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Circadian Variation in Ionized Calcium and Intact Parathyroid Hormone: Evidence for Sex Differences in Calcium Homeostasis*

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ABSTRACT. The rate of bone loss with age and the incidence of osteoporosis are greater in women than men, which led us to question whether subtle sex differences may occur in the circadian variation of serum ionized calcium (iCa) and PTH. We measured iCa hourly and intact PTH every 2 h for 26 h in 25 women (21–69 yr) and 24 men (20–67 yr) consuming self-selected diets. Urine was collected at 0800–1600, 1600–2400, and 2400–0800 h. Serum iCa levels followed a circadian rhythm in both sexes ($P \leq 0.01$), and the patterns differed between sexes, notably during early morning, when serum iCa levels were lower in women ($P = 0.02$). Urinary calcium excretion and fractional excretion of calcium declined in both sexes at night (2400–0800 h), but the decline in men was significantly greater ($P = 0.02$). Similarly, the percent reduction in urinary calcium excretion at night was greater in men than in women (34% *vs.* 17%; $P \leq 0.05$). In women, 26-h mean serum iCa values correlated significantly with total daily calcium intake ($r = 0.44$; $P = 0.03$). Serum intact PTH levels showed a significant circadian pattern in both sexes ($P \leq 0.001$). The patterns of serum intact PTH differed between the sexes ($P = 0.05$), with an earlier and greater increase at night in men. This blunted nocturnal rise in PTH in women may explain the poor nocturnal adaptation to fasting found in women who, despite lower calcium intake, did not reduce urinary calcium loss at night as effectively as men. (*J Clin Endocrinol Metab* **72:** 69–76, 1991)

BONE loss is an inevitable fact of aging, but the rate of bone loss with age and the incidence of osteoporotic fracture are greater in women than men (1, 2). No apparent differences exist between men and women when serum factors that regulate calcium homeostasis, such as ionized calcium (iCa) or PTH, are measured in fasting subjects (3). iCa and PTH as well as measurements of bone turnover follow circadian rhythms (4–14). By limiting blood sampling to one point along a fluctuating pattern, subtle but important sex differences in the natural variation in these regulators may go undetected.

Recently reported differences in the circadian pattern of iCa in healthy postmenopausal women (6) compared to the pattern observed in young men (4) led us to question the influence of sex and age on the normal circadian rhythm of the calcium-parathyroid axis. The magnitude of change reported in the oscillation of iCa over 24 h is relatively small (0.02 mmol/L, 0.08 mg/dL), but changes of this magnitude have been associated with changes in the secretion of intact PTH (4, 15).

The technology now exists to allow accurate monitoring of such small changes in iCa in serum, plasma, or whole blood (16). Past studies characterizing the circadian variation in PTH were hindered by the inability of the assay to distinguish biologically active or intact PTH from biologically inert fragments that are retained as the kidney ages (9, 10). Recent development of two-site radioimmunometric assays specific for intact PTH-(1–84), the biologically active form, resolves this dilemma (17). Knowledge of possible sex differences that may occur in the circadian rhythm of iCa and intact PTH could provide insight into the underlying sex differences in the rate of bone remodeling and may help to under-

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stand the cause of osteoporosis.

In the present study our goal was to characterize the circadian pattern of serum iCa, intact PTH, phosphorus (Pi), and other electrolytes in a large sample of normal subjects. Our specific aim was to determine if sex or age, or both, influence the circadian rhythmicity of the calcium-parathyroid axis in healthy subjects consuming their usual dietary levels of calcium and Pi, and if calcium intake had any influence on these rhythms.

Materials and Methods

Subjects

We studied 49 healthy normal volunteers, 25 women and 24 men, ranging in age from 20–69 yr. For each sex and decade of life within this range, we recruited 5 subjects, with the exception of men 60–69 yr of age, for which we studied 4 subjects. All postmenopausal women were older than 50 yr (mean yr postmenopause, 12.3; range, 1–22 yr). The protocol was approved by the Institutional Review Board of the Mayo Clinic, and each subject gave written informed consent before the study.

Each subject was studied as an in-patient in the Clinical Research Center (CRC) of the Mayo Clinic. A preliminary interview and physical examination familiarized subjects with the facility and protocol and ensured that all subjects were normal and did not suffer from any diseases or peculiarities of diet and that they were not receiving any medications known to influence calcium metabolism (vitamin and/or mineral supplements, estrogens, thiazide diuretics, or anticonvulsants). All patients had normal values for serum iCa, total Ca, Pi, alkaline phosphatase, creatinine, and albumin measured in morning fasting serum. Before study, diet histories were taken verbally by an experienced research dietician, and each subject was instructed on how to record dietary intake for a required 7-day food record. On the day of the study, each subject was instructed to select a variety of foods from a specially prepared menu in the quantity that they would usually consume during a typical day. For each subject, the meals were served exactly at 0800, 1200, and 1800 h with a snack at 2000 h, and each meal was consumed within 30 min. Because all menu items were prepared and carefully weighed in our research kitchen, accurate levels of Ca and Pi could be estimated from each subject's intake on the day of study using Nutritionist III software program (N-Squared Computing, Silverton, OR). Deionized water, tea, or decaffeinated coffee made with deionized water was allowed *ad libitum*.

Study protocol

Subjects were admitted to the CRC at 0630 h, after an overnight (12-h) fast. At this time, an indwelling venous catheter was placed in a forearm vein. Care was taken to maintain each subject in the proper body posture throughout the entire 26-h study. All subjects were recumbent (head and limbs at the same level) from arrival to 0800 h and from 2230–0800 h of the following day. Subjects were ambulant from 0800–2230 h; they could sit or walk within the confines of the CRC, but not recline. Urine was collected during the 24 h of the study in three separate 8-h collections as follows: 0800–1600, 1600–2400, and 2400–0800 h. Blood was drawn hourly from 0700–0800 h of the following day.

Catheter patency was maintained for the study duration by flushing with 2 mL normal saline after each blood withdrawal and restyletting the catheter. Heating pads were used to facilitate blood withdrawal and to avoid the use of tourniquets or heparin, which are known to interfere with measurement of iCa. The presence of 25 U heparin/mL has been shown in our laboratory to decrease iCa levels (Burritt, M. F., unpublished data). For each withdrawal, 14 mL blood were drawn with a plastic syringe, and the blood was immediately dispensed anaerobically into serum separator tubes (Becton Dickinson, Inc., Rutherford, NJ) for determination of iCa values and then into plain (no anticoagulant) tubes. Serum in tubes was allowed to clot for 30 min at room temperature before centrifugation. For each subject, the iCa value was determined within 8 h of the last blood withdrawal (18); serum samples were stored at −70 C for later analyses. All analyses for a given subject were performed in the same assay to minimize interassay variation. Total urine volumes were measured at the end of each collection period, and aliquots were frozen for later analyses.

Analytical methods

Serum iCa values were measured in anaerobic samples by a Radiometer ICA 1 Analyzer (Radiometer Copenhagen, Copenhagen, Denmark) (16). The inter- and intraassay coefficients of variation for serum iCa with aqueous controls and a normal serum pool (adjusted to pH 7.4) were 1.6% or less and less than 0.8%, respectively. All values for iCa are reported at their actual pH and were not adjusted to pH 7.4. We measured serum and urinary total Ca (normal serum range, 8.9–10.1 mg/dL) by atomic absorption spectroscopy (IL 751 Atomic Absorption Spectrophotometer, Instrumentation Laboratories, Lexington, MA). The day to day variations in total serum Ca measurement were 0.9% and 1.3% for high and low serum pools, respectively. Intact PTH was measured in duplicate in serum drawn every other hour by a two-site immunoradiometric assay, the Allegro Intact PTH kit (Nichols Institute, San Juan Capistrano, CA) (17). The assay limit of detection in our laboratory is 1 pg human PTH-(1–84)/mL, and the normal range for men and women (n = 70), aged 21–70 yr, is 8–57 pg intact PTH/mL. Intra- and interassay coefficients of variation are less than 6%. Serum and urinary creatinine values were determined using a Beckman Astra 8 Analyzer (Beckman Instruments, Inc., Brea, CA). Fractional excretion of calcium (FeCa) was calculated by using the mean serum iCa values averaged over each of the 8-h periods of urine collection in the standard formula and expressed as a ratio of creatinine clearance. We measured serum albumin and serum and urinary Pi levels on a Rotochem Centrifugal Analyzer (Travenol Laboratories, Inc., Round Lake, IL), which uses the methods of Robertson (19) and Morin and Prox (20), respectively. FFA levels were measured before and after the noon and evening meals in six men according to the method of Dole (21).

Statistical methods

Throughout our statistical analyses, we used the SAS system (SAS Institute, Inc., SAS User's Guide: Basics, version 5, 1985,

Cary, NC), and all significance testing was considered to be two tailed. To evaluate whether a true circadian rhythm occurred for these variables, the data from each subject were standardized as follows. The mean and SD for the 26 time points were determined for each subject. Standardized scores were then calculated for each time point by subtracting the mean from each observation and dividing by the SD. In the absence of a true distinct circadian rhythm, the mean over subjects of these standardized scores would be zero at each time point.

Three statistical approaches were used to evaluate the rhythmic nature and differences in the overall pattern of the time series of standardized scores. First, a white noise test (22) globally tested whether the variations in standardized scores over time were merely random fluctuation. Second, each subject's standardized scores were separately fit to the same degree high order polynomials and Hotellings's multivariate T^2 test was applied to the vector of regression coefficients. In the absence of any pattern, the mean vector would consist of zeros. Multivariate T^2 analyses were also applied to the coefficients of the mathematical models for men and women to test for sex differences. To identify areas of the curves that were significantly different, we used a two-sample t test to compare mean standardized values between sexes at a given time. The association between circadian pattern and age was assessed using the correlations between the individual polynomial coefficients and age. The analyses described above were performed for serum iCa, intact PTH, Pi, total Ca, albumin, and pH.

We used Pearson's correlation coefficient to determine whether the variation in one serum variable was correlated with another variable at the same time point or lagged time points. The correlation was estimated for each subject, with association tested with the one-sample t test for the null hypothesis that the mean correlation is equal to zero.

$P < 0.05$ or less was considered statistically significant. All statistical tests were two sided.

Results

The standardized iCa scores *vs.* time are plotted for women and men in Fig. 1 (*top*). The white noise test confirmed a circadian rhythm in serum iCa for women ($P \leq 0.01$) and men ($P \leq 0.01$). Changes in mean iCa with time were best described by a seven-term polynomial model, which provided a good fit to the mean data, with $r^2 = 0.74$ for women and $r^2 = 0.70$ for men ($P \leq 0.0001$ for both sexes). The model for women differed significantly from that for men ($P = 0.02$). The standardized scores in women were lower at night than in men, notably between 0600–0800 h. The mean maximal change in serum iCa (high to low) over the 26 h was 0.066 mmol/L for women (within-women range, 0.03–0.12 mmol/L) and 0.058 mmol/L for men (within-men range, 0.03–0.10 mmol/L).

When the data were classified according to dietary calcium intake on the day of the study, serum iCa showed no changes in the overall shape of the circadian rhythm

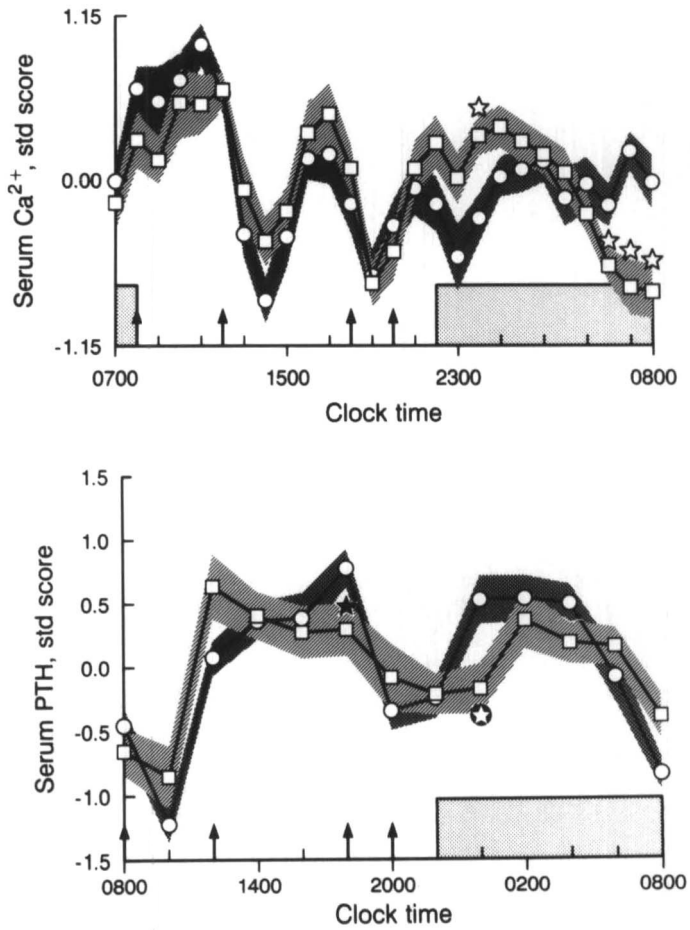

FIG. 1. *Top*, Circadian variation in serum iCa, expressed as standardized scores. The group mean ± SEM (*shaded area*) is shown in women (□–□) and men (○–○). ☆, $P < 0.04$, by two-sample t test, men *vs.* women at each time point. *Bottom*, The circadian variation in serum intact PTH measured every other hour is shown for women (□–□) and men (○–○). The group mean ± SEM (*shaded area*) for the standardized data are shown. ★, $P = 0.03$; ●, $P = 0.008$ (by two-sample t test, men *vs.* women at each time point). Meals were taken at times indicated by *arrows*, and a snack was consumed at 2000 h in some, but not all, subjects. The *stippled horizontal bar* indicates that the subjects were recumbent.

for either sex. There was a greater 24-h mean or global mean iCa level for women who ingested more than 800 mg calcium, but this was not significant ($P = 0.08$; Fig. 2, *top*). Correlation of calcium intake on the day of the study and global mean iCa level was positive and significant in women ($r = 0.44$; $P = 0.03$), but not in men ($r = -0.12$; P = NS). The correlation between calcium intake assessed on the day of the study and the mean intakes estimated from 7-day food records was computed to determine if the former reflects estimates of the subjects' habitual calcium intake. Despite the inherent weakness in values obtained from 7-day food records, there was significant agreement between these estimates of calcium intake in women ($r = 0.48$; $P = 0.02$), but there was a weaker correlation in men ($r = 0.35$; $P = 0.09$). Multi-

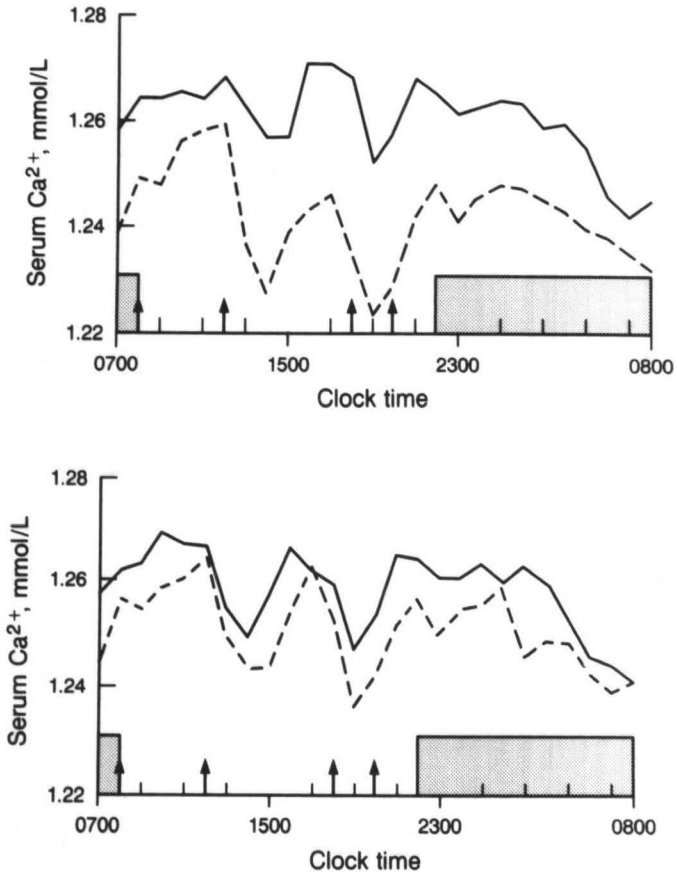

FIG. 2. *Top*, Serum iCa rhythm in women consuming more than 800 mg Ca on the day of the study (—-; $n = 15$) and in women consuming less than 800 mg Ca (- - -; $n = 10$). *Bottom*, Mean serum iCa levels in the menopausal women (- - -; ≥50 yr; n = 10) and in the remaining premenopausal subjects (—-; <50 yr; n = 15). *Arrows* indicate the times of meals, and the *stippled horizontal bar* indicate the period of recumbency.

variate analysis revealed no significant association between iCa and age in either sex (Fig. 2, *bottom*).

Serum iCa levels reached a plateau in men and declined in women after 0500 h, and similar declines were observed in urinary calcium excretion during the third 8-h collection (Table 1). The percent decrease in urinary calcium during the night (expressed as a percentage of the daytime mean values) was greater in men than in women (34% *vs.* 17%; $P \leq 0.05$). The FeCa showed a similar pattern of nocturnal decline for both men and women (30% *vs.* 16%; $P = 0.08$). However, despite their lower intake of calcium (mean ± SD, 916 ± 338 *vs.* 1087 ± 466 mg Ca/day), the FeCa was significantly greater in women, notably at night ($P = 0.02$). Overall, women excreted a higher percentage of their total daily Ca intake in urine than men; however, the difference was not statistically significant (median ratio of urinary calcium to dietary calcium in women and men, respectively, 0.21 *vs.* 0.17). A significant correlation between dietary calcium intake and total urinary calcium loss was observed in women (r = 0.48; $P = 0.017$), but not in men (r = 0.32; P = NS).

Intact PTH levels measured every other hour also showed a significant circadian rhythm in men and women, as assessed by the white noise test (P 0.05 for both). The mean hormone profiles were best fit by a six-term polynomial, with $r^2 = 0.80$ for women and $r^2 = 0.86$ for men. As shown in Fig. 1 (*bottom*), the standardized scores followed a biphasic profile, with peaks at 1800 and 0200 h and troughs at 1000 and 2000 h. Figure 3 (*bottom*) plots the mean intact PTH levels over time for both sexes. The range of individual peak to trough differences for intact PTH was 8–55 pg/mL or 23–67% of individual peak values. Although age had no apparent effect on the circadian variation in intact PTH, the difference between the circadian rhythm in men and women reached significance based on Hotellings' multivariate analyses (P = 0.05), specifically at 1800 and 2400 h based on two-sample t tests (Fig. 1, *bottom*).

When intact PTH levels were correlated with iCa levels at concurrent time points, there was a significant trend for these values to move in opposite directions for men (mean r = −0.44; $P = 0.0001$). In women, a similar, although slightly weaker, relationship was evident (mean r = −0.28; $P = 0.0026$). Global mean intact PTH did not correlate with daily calcium intake on the day of the study for either men or women.

Fluctuations in serum Pi followed a biphasic pattern, with troughs occurring at 0900 and 2000 h and peaks at 1600 h and in the late evening (2200–0200 h). The circadian pattern of serum Pi was not detectably different between men and women, nor was it affected by age. To determine if there was an association between iCa and Pi, standardized iCa scores were correlated with standardized serum Pi scores at concurrent time points. There was no significant correlation between iCa and Pi at any of these time points in women. For men, the average correlation was −0.15 ($P = 0.02$), *i.e.* a slight tendency for iCa to decrease when Pi increased and *vice versa*. Figure 3 illustrates the striking similarities between the patterns of serum Pi and intact PTH in men and women.

Serum total Ca, albumin, and pH also showed distinct circadian patterns in men and women, but no apparent differences in these serum rhythms occurred between sexes (data not shown). The circadian variations in total Ca paralleled those in albumin. Unlike iCa, both serum total Ca and albumin levels were affected by postural change, showing a marked decrease during recumbency (data not shown). Within-subject correlations between iCa and serum albumin at concurrent time points indicated a significant positive correlation for men and women (mean r = 0.17; $P = 0.018$ and mean r = 0.26; P = 0.0004, respectively), whereas the correlation between

TABLE 1. Diurnal changes in urinary calcium excretion (mean ± SEM)

Variable	Time of collection			P^a	% Decrease at night	
	Daytime		Nighttime (2400–0800 h)		Mean	P
	0800–1600 h	1600–2400 h				
Urinary Ca (mg/8 h)						
Women	70 ± 6	59 ± 6	49 ± 4	0.005	17 ± 7	0.01
Men	78 ± 8	71 ± 7	47 ± 5	<0.001	34 ± 6	0.0001
P between groups	NS	NS	NS		0.05	
FeCa (%)[b]						
Women	0.12 ± 0.01	0.11 ± 0.01	0.09 ± 0.01	0.01	16 ± 6	0.02
Men	0.10 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.01	30 ± 5	<0.001
P between groups	0.05	NS	0.02		NS	

[a] Within sex comparisons, daytime *vs.* nighttime.
[b] FeCa calculated from the mean of the serum iCa values for the 8-h urine collection.

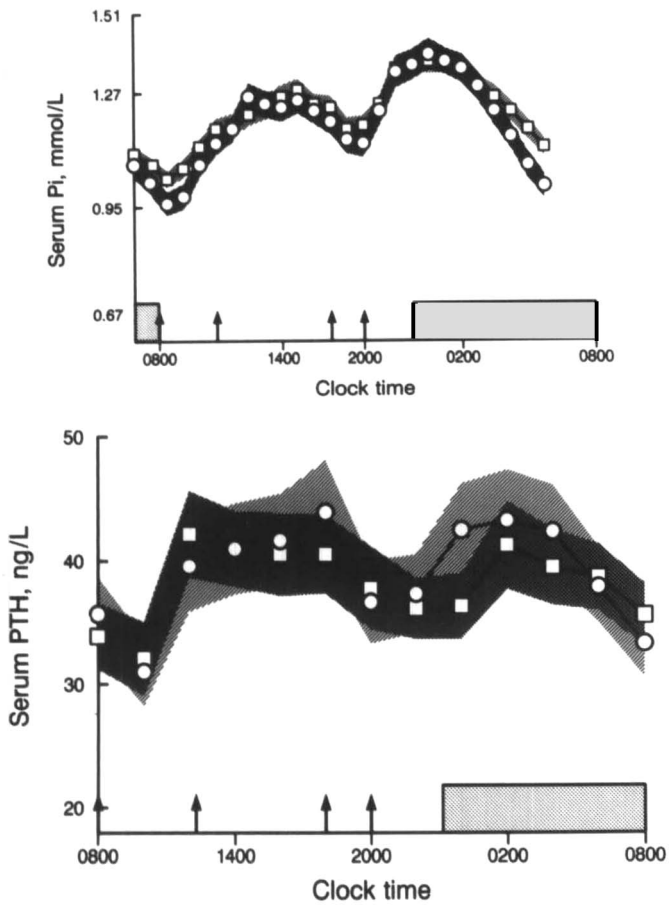

FIG. 3. *Top*, Circadian variation in serum Pi measured hourly in women (□–□; n = 25) and men (○–○; n = 24). *Bottom*, Circadian variation in serum intact PTH measured every other hour in women (□–□; n = 25) and men (○–○; n = 24). Group mean values are shown. Meals and snacks are indicated by the *arrows*, and the *stippled bar* indicates recumbent posture.

serum iCa and pH was significant, but negative, for both sexes (mean r = −0.25; $P = 0.0017$ for men and mean r = −0.37; $P = 0.001$ for women). The circadian patterns for pH in men and women were identical when either the raw data or standard scores were plotted. Notably, no sex differences were observed between 0100–0800 h, the period where sex differences in serum iCa were observed.

Serum fluctuations in pH were small, but the patterns were identical in both sexes and showed some influence of meal consumption through an increase in pH up to 3 h postprandially. Although this relationship was not always evident, postprandial increases in pH were related to decreases in iCa, most notable after the evening meal. However, pH was also observed to increase in the early morning, an event apparently independent of postprandial alkaline tide. No pre- or postmeal patterns were observed for FFA levels, nor were we able to detect a relationship between serum levels of FFA and iCa (data not shown).

Discussion

Our finding of a significant circadian variation in iCa in normal adult men and women consuming varied diets confirms some (4–8), but not all, previous reports (11). Our results, however, differed from those of earlier studies, with regard to the exact timing of peaks and troughs in serum iCa. More importantly, we found a statistically significant difference between the circadian rhythms of men and women, which was most notable during the early morning, when serum iCa levels remained constant in men and declined in women.

Of the earlier studies, the most relevant to our results include that of Perry III *et al.* (6), who studied healthy postmenopausal women, and that of Markowitz *et al.* (4), who studied normal young adult men. Both researchers reported evening troughs in iCa around 1600–1800 h, which is in agreement with our findings, but the peak iCa differed, with serum iCa level peaking in the late

evening to early morning (0200–0400) in postmenopausal women and in the midmorning in young men. It is difficult to make meaningful comparisons of the findings of these studies given the different approaches to handling the data, such as the use of running means *vs.* standardized data, differences in analytical methods (serum *vs.* blood), different frequencies of blood sampling, and variability in nutrient content and timing of meals as well as age and sex differences in subjects.

We also found significant sex differences in the circadian pattern of urinary calcium. The variation between diurnal and nocturnal excretion of calcium in urine is well established (23–25), and our findings confirm previous studies showing a nocturnal decline. Although men and women showed the anticipated nocturnal decrease in urinary calcium loss, women showed a significantly smaller decline than men, despite the observed decline in serum iCa during the fasting early morning hours and their lower calcium intake.

We confirmed the existence of a significant circadian variation in intact PTH in men and women (9–12). The frequency of our blood sampling, however, did not allow us to detect the recently described pulsatile secretion of intact PTH (26, 27). It is important to note that this rapid fluctuation in PTH could potentially influence the interpretation of our results. Our findings in men agreed closely with the recent study by Markowitz *et al.* (28), who reported a biphasic PTH rhythm, with peaks at 2000 and 0400 h and troughs in the midmorning and late evening. The circadian rhythm of serum intact PTH in women showed a similar biphasic pattern, but it differed significantly from that in men, and neither sex showed a significant effect of age on this hormonal pattern as has been shown in single samples from fasting subjects (3, 29). In women, the late evening increase in serum intact PTH was blunted and lagged behind that in men. To our knowledge, this is the first report demonstrating a significant circadian rhythm of intact PTH in both men and women and the first to indicate subtle sex differences in the circadian fluctuation of this hormone.

Our findings and those of Markowitz *et al.* (28) differ from previously reports of circadian PTH rhythms (9–12) that describe a single nocturnal peak. Early assays for PTH used antisera that recognized intact PTH as well as inert fragments, unlike the assay we used which is specific for the intact PTH-(1–84) (17). The assay used by Markowitz *et al.* recognized intact and midregion, 44–68, fragments, but not amino- or carboxy-terminal fragments of PTH. Measurement of intact PTH in our study dismisses the possibility that the observed nocturnal increase in PTH level is due to decreased renal clearance of inert PTH fragments resulting from the usual decrease in renal creatinine clearance at night. Our findings confirm a true increase in the intact hormone at night that is apparently greater in men than women. Recently, Logue *et al.* (12) described a circadian rhythm for intact PTH in adult men that is characterized by a single nocturnal peak. However, examination of subject's individual curves showed the presence of smaller peaks during the day, a feature that was lost when the data were averaged over subjects. These researchers found that the peak in nephrogenous cAMP, an indicator of biological activity of PTH in the kidney, was associated with the nocturnal peak in intact PTH.

Examining the interrelationship between iCa and intact PTH at concurrent time points, we saw a significant negative correlation in men, but a weaker negative correlation in women. Similarly, Markowitz *et al.* (28) found that these variables were highly correlated at concurrent time points, although they reported that serum iCa was best correlated with PTH when there was a 2-h lag in PTH (28).

The sex difference in urinary excretion of Ca during the early morning period may be mediated in part by the sex differences in intact PTH secretion or action. The delayed and blunted nocturnal rise in serum intact PTH in women may contribute to their observed greater urinary loss of calcium, because PTH action increases renal tubular reabsorption of calcium. A similar relationship was found between normal postmenopausal and osteoporotic women (30, 31). These studies reported an abnormal circadian rhythmicity in the calcium-parathyroid axis in osteoporotic women. Eastell *et al.* (30) and Dubé *et al.* (31) found no nocturnal increase in PTH level and a failure to decrease urinary Ca excretion at night in osteoporotic women compared to normal postmenopausal women. These investigators reasoned that this abnormality in the circadian rhythm of the calcium-parathyroid axis may be an important factor in the pathogenesis of osteoporosis.

For the majority of the variables we studied, age had no apparent effect on the circadian rhythm, but we cannot dismiss the possibility that the use of five or fewer subjects per decade limited our ability to observe an age effect. Furthermore, the subtle differences in calcium homeostasis that reportedly intensify during menopause may occur earlier than previously suggested, thus obscuring an age effect in women.

In addition to the endogenous or intrinsic factors that are thought to drive the circadian rhythm of iCa and PTH (4, 32), specific extrinsic factors, such as posture and food intake, superimpose their effects on the normal circadian fluctuations in calcium homeostasis (33, 34). Portale *et al.* (35) demonstrated that variations in the dietary level of Pi can modulate the endogenous circadian rhythm in serum Pi and iCa, and we have shown that modifying mineral intake can modulate the circadian pattern of serum iCa and PTH (36). Dietary restriction

of Pi abolished one periodic component of the Pi rhythm, whereas Pi supplementation exaggerated it (35). In both diet studies, the circadian rhythm persisted, but the 24-h mean for serum iCa, Pi, or PTH levels increased or decreased appropriately. These studies demonstrate the importance of the timing of blood sampling and how the use of a circadian design can enhance the detection of changes that usual blood sampling in fasting subjects misses. In both studies (35, 36), no changes in serum iCa, Pi, or PTH levels were observed in blood collected between 0800–0900 h, but differences were observed later in the day and night.

Differences in dietary calcium intake influenced the circadian variation of iCa in the present study, as evidenced by the significant correlation between calcium intake and global mean serum iCa levels in women. This relation was not apparent in men, which suggests that women may be more dependent on calcium intake to maintain calcium homeostasis than men.

Other epiphenomena of food intake had less apparent effect on the circadian rhythm of calcium metabolism, which included increased pH associated with postprandial alkaline tide, increased gastric and intestinal secretions (37), and postprandial increase in serum FFA levels (38). Serum iCa did correlate significantly with changes in serum pH, but our study design did not allow us to distinguish meal effects from those attributed to the endogenously driven circadian rhythm of gastric acid secretion (39). Serum iCa did not fluctuate with the small changes in FFA after a meal.

In conclusion, we demonstrated the existence of distinct circadian rhythms of iCa and intact PTH that show subtle but significant differences between men and women. Although diet and other extrinsic factors showed some influence on these patterns, unknown endogenous factors are thought to drive these rhythms. Our findings indicate a sex difference in the nocturnal adaptation to fasting, with women showing a greater excretion of urinary calcium and lower serum iCa levels after 0500 h. If the poor nocturnal adaptation to fasting results from the observed blunting of the nocturnal increase in PTH, then these differences in the circadian variation of iCa and PTH suggest that women may be less effective at maintaining calcium homeostasis than men. Although our reported differences between men and women are small, poor calcium retention resulting in daily differences of approximately 20 mg Ca/day over decades may contribute to the observed sex differences in the rate of bone loss.

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