults consuming high phosphorus, low calcium diets assembled from common foods. J Clin Endocrinol Metab. 1988;66:823–9.
21. LoCascio V, Cominacini L, Adami S, Galvanini G, Davoli A, Sivro LA. Relationship of total and ionized serum calcium circadian variations in normal and hyperparathyroid subjects. Horm Metab Res. 1982;14:4430.
22. Ishida M, Seino Y, Yamaoka K, et al. The circadian rhythms of blood ionized calcium in humans. Scand J Clin Lab Invest. 1983;43(Suppl):83–6.
23. Jubiz W, Canterbury JM, Reiss E, Tyler FH. Circadian rhythm in serum parathyroid hormone concentration in human subjects: correlation with serum calcium, phosphate, albumin, and growth hormone levels. J Clin Invest. 1972;51:2040–6.
24. Carruthers BM, Copp DH, McIntosh HW. Diurnal variation in urinary excretion of calcium and phosphate and its relation to blood levels. J Lab Clin Med. 1964;63:959–68.
25. Hansen JW, Gordan GS, Prussin SG. Direct measurement of osteolysis in man. J Clin Invest. 1973;52:304–15.
26. Halloran BP, Portale AA, Castro M, Morris RC, Goldsmith RS. Serum concentration of 1,25-dihydroxyvitamin D in the human: diurnal variation. J Clin Endocrinol Metab. 1985;60:1104–10.
27. Moore-Ede MC. Physiology of the circadian timing system: predictive *versus* reactive homeostasis. Am J Physiol. 1986;19:R735–52.
28. Nussbaum SR, Zahradnik RJ, Lavigne JR. Highly-sensitive two-site immunoradiometric assay of parathyrin, and its clinical utility in evaluating patients with hypercalcemia. Clin Chem. 1987;33:1364–7.
29. Markowitz ME, Arnaud S, Rosen JF, Thorpy M, Laximinarayan S. Temporal interrelationships between the circadian rhythms of serum parathyroid hormone and calcium concentrations. J Clin Endocrinol Metab. 1988;67:1068–73.
30. Rosenblatt M, Kronenberg HM, Potts JT. Parathyroid hormone: physiology chemistry, biosynthesis, secretion, metabolism, and mode of action. In: DeGroot LJ, Besser GM, Cahill GF, et al, eds. Endocrinology. Philadelphia: Saunders; 1989;848–91.
31. Simmons DJ, Nichols G. Diurnal periodicity in the metabolic activity of bone tissue. Am J Physiol. 1966;210:411–8.
32. Simmons DJ, Whiteside LA, Whitson SW. Biorhythmic profiles in the rat skeleton. Metab Bone Dis Rel Res. 1979;2:49–64.
33. Russell JE, Grazman B, Simmons DJ. Mineralization in rat metaphyseal bone exhibits a circadian stage dependency. Proc Soc Exp Biol Med. 1984;176:342–5.
34. Wong KM, Klein L. Circadian variations in contributions of bone and intestine to plasma calcium in dogs. Am J Physiol. 1984;246:R688–92.
35. Gundberg CM, Markowitz ME, Mizruchi M, Rosen JF. Osteocalcin in human serum: a circadian rhythm. J Clin Endocrinol Metab. 1985;60:736–9.
36. Couch M, Woods DA, Gallagher JA, Poser JW, Russell RGG. Serum osteocalcin in British subjects [Abstract]. Calcif Tissue Int. 1984;26:S3.
37. Delmas PD, Lacroze S, Chapuy MC, Malaval L. Circadian rhythm in serum bone Gla-protein concentration in the human: correlation with serum 1,25-dihydroxyvitamin D. Proc of the 9th Int Conference on Calcium Regulating Hormones and Bone Metabolism. 1986;447:342.
38. Pietschmann P, Resch H, Woloszczuk W, Willvonseder R. A circadian rhythm of serum osteocalcin levels in postmenopausal osteoporosis. Eur J Clin Invest. 1990;20:310–2.
39. Heaney RP. A unified concept of osteoporosis. Am J Med. 1965;39:877–80.
40. Kotowicz MA, Klee GG, Kao PC, et al. Relationship between serum intact parathyroid hormone concentrations and bone remodeling in type I osteoporosis: evidence that skeletal sensitivity is increased. Osteoporosis Int. 1990;1:14–22.
41. Horowitz M, Need AG, Philcox JC, Nordin BEC. Effect of calcium supplementation on urinary hydroxyproline in osteoporotic postmenopausal women. Am J Clin Nutr. 1984;3a:857–9.