Nyctohemeral changes in bone turnover assessed by serum bone Gla-protein concentration and urinary deoxypyridinoline excretion: effects of growth and ageing

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1. To investigate whether there is a nyctohemeral rhythm in bone turnover, we measured serum bone Gla-protein (osteocalcin, an index of osteoblast activity) concentration every 2 h and urinary deoxypyridinoline (a marker of bone collagen resorption) excretion for 8 h periods in 10 pubertal girls (aged 10–14 years), 15 premenopausal women (aged 20–49 years) and 17 postmenopausal women (aged 50–75 years).

2. The serum concentration of bone Gla-protein and the urinary excretion of deoxypyridinoline were five times higher in the pubertal girls than in the premenopausal women. The urinary excretion of deoxypyridinoline in the postmenopausal women was twice that in the premenopausal women.

3. There was a nyctohemeral pattern in all age groups with mean night-time increases of 28% ($P<0.001$) in the urinary excretion of deoxypyridinoline and of 5% ($P<0.001$) in the serum bone Gla-protein concentration.

4. There also were nyctohemeral patterns in the urinary excretion of calcium ($P<0.02$), sodium ($P<0.001$) and potassium ($P<0.001$), with decreases at night. There was a negative correlation between the night-time changes in the urinary excretion of deoxypyridinoline and calcium, especially in adult women ($P<0.01$).

5. The serum level of parathyroid hormone increased with age, but this effect was only observed at night (01.00 to 07.00 hours). There was a nyctohemeral rhythm of the serum intact parathyroid hormone level at all ages, with a peak in the afternoon and night.

6. Thus, at night, there is a large increase in bone resorption and a small increase in osteoblastic activity, representing a nyctohemeral rhythm of bone turnover. Although the amplitudes of bone formation and bone resorption are greater during growth, the pattern of nyctohemeral changes present during growth continues up to the age of 75 years.

INTRODUCTION

There is evidence in experimental animals of a nyctohemeral rhythm in bone remodelling, where nyctohemeral refers to a variation associated with the sleep/wake daily cycle. In rats, there is a peak in collagen synthesis [1] in bone during the inactive (light) period and a peak in metaphyseal mineralization rate [2] during the active (dark) period. Bone resorption activity in rats was found to be increased in bone removed during the inactive period [3], and radiocalcium kinetic studies in dogs have shown increased ^{45}Ca release from bone during the inactive period [4]. Thus, bone matrix synthesis and bone resorption increase during inactive periods and bone mineralization increases during active periods.

The previous techniques used to assess bone turnover, radiocalcium kinetics and bone histomorphometry, estimate bone turnover over a period of 2–3 weeks and so are not suitable for nyctohemeral studies. The available biochemical markers for bone turnover, serum total alkaline phosphatase activity and urinary hydroxyproline excretion, are non-specific and are relatively insensitive.

The recent availability of specific biochemical markers of bone turnover make nyctohemeral studies feasible in humans. The level of bone Gla-protein (BGP, osteocalcin), a 49-amino acid peptide secreted by osteoblasts, in the serum reflects bone formation in normal subjects as assessed independently by bone histomorphometry and radiocalcium kinetics [6]. It is a particularly suitable marker for nyctohemeral studies because its half-life in the plasma is about 1 h [7]. Pyridinoline (Pyr) and deoxypyridinoline (Dpyr) represent the non-reducible cross-links of bone collagen. They are present in the diet but are not absorbed [8]. Pyr is found in collagen from a number of tissues including cartilage, tendon, ligament and aorta, but Dpyr is specific to bone collagen [5]. There is a strong correlation between urinary Dpyr excretion and

Key words: ageing, bone remodelling, growth, nyctohemeral, osteocalcin, osteoporosis, pyridinium cross-links of collagen.

Abbreviations: BGP, bone Gla-protein; Dpyr, deoxypyridinoline; PTH, parathyroid hormone; Pyr, pyridinoline.

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bone resorption rate as measured by radiostrontium kinetics [9].

The aim of the present study was to determine: (1) if there is a nyctohemeral rhythm of bone turnover in normal women; (2) how it relates to the well-described rhythms of mineral excretion and parathyroid hormone (PTH) secretion; and (3) if so, whether these rhythms are present during growth and with ageing. If the pattern of bone turnover in normal subjects can be established, it will be possible to examine for abnormalities of this rhythm in disease states.

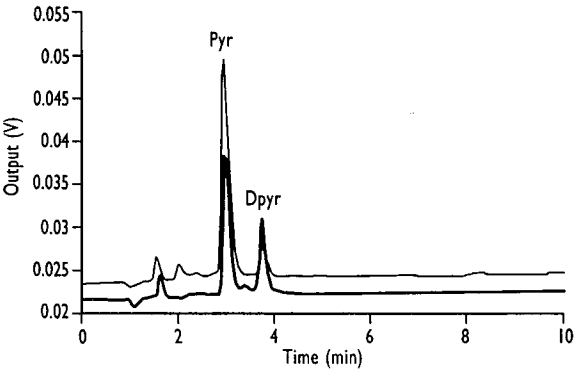

Fig. 1. H.p.l.c. of a urine sample from a pubertal girl and the standard prepared from the urine of patients with Paget's disease of bone. Chromatography conditions: solvent A, 20 mmol/l ammonium chloride at pH 3.5, containing 1.4 mmol/l n-heptafluorobutyric acid (HFBA); solvent B, 20 mmol/l ammonium chloride, pH 3.5, acetonitrile containing 4 mmol/l HFBA, 1:3. Gradient conditions: flow rate, 1.0 ml/min; elution, 10% solvent B, then linear gradient for 6 min to 22% B, 100% B wash for 4 min, and re-equilibrium at 10% for 6 min.

METHODS

Subjects

Three groups were studied. The first comprised 10 pubertal girls, 10–14 years old (mean age 12.1 years, SD 1.4 years), with Tanner breast stage 1 in one girl, 2 in two girls, 3 in four girls and 4 in three girls. Tanner pubertal hair stages were 1 in three girls, 2 in one girl, 3 in two girls, 4 in three girls and 5 in one girl. Two girls had gone through the menarche 4 and 18 months previously.

The second group contained 15 premenopausal women, 20–49 years old (mean age 35.1 years, SD 8.1 years). All had regular menses and none was taking the contraceptive pill.

The third group comprised 17 postmenopausal women, 50–75 years old (mean age 63.0 years, SD 7.4 years), who had experienced the menopause 1–24 years previously. All had normal spine radiographs and a lumbar spine bone mineral density above the 'fracture threshold', 0.98 g/cm^2, as measured by dual-photon absorptiometry.

None of the subjects had recently undergone transmeridian travel and none worked at night. None had any illness or was taking any drug known to affect calcium metabolism. Data on calcium homoeostasis (but not bone turnover) in the 15 premenopausal women and 10 of the postmenopausal women have been reported elsewhere [10].

Study protocol

Dietary calcium intake was estimated from a 7-day diet record and an interview with a trained dietitian. The study diet was planned to provide the habitual intake of calcium. Meals were served at 08.00, 12.00 and 18.00 hours and were consumed within 30 min.

Each participant was admitted to the Mayo General Clinical Research Center at 06.15 hours and an intravenous cannula was inserted. Blood was drawn starting at 07.00 hours and every 2 h thereafter for 24 h. The serum was separated and stored at −70°C. Urine was collected over 8 h periods for 24 h starting at 07.00 hours (pubertal girls and seven postmenopausal women) or at 08.00 hours (premenopausal women and 10 postmenopausal women). Concentrated acetic acid (50 ml) was used as a preservative. Samples of the collections were stored at −70°C until transferred to Sheffield for measurement of pyridinium cross-links.

All subjects were ambulatory (sitting or walking) from 08.00 to 22.30 hours and were recumbent from 22.30 to 07.00 hours.

All subjects gave their informed written consent, and the study was approved by the Institutional Review Board.

Biochemical measurements

Pyr and Dpyr were measured in Sheffield by modifications of the methods of Black *et al.* [11] for sample preparation and the method of Eyre *et al.* [5] for h.p.l.c. in duplicate.

H.p.l.c. separation was achieved with a Waters Wisp autosampler, a Waters 720 system controller for gradient elution and a 3.3 cm by 4.6 cm octadecyldimethylsilyl column with 3 μm packing (LC-18-DB; Supelchem U.K. Ltd, Saffron Walden, Essex, U.K.) The separation conditions were as stated in the legend to Fig. 1, and the compounds were detected by measuring their natural fluorescence (excitation 295 nm, emission 395 nm) in a Jasco FP 820 spectrofluorimeter (Jasco International Co. Ltd, Tokyo, Japan). A Waters 810 baseline computer system was used for data acquisition (Millipore Waters Chromatography Division, Milford, MA, U.S.A.).

Quantification was based on an external standard purified by h.p.l.c. from urine from patients with Paget's disease of bone. This standard was calibrated against a standard (a gift from Dr D. Black

[11]) prepared from bone. The intra- and inter-assay variations were less than 11%.

Serum BGP concentration was measured by radioimmunoassay [12] using a rabbit antiserum to bovine BGP. Homogeneous bovine BGP was used for tracer and standard. Antibody-bound and -free ^{125}I-labelled BGP were separated by the double-antibody method. The intra-assay variation was $<7\%$ and the inter-assay variation was $<10\%$. All measurements were made in duplicate. Any samples that were haemolysed were discarded (6% of samples).

Serum ionized calcium concentration was measured in duplicate anaerobic samples with a Radiometer ICA analyser (Radiometer, Copenhagen, Denmark). Intact PTH in serum was measured in duplicate by a two-site immunoradiometric assay (Allegro intact PTH kit; Nichols Institute, San Juan Capistrano, CA, U.S.A.) [13]. The results for the serum ionized calcium and intact PTH concentrations in the adult women were reported previously [10]. However, the serum PTH concentration was re-measured so that adult and teenage samples were run in the same assays.

Urinary calcium was measured by atomic absorption spectroscopy (IL 751 atomic absorption spectrophotometer; Instrumentation Laboratories, Lexington, MA, U.S.A.). Urinary sodium, potassium and creatinine were measured by an automated analyser (Beckman Astra 8 analyser; Beckman Instruments, Inc., Brea, CA, U.S.A.). All measurements were made in duplicate.

Statistical analysis

The three groups were compared by one-way analysis of variance followed by a *t*-test. The variables were tested for normality of distribution by the Kolmogorov–Smirnov one-sample test for goodness-of-fit. If the distribution was not normal, the 95% confidence intervals were calculated non-parametrically.

The effect of the time period on urine and serum measurements was tested by multifactor analysis of variance after standardization of the data. The data were standardized by calculating the mean and SD for each individual, and the standardized score (*R* score) for each of that individual's three results was calculated by dividing the difference between the actual value and the mean by the SD. This approach allows each individual to contribute equally to the pattern and takes away the effect of level. As there were 13 periods for serum measurement, a Bonferonni correction was made.

Day/night difference was expressed as a percentage: the mean of night-time samples (23.00 to 07.00 hours) minus the mean of daytime samples (09.00 to 21.00 hours) divided by the mean of daytime samples and multiplied by 100. A one-sample *t*-test was used to test the difference of this percentage from zero.

The night-time standardized scores for each urine variable were compared by simple linear regression. All analyses were performed using Statgraphics software (STSC, Inc., Rockville, MD, U.S.A.).

RESULTS

24 h values for bone resorption

The urinary excretion of Dpyr was five-fold higher in the pubertal girls than in the adult women (Fig. 2). The mean value in premenopausal women (7.6, SD 1.6, 95% confidence interval 4.1–10.1 nmol/mmol of creatinine) was significantly different from that in the postmenopausal women (15.2, SD 7.7, 95% confidence interval 8.7–40.5 nmol/mmol of creatinine, $P=0.001$). In the pubertal girls the mean urinary excretion of Dpyr was 39.5 nmol/mmol of creatinine (95% confidence interval 16.0–70.0 nmol/mmol of creatinine, determined non-parametrically). The four girls in Tanner (pubertal hair) stage 1 or 2 had a lower serum oestradiol level than those in stages 3–5 (71.5 versus 100.5 pg/ml, $P<0.05$) and tended to have higher urinary Dpyr excretion (50.0 versus 32.5 nmol/mmol of creatinine), although this difference did not reach significance.

The urinary excretion of Pyr was about five-fold higher in the pubertal girls than in the adult women. The mean value for premenopausal women (31.3, SD 8.3, 95% confidence interval 14.7–47.7 nmol/mmol of creatinine) was significantly different

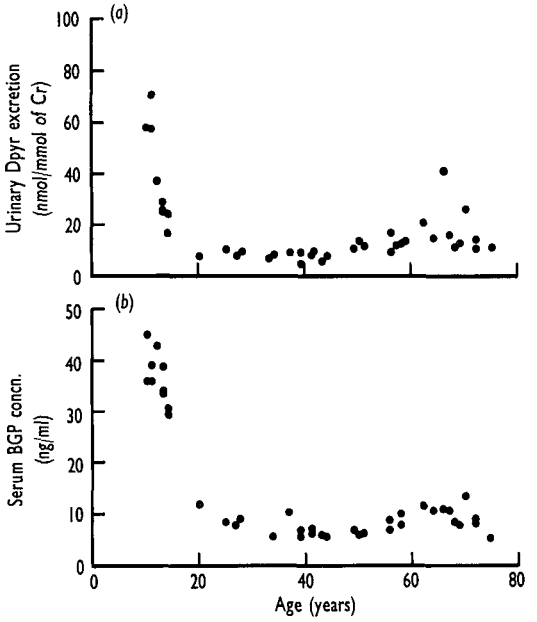

Fig. 2. Effect of age on the 24 h urinary excretion of Dpyr (expressed as a ratio to creatinine excretion) (*a*) and the serum BGP concentration (mean of 13 values) (*b*). Abbreviation: Cr, creatinine.

from that in the postmenopausal women (60.0, SD 33.2, 95% confidence interval 29.2–179.1 nmol/mmol of creatinine, $P=0.004$). In the pubertal girls, the mean urinary excretion of Pyr was 149.9 nmol/mmol of creatinine (95% confidence interval 73–292 nmol/mmol of creatinine, determined non-parametrically).

The urinary excretions of Pyr and Dpyr expressed in nmol/24 h were correlated in the group as a whole ($r=0.96$; $P<0.0001$) and for the pubertal girls ($r=0.95$; $P<0.0001$), the premenopausal women ($r=0.68$; $P<0.01$) and the postmenopausal women ($r=0.84$; $P<0.001$). The ratio of Pyr to Dpyr did not differ between groups (by analysis of variance); the mean ($\pm$SD) ratio was 4.1 ± 0.87.

24h values for bone formation

The 24 h mean serum level of BGP was also five-fold higher in the pubertal girls than in the adult women (Fig. 2). The mean$\pm$SD was 7.1 ± 1.9 ng/ml in premenopausal women and 8.7 ± 2.2 ng/ml in the postmenopausal women ($P<0.01$). The data were normally distributed in both groups and the 95% confidence interval was 3.5–10.7 ng/ml for premenopausal women and 4.9–13.2 ng/ml for postmenopausal women. In the pubertal girls, the mean serum BGP level was 36.2 ng/ml and the 95% confidence interval was 26.4–46.0 ng/ml. The four girls who were in Tanner hair stages 1 and 2 had levels similar to those in stages 3–5 (37.4 and 35.4 ng/ml, respectively).

In all participants there was a positive correlation between mean serum BGP level and urinary Dpyr excretion ($r=0.81$, $P<0.0001$). When each of the groups was analysed separately, the correlation was significant for the pubertal girls ($r=0.66$, $P<0.05$) and for the postmenopausal women ($r=0.69$, $P<0.01$), but not for the premenopausal women ($r=0.23$, not significant).

Nyctohemeral changes in bone turnover

Urinary excretion of Dpyr was higher at night in all three groups (Fig. 3*a*). This was shown best by evaluating the effect of the time period on standardized score (Fig. 3*b*). There was a nyctohemeral pattern in all groups whether or not urinary Dypr excretion was corrected for creatinine excretion ($P<0.0001$), and this pattern did not differ among the groups. All groups had a significant increase in urinary Dpyr excretion at night ($P<0.05$).

There was a significant effect of the time of day on standardized scores (R score) in all groups for serum BGP level (Fig. 4, $P<0.00001$). The lowest value was at 13.00 hours ($P<0.001$) and the highest value was at 03.00 hours ($P<0.001$). The pattern did not differ significantly between groups.

When the results were expressed as the day/night difference, the mean increase in the three groups at night was 28.0% for urinary Dpyr excretion ($P=0.001$, 95% confidence interval 11.6–44.5%) and only 5.3% for serum BGP level ($P<0.001$, 95% confidence interval 2.6–8.0%). The day/night difference was not statistically different among the groups.

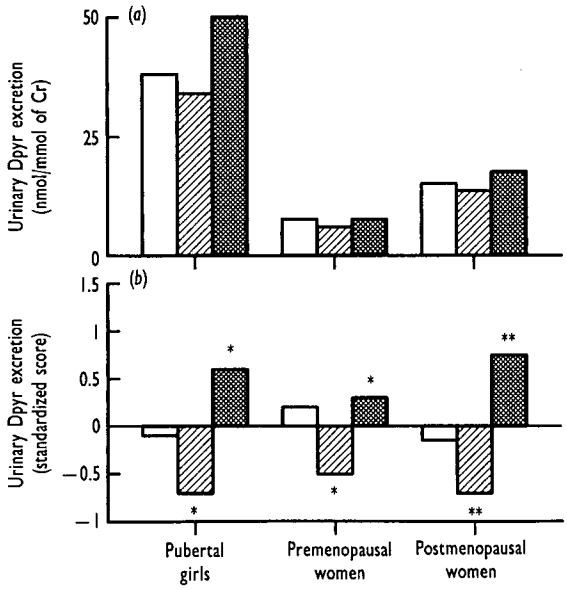

Fig. 3. Nyctohemeral variations in the urinary excretion of Dpyr in pubertal girls, premenopausal women and postmenopausal women (*a*) expressed as a ratio to creatinine (Cr) excretion and (*b*) expressed as a standardized score (*R* score). Statistical significance: $^*P<0.05$, $^{**}P<0.01$ compared with zero. Time periods: □, 07.00–15.00 hours; ▨, 15.00–23.00 hours; ▩, 23.00–07.00 hours.

Nyctohemeral changes in mineral excretion

The urinary excretion of calcium, sodium and potassium was lower at night in all three groups (Fig. 5). In the standardized data, the effect of period was significant for calcium ($P=0.01$), sodium ($P=0.0001$) and potassium ($P=0.0001$). The pattern did not differ between the groups.

There was a negative correlation between nocturnal urinary Dpyr and calcium excretion in the premenopausal women ($r=-0.73$; $P=0.01$), but no significant correlation in the postmenopausal women ($r=-0.43$) or in the pubertal girls ($r=-0.56$) (Table 1). The standardized score for urinary Dpyr excretion in the nocturnal period did not correlate with that for urinary sodium or potassium excretion. The standardized score for urinary calcium excretion in the nocturnal period did show a positive correlation with urinary sodium and potassium excretion (Table 1).

The mean 25 h serum ionized calcium concentration was similar in the pubertal girls (1.26 mmol/l), the premenopausal women (1.26 mmol/l) and the postmenopausal women (1.25 mmol/l) (Fig. 6). The

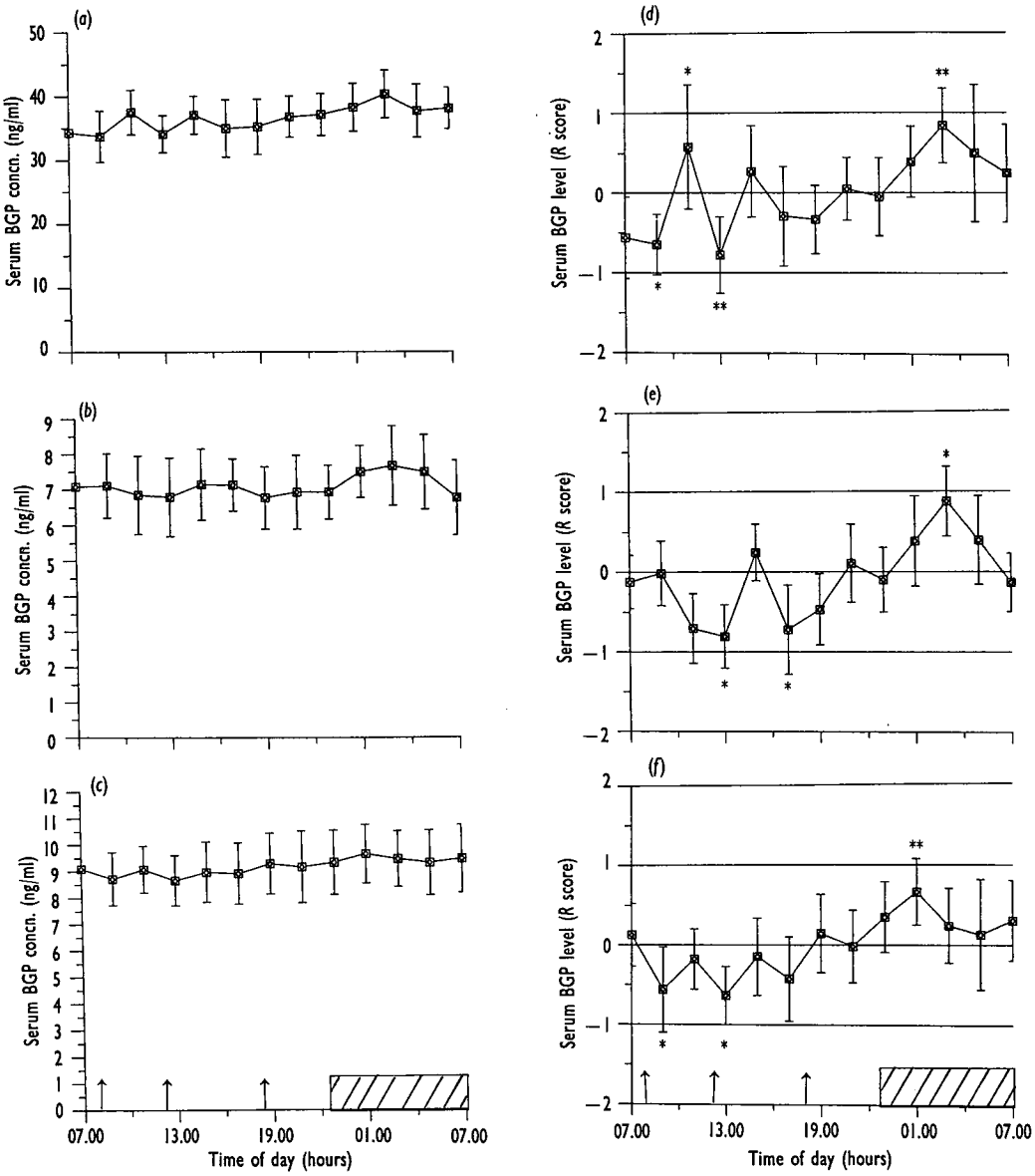

Fig. 4. Nyctohemeral variation in the serum BGP level. The arrows indicate the timing of three meals; the horizontal bar is the period of bed rest. Left-hand panels show serum BGP concentrations for pubertal girls (*a*), premenopausal women (*b*) and postmenopausal women (*c*). Right-hand panels show standardized scores (*R* scores) for serum BGP concentrations in pubertal girls (*d*), premenopausal women (e) and postmenopausal women (*f*). Values are means ± 2 SEMs. Statistical significance (one-sample *t*-test): $*P<0.05$, $**P<0.01$ compared with zero.

mean 25 h serum PTH concentration in the pubertal girls (mean ± SEM 33.2 ± 3.0 pg/ml) was similar to that in the premenopausal women (33.0 ± 2.7 pg/ml) and that in the postmenopausal women (38.6 ± 1.9 pg/ml) (Fig. 6).

To examine whether any relationship between serum PTH concentration and age was influenced by the time of day, these two variables were correlated at each of the 13 time points (Table 2). A significant effect of age on serum PTH levels was observed at night only (01.00–07.00 hours). Also, there was an increase in the fractional excretion of calcium at night with age ($r=0.45$, $P<0.01$). However, this increase was also found during the periods 07.00–15.00 hours ($r=0.35$, $P<0.05$) and 15.00 and 23.00 hours ($r=0.40$, $P<0.01$).

DISCUSSION

Although urinary excretion of hydroxyproline has been used as a biochemical marker of bone resorption, it is non-specific (only 50% is derived from bone [14]) and is affected by dietary collagen. Thus, it is not surprising that studies of the nyctohemeral rhythm of urinary hydroxyproline excretion have given conflicting results [15–17]. In contrast, Dpyr in the urine is almost exclusively from bone [5]. In preliminary studies we were unable to find an effect

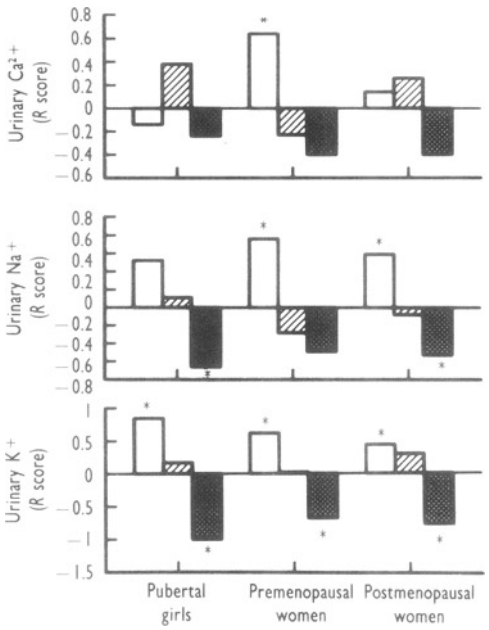

Fig. 5. Nyctohemeral variations in mineral excretion in pubertal girls, premenopausal women and postmenopausal women. Statistical significance: $*P<0.05$ compared with zero. Time periods: □, 07.00–15.00 hours; ▨ 15.00–23.00 hours; ▩, 23.00–07.00 hours.

Table 1. Correlation matrix of nocturnal standardized scores for markers of bone resorption and mineral excretion. Statistical significance: $*P<0.05$, $**P<0.01$, $***P<0.001$.

	Urinary Ca^{2+} excretion	Urinary Na^+ excretion	Urinary K^+ excretion
Urinary Dpyr excretion	−0.56**	−0.28	−0.12
Urinary Ca^{2+} excretion	—	0.52***	0.32*
Urinary Na^+ excretion	—	—	0.49**

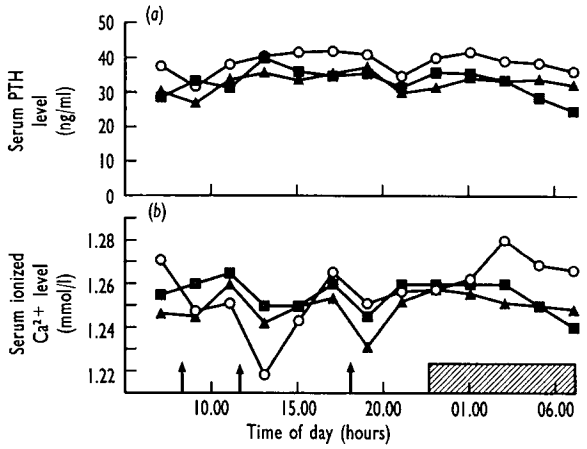

Fig. 6. Nyctohemeral variations in serum intact PTH (*a*) and ionized calcium (*b*) levels in pubertal girls (□), premenopausal women (△) and postmenopausal women (○). The arows indicate the timing of the three meals. The hatched bar is the period of sleep.

Table 2. Effect of time of day on the relationship between serum PTH concentration and age. Abbreviation: NS, not significant.

Time of day (hours)	*r*	*P*
07.00	0.35	0.02
09.00	0.04	NS
11.00	0.28	NS
13.00	0.07	NS
15.00	0.21	NS
17.00	0.23	NS
19.00	0.19	NS
21.00	0.18	NS
23.00	0.24	NS
01.00	0.32	0.04
03.00	0.27	0.09
05.00	0.49	0.001
07.00	0.50	0.001

of diet on the urinary excretion of Dpyr [8]. Using this biochemical marker, we documented a nyctohemeral rhythm that is present between the ages of 10 and 75 years, and age appears to have no influence on its magnitude.

The day/night difference in urinary Dpyr excretion was six-fold greater than that in serum BGP concentration; the former is a marker of bone resorption and the latter is a marker of bone formation. This major increase in bone collagen resorption undoubtedly is associated with a comparable release of calcium from bone. It is likely that the mechanisms whereby the serum ionized calcium concentration is maintained constant throughout the night include this calcium efflux from bone combined with a decrease in urinary calcium excretion. It might be speculated that bone loss occurs mainly at night and that one approach to halt the bone loss with age would be to inhibit the nocturnal increase in bone resorption, e.g. with a calcium supplement at bedtime.

There have been previous reports of a nyctohemeral pattern of the serum BGP level in young adults, with a peak at night [18–20]. We now find that age has no effect on the day/night difference despite the increase in mean serum BGP level with age. The nocturnal increase in the serum BGP level was smaller and more difficult to detect than that in urinary Dpyr excretion. There may be a small increase in bone matrix synthesis at night, but because there is no biochemical marker of bone mineralization it is not known whether this shows a nyctohemeral rhythm in humans; in experimental animals, mineralization increases during the night (the active period) [2].

Serum intact PTH concentration follows a nyctohemeral rhythm with peaks in the afternoon and night [10, 21]. This was found to be present at all ages (Fig. 6). The well-described age-related increase in serum PTH level was only present at night (Table 2). This increase has been considered to be a

response to a decrease in fractional calcium absorption. As it is only present during fasting, it could also result from the age-related increase in nocturnal fractional calcium excretion. Nordin *et al.* [23] have reported a decrease in fasting fractional calcium excretion with age, and they consider this to be the result of oestrogen deficiency.

The mechanism of the nyctohemeral rhythm has not yet been determined. It has been proposed that the nocturnal peak in serum BGP level results from cortisol deficiency because it can be blocked by the oral administration of 'physiological' doses of prednisone [22]. It may result from a nocturnal increase in growth hormone. Delmas *et al.* [24] reported low serum BGP levels in growth hormone-deficient children and a return to normal with administration of growth hormone. Ebeling *et al.* [25] reported that blocking the nocturnal peak in growth hormone with somatostatin had no effect on the peak in serum BGP level. There have been no reports on the mechanism of the increase in bone resorption at night. PTH is a potent simulator of bone resorption and its serum level increases at night. However, using an assay for intact PTH we found that, in addition to the nocturnal peak, there was a peak of similar magnitude during the afternoon in healthy women [10]. Also, in the pubertal girls we observed a greater absolute increase in bone resorption but the nyctohemeral pattern and mean serum levels of PTH were similar to those in the women. It would be necessary to propose that in pubertal girls there might be increased sensitivity to the action of PTH.

In experimental animals there is evidence that the nyctohemeral variation in bone turnover may be influenced by the timing of meals [1]. There also is evidence that the rhythms in cellular activity persist in bone organ culture [3]. This suggests that the rhythm may be an endogenous property of tissues. This concept also is suggested by studies of the mechanism of other nyctohemeral rhythms. Many of these are endogenous, including the nyctohemeral rhythm of mineral excretion [7]. Moore-Ede [26] was unable to abolish the nyctohemeral rhythm of calcium excretion in humans, either by maintaining subjects supine or by feeding them throughout the night. He proposed that the endogenous rhythms are the basis of predictive homoeostasis. Thus, during the nocturnal calcium fast, the increase in bone resorption and decrease in calcium excretion prevent a decrease in serum ionized calcium concentration.

There was a negative correlation between urinary Dpyr excretion and urinary calcium excretion. If there was no rhythm in the fractional excretion of calcium, then the increase in bone resorption would result in an increase in urinary excretion of calcium and there would be a positive correlation. The negative correlation may be a result of a common hormonal influence such as a nocturnal increase in serum PTH concentration. Alternatively, individuals with a pronounced rhythm of bone resorption also may have a pronounced rhythm of calcium excretion. We have preliminary evidence that the day/night difference in urinary calcium (and sodium) excretion is less pronounced in patients with osteoporosis, and it is possible that accelerated bone loss may result from disturbances in these rhythms [27].

The 24 h excretion of collagen cross-links in normal women reported here is similar to that reported by other groups [11, 28–30]. The five-fold increase in urinary Pyr and Dpyr excretion and in the serum BGP level is in keeping with previous observations made on urinary hydroxyproline excretion [31], serum BGP concentration [24] and serum alkaline phosphatase activity [32]. Thus, the pubertal growth spurt is accompanied by a large increase in bone turnover but no apparent change in the nyctohemeral rhythm of bone turnover. However, although the pattern does not differ, there is much larger absolute increase at night in puberty, and this may affect the relationship between urinary calcium excretion and urinary Dpyr excretion found in adults.

Bone formation and bone resorption are coupled processes at the level of the bone remodelling unit. Therefore, it was not surprising to find a positive correlation between urinary Dpyr excretion and the mean serum BGP level. This correlation was significant in the postmenopausal women and so it may be feasible to use this relationship to identify 'fast losers', such as has been done with less specific biochemical markers of bone turnover [33]. The relationship was not significant in the premenopausal women; this may result from positive calcium balance in women in their 20s and negative calcium balance in women in their 40s. The relationship would best be studied in a narrower age range.

Thus, bone turnover and mineral excretion in females undergo a nyctohemeral rhythm that appears to be unaffected by growth and ageing. The rhythm appears to be independent of the absolute level of bone turnover. On a practical level, it is important to be aware of this rhythm in considering the timing of sample collection. On a theoretical level, it may be that disturbances of this rhythm may play a role in the pathogenesis of involutional osteoporosis.

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