Evaluation of a nonhuman primate model to study circadian rhythms of calcium metabolism

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Hotchkiss, Charlotte E., and Christopher P. Jerome. Evaluation of a nonhuman primate model to study circadian rhythms of calcium metabolism. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R494–R501, 1998.—We evaluated primate models for the study of circadian rhythms in calcium and bone metabolism. Blood and urine were collected from two cynomolgus macaques every 4 h for 24 h. Studies were initiated at three different clock times to separate the effects of repeated experimental sampling from circadian effects. Also, samples were collected from seven monkeys at times of expected maxima and minima. Some parameters exhibited the expected circadian rhythm with increases at night (serum total calcium) or in the early morning (urinary collagen cross-links). Others displayed the effects of the experimental procedure, either increasing (urinary creatinine and phosphorus) or decreasing (osteocalcin, urinary calcium) with repeated sampling. Serum phosphorus, cortisol, and type I procollagen were influenced by both clock time and experimental procedures. Alkaline phosphatase and parathyroid hormone did not show any differences with time or sampling. This data is consistent with findings in humans that bone resorption increases at night and that endogenous corticosteroids decrease bone formation. The usefulness of the monkey model is limited by the physiological stress of sample collection in these subjects.

Bone; monkey; biomarkers; physiological stress

CIRCADIAN RHYTHMS IN BONE formation and resorption have been demonstrated in humans. Markers of bone formation [osteocalcin (11, 29), alkaline phosphatase (11, 29, 33), and the carboxy terminal propeptide of type I procollagen (PICP) (12)] increase late at night. Markers of bone resorption [urinary pyridinoline, deoxypyridinoline (38), and the carboxy terminal pyridinoline cross-linked telopeptide of type I collagen (12, 37)] also increase late at night and in the early morning hours. These changes are reflected by changes in overall calcium metabolism. Although there is disagreement as to whether serum calcium varies in a circadian manner, it is clear that parathyroid hormone (PTH) increases at night (5, 7, 9–11, 30), with a minor peak in the late afternoon (7, 11). Urinary calcium excretion decreases at night in normal individuals, both associated with the general decrease in excretion of water and minerals at night (23) and also by decreased fractional excretion of calcium as related to creatinine clearance (5, 7, 8). The increased renal tubular reabsorption of calcium in men and premenopausal women appears to be secondary to the increase in serum PTH at night (7, 8, 16). These rhythms may be important in the normal maintenance of bone. In women with postmenopausal osteoporosis, the nighttime PTH increase is blunted (7). Because estrogen decreases skeletal sensitivity to PTH, it has been suggested that increased sensitivity following estrogen deprivation could result in a diminished feedback response, preventing the high nocturnal rise necessary to increase renal tubular absorption (6). Without this increase, renal reabsorption of calcium is not stimulated, and urinary calcium losses at night are markedly higher in women than in men (5), higher in older than in younger women (16), and higher in osteoporotic women than in normal women (7).

Based on these findings, two methods of increasing bone density in women have been attempted. In the first, calcium supplements are given at bedtime to compensate for upcoming renal losses. Mixed results have been obtained with this method (2, 36). The second involves reestablishment of the PTH rhythm by timed dietary supplements of calcium and phosphate, for which the preliminary data look promising (35). The possibility of increasing bone density with timed mineral supplements is appealing, in that side effects associated with hormonal drugs are eliminated.

A great deal has been learned about these circadian rhythms from clinical trials. However, there are significant drawbacks to human studies. There are limitations to the invasive techniques that can be performed on humans, and there is also a limit to the amount of time that humans are willing and able to spend as experimental subjects. In the long term, differences in diet, feeding regimen, activity, and other medications become apparent as confounding factors. These problems can be eliminated with animal models.

Because of the similarities in bone structure and reproductive parameters, cynomolgus macaques, after ovariectomy (14, 15) or treatment with a gonadotropin-releasing hormone agonist (19), have proven to be excellent models for the study of the pathogenesis and treatment of postmenopausal osteoporosis in women. However, the day-to-day changes in bone and calcium metabolism have not been examined. This study was designed to evaluate the usefulness of cynomolgus macaques maintained under standardized conditions as physiological models for circadian rhythms of bone and calcium metabolism.

MATERIALS AND METHODS

Monkeys. All procedures were approved by the Bowman Gray School of Medicine Animal Care and Use Committee.
Twenty-four-hour studies. Three 24-h series of blood and urine collections were performed in two monkeys (monkeys 5,171 and 5,172). To distinguish the effects of the experimental procedure from true circadian changes, each experiment was initiated at a different time of day. The first began at 0800, the second at 1600, and the third at 2400. Before experimentation, the monkeys were acclimated to short-term restraint in a Primate Products chair for sample collection. Each was surgically implanted with a vascular access port in the femoral vein.

Blood was collected every 4 h. For the first series, patency of the catheter was maintained between collections with saline flush and heparin lock (1,000 U/ml). Because of the possible effects of heparin on calcium metabolism, a lock solution of 50% dextrose was used for the second series. The vascular access ports were removed after the second series because of skin erosion over the catheters; in the third series, blood was collected by direct venipuncture.

Urine was collected from the metabolism pan every 4 h, and a clean sample was obtained every 8 h. Clean urine samples were obtained by free catch or catheterization. In the first series, food was offered for only a 2-h period at the normal feeding time (1300–1500). Because no food was eaten during this time, food was provided for the entire time in the subsequent series, and consumption was recorded every 4 h. Water was available ad libitum. Experiments were performed with lights on from 0700 to 2100. Samples were collected at night using a darkroom safety light.

Day/night comparisons. After examination of data from the 24-h experiments, it was apparent that the experimental procedure affected the physiology of the monkeys. To minimize blood loss and physiological stress to the monkeys, a protocol involving only two sample collections at expected maxima and minima for several parameters was developed. Blood was collected from seven intact female cynomolgus monkeys sedated with ketamine (30 mg/kg) at 1200 and 2400. Previous experiments demonstrated that ketamine anesthesia does not affect calcium metabolism (13). Eight-hour urine samples were collected from metabolism cages at 2000 and 0800.

Body temperature. Subcutaneous microchips (Implantable Programmable Temperature Transponders, BioMedic Data Systems) provided body temperature readings. Pilot experiments showed a correlation between microchip temperature and rectal temperature (r = 0.86 for monkey 5,171, 0.92 for monkey 5,172; P < 0.001), although the absolute temperature was not necessarily accurate. Therefore, the microchips provided a reliable measurement of changes in body temperature without disturbing the animal.

Biochemical analyses. Ionized calcium samples were maintained anaerobically until analysis. An AVL 988–4 Electrolyte Analyzer (AVL Scientific, Roswell, GA) was used to measure ionized calcium corrected to pH 7.4. Total serum calcium, phosphorus, albumin, creatinine, and total alkaline phosphatase were measured by standard biochemical techniques in an automated system (Cobas Fara II; Roche Diagnostics, Montclair, NJ), as were urinary calcium, phosphorus, and creatinine. The fractional excretion of calcium (FE Ca) was calculated using the formula FE Ca = (urinary Ca/serum Ca2+) × (serum creatinine/urinary creatinine).

Serum parathyroid hormone, osteocalcin, cortisol, and urinary carboxy-terminal pyridinoline cross-linked telopeptide of type I collagen (CrossLaps) were measured using ELISA (Active I-PTH, Diagnostic Systems Laboratories; Midt- 

Twenty-four-hour data for individual monkeys were analyzed by the cosinor method (26) using an Excel spreadsheet (3). Evaluation of clock time vs. order in which the samples were collected (sample number) for both monkeys together was performed using the SAS procedure MIXED (SAS Institute, Cary, NC), with the individual monkeys and collection days as random variables. R scores (7) were calculated by subtracting the mean for all time points in a session for a particular monkey by the data obtained at each time point and dividing by the standard deviation for that monkey during that session. Analysis of R scores did not change the interpretation of the data; however, R scores are used for some parameters to increase clarity of data presentation. Day/night samples were compared using a paired Student’s t-test, and Pearson correlation coefficients were calculated for selected variables.

RESULTS

Circadian rhythm markers. Body temperature demonstrated a significant circadian rhythm for both monkeys by cosinor analysis, with acrophase time at 1313 for monkey 5,171 and 1357 for monkey 5,172 (Fig. 1). Monkey 5,171 exhibited a significant circadian rhythm for albumin with an acrophase at 1620 (Fig. 2). Monkey 5,172 demonstrated a significant circadian rhythm for cortisol, with an acrophase time of 0746. Using the mixed statistical model, we found a significant effect of time but not order of sample collection for these three variables for both monkeys. However, all cortisol values were approximately twofold higher than those reported for tethered cynomolgus monkeys (4), the early morning peak was blunted, and there was a trend toward increasing cortisol with repeated sample collection (P = 0.053; Fig. 3).

Fig. 1. Body temperature as a function of time in cynomolgus macaques (means ± SE for 3 sessions in 2 subjects).
Food and water consumption. Water consumption was not recorded in the 24-h studies. When urine samples were collected at 2000 and 0800, the water consumption was significantly higher during the day than during the night (Table 1). There was no clear pattern of food consumption in either study, although food consumption was significantly correlated with water consumption in the day/night study ($r = 0.79$, $P < 0.001$). After ingestion of food, blood pH increased ($r = 0.42$, $P < 0.05$). Ionized calcium at pH 7.4 was directly correlated with food consumption during the previous 4-h period ($r = 0.51$, $P < 0.05$).

Serum calcium and phosphorus. Total serum calcium was at acrophase at 2136 h for monkey 5,171 by cosinor analysis. The mixed model demonstrated a significant time effect and no sample collection effect for both monkeys (Fig. 2). Although no significant circadian changes were seen in ionized calcium in these monkeys (Fig. 2), both ionized and total calcium were higher at night in seven monkeys bled at 1200 and 2400 (Table 1). Although the number of samples was low, the statistical power for the paired $t$-test was 90% for these parameters.

Phosphorus had a significant acrophase time of 2313 for monkey 5,172. Under the mixed model, both time and order of sample collection caused significant changes in phosphorus (Table 2). Although phosphorus was higher at night, it also increased with repeated sample collection.

PTH. In this study, PTH did not demonstrate any statistically significant changes as a result of time or order of sample collection (Table 2).

Bone biomarkers. Osteocalcin, a marker of bone turnover that primarily reflects formation in the assay used, decreased with increasing sampling in the 24-h study (Fig. 3) and was lower at night (the second sample collected from the monkeys) in the day/night study (Table 1). Total alkaline phosphatase, another marker of bone formation, did not demonstrate any significant changes as a result of time or sample collection (Table 2). PICP was not performed for the first series due to lack of serum; however, cosinor analysis detected a circadian rhythm for monkey 5,171 with an acrophase at 0148 for the last two series, and there was a trend ($P = 0.059$) toward a significant time effect overall (Table 2). On the other hand, in the day/night study, PICP, like osteocalcin, was significantly lower at 2400 than at 1200 (Table 1).

As to markers of bone resorption, uncorrected urinary collagen cross-links increased with repeated sample collection, in conjunction with urinary creatinine (Table 2). However, the collagen cross-link-to-creatinine ratio demonstrated an acrophase time of 0705 by

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**Table 1. Day/night comparisons**

<table>
<thead>
<tr>
<th>Time</th>
<th>1200</th>
<th>2400</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total calcium, mg/dl</td>
<td>8.9</td>
<td>9.5</td>
<td>0.001</td>
</tr>
<tr>
<td>Ionized calcium, mg/dl</td>
<td>4.8</td>
<td>5.2</td>
<td>0.008</td>
</tr>
<tr>
<td>Phosphorus, mg/dl</td>
<td>3.8</td>
<td>3.8</td>
<td>0.972</td>
</tr>
<tr>
<td>PTH, pg/ml</td>
<td>31.7</td>
<td>26.6</td>
<td>0.197</td>
</tr>
<tr>
<td>Osteocalcin, ng/ml</td>
<td>8.6</td>
<td>6.8</td>
<td>0.006</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/l</td>
<td>204</td>
<td>207</td>
<td>0.529</td>
</tr>
<tr>
<td>PICP, ng/ml</td>
<td>120</td>
<td>96</td>
<td>0.012</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cr, mg/dl</td>
<td>58.5</td>
<td>51.6</td>
<td>0.381</td>
</tr>
<tr>
<td>Calcium, mg/dl</td>
<td>14.2</td>
<td>13.9</td>
<td>0.872</td>
</tr>
<tr>
<td>Ca/Cr</td>
<td>0.26</td>
<td>0.39</td>
<td>0.266</td>
</tr>
<tr>
<td>Phosphorus, mg/dl</td>
<td>38.7</td>
<td>24.3</td>
<td>0.094</td>
</tr>
<tr>
<td>P/Cr</td>
<td>0.63</td>
<td>0.42</td>
<td>0.003</td>
</tr>
<tr>
<td>Collagen cross-links, µg/mM Cr</td>
<td>389</td>
<td>489</td>
<td>0.072</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Water consumed, ml</td>
<td>139</td>
<td>46</td>
<td>0.032</td>
</tr>
<tr>
<td>Food consumed, g</td>
<td>51</td>
<td>36</td>
<td>0.186</td>
</tr>
</tbody>
</table>

PTH, parathyroid hormone; PICP, type I procollagen; Cr, creatinine.
cosinor analysis for monkey 5,171 and showed a significant time effect for both monkeys using the mixed model (Fig. 4). There was a trend to a higher cross-link-to-creatinine ratio in urine collected at 0800 than at 2000 (Table 1).

Urinary electrolyte excretion. Urinary excretion of creatinine increased with repeated sample collection but did not exhibit a circadian rhythm (Table 2). Both urinary calcium concentration and FE<sub>Ca</sub> decreased but did not exhibit a circadian rhythm (Table 2). Both calcium and phosphorus concentration and fractional excretion of phosphorus (FE<sub>P</sub>) increased with repeated sampling (Fig. 5). Circadian rhythms were not detected in the excretion of either element, although in the day/night study, the urinary phosphorus-to-creatinine ratio was significantly lower during the night (Table 1). FE<sub>Ca</sub> and FE<sub>P</sub> were significantly inversely correlated in the 24-h period (Fig. 5). There was a trend to a higher cross-link-to-creatinine ratio in urine collected at 0800 than at 2000 (Table 1).

**DISCUSSION**

Changes in circadian rhythms of calcium metabolism may play a role in the development of osteoporosis. Development of an animal model would allow for controlled study of these rhythms, which causes them, and how they can be altered. Because of the similarity of nonhuman primates to humans, the cynomolgus macaque was evaluated as such a model. In this preliminary study, we found evidence of circadian rhythms similar to those of humans in some parameters; however, the data was also strongly influenced by the effects of the experimental procedures on the monkeys.

**Table 2. Values by time and sample number**

<table>
<thead>
<tr>
<th>Time</th>
<th>P&lt;sub&gt;m/dl&lt;/sub&gt;</th>
<th>PTH, pg/ml</th>
<th>Alk Phos, U/L</th>
<th>PICP, mg/dl</th>
<th>Urine Cr, mg/dl</th>
<th>GFR, ml/min</th>
<th>Urine Ca, mg/dl</th>
<th>Urine P, mg/dl</th>
<th>Collagen Cross-Links, µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>0400</td>
<td>3.1 ± 0.4</td>
<td>31 ± 9</td>
<td>221 ± 30</td>
<td>90 ± 13</td>
<td>47 ± 10</td>
<td>5.9 ± 0.6</td>
<td>20 ± 5</td>
<td>23 ± 19</td>
<td>551 ± 84</td>
</tr>
<tr>
<td>0800</td>
<td>2.7 ± 0.4</td>
<td>21 ± 4</td>
<td>237 ± 24</td>
<td>74 ± 16</td>
<td>59 ± 8</td>
<td>6.4 ± 0.7</td>
<td>14 ± 4</td>
<td>24 ± 9</td>
<td>887 ± 219</td>
</tr>
<tr>
<td>1200</td>
<td>3.3 ± 0.6</td>
<td>18 ± 4</td>
<td>217 ± 24</td>
<td>64 ± 15</td>
<td>57 ± 12</td>
<td>4.1 ± 0.7</td>
<td>20 ± 7</td>
<td>12 ± 10</td>
<td>725 ± 153</td>
</tr>
<tr>
<td>1600</td>
<td>3.1 ± 0.4</td>
<td>21 ± 7</td>
<td>206 ± 22</td>
<td>64 ± 14</td>
<td>78 ± 14</td>
<td>5.3 ± 0.7</td>
<td>20 ± 7</td>
<td>23 ± 7</td>
<td>854 ± 197</td>
</tr>
<tr>
<td>2000</td>
<td>3.7 ± 0.2</td>
<td>27 ± 12</td>
<td>223 ± 30</td>
<td>80 ± 18</td>
<td>66 ± 10</td>
<td>5.4 ± 1.2</td>
<td>27 ± 12</td>
<td>21 ± 11</td>
<td>565 ± 134</td>
</tr>
<tr>
<td>2400</td>
<td>3.7 ± 0.3</td>
<td>23 ± 8</td>
<td>211 ± 25</td>
<td>105 ± 15</td>
<td>52 ± 7</td>
<td>5.4 ± 1.1</td>
<td>29 ± 7</td>
<td>24 ± 11</td>
<td>565 ± 88</td>
</tr>
</tbody>
</table>

Values are means ± SE. Alk Phos, alkaline phosphatase; GFR, glomerular filtration rate.

Fig. 4. Ratio of carboxy-terminal cross-linked telopeptide of type I collagen (CrossLaps) to creatinine (Cr) in urine as a function of time in cynomolgus macaques (means ± SE for 3 sessions in 2 subjects).

Fig. 5. Effect of repeated sample collection on the fractional urinary excretion of calcium and phosphorus in cynomolgus macaques (means ± SE for 3 sessions in 2 subjects). First sample collection was at 0800, 1600, or 2400, and repeated samples were taken at 4-h intervals.
keys. The sample collection procedure caused an endocrine stress response, as reflected in an increase in cortisol evident after the second time point, and also caused changes in food consumption, as well as blood loss (0.5–1.0% of body weight).

The generation of circadian rhythms is multifactorial and may involve endogenous rhythms (25), light and dark (40), feeding schedules (9, 25), activity level (9), posture (24), cortisol levels (27), and feedback loops (20). The fact that certain rhythms were robust in these monkeys while others were altered by the experiment provides further insight into the source of these rhythms. In this study, the light cycle was not changed and posture was normal, but food intake was reduced, activity level was decreased, and cortisol levels were increased. The monkeys’ sleep patterns were altered, but that should not have had much effect, because sleep deprivation or sleep shift does not change circadian rhythms of osteocalcin or PTH (17, 28).

Circadian rhythm markers. The circadian rhythm of body temperature has been well described for both human and nonhuman primates (4, 10, 23), as has the early morning peak in serum cortisol (4, 23, 27). In addition, less urine is produced at night, resulting in hemodilution and a decrease in albumin concentration in the early morning hours (23). Rhythms in all three parameters were detected in these monkeys; the experimental procedures did not abolish circadian periodicity. However, physiological stress can affect the circadian rhythm of cortisol (31). Although a cortisol rhythm could be detected in these monkeys, it was not as pronounced as expected, and all values were approximately two times normal for this species (4). Thus it is clear that stress was also a factor in the results obtained. Physiological stress could be decreased to some extent by use of hand-reared rather than wild-caught animals. It could be eliminated by use of a tethering system, but that would limit the mobility of the animals.

Calcium and albumin. In this study there was a significant circadian rhythm in total calcium that reflected the changes in albumin, as has been reported in humans (5, 7, 20, 36). No circadian rhythm could be detected in ionized calcium, although it was significantly higher at night in the day vs. night experiment. This data is consistent with human studies. Although some investigators did not detect a rhythm in ionized calcium (10, 28), others have found peaks in the mid-morning (20, 30) or fluctuations during the day with a generalized increase at night (5, 7, 8). It has been reported that the peak is blunted in women and occurs earlier than in men (5, 7), which may be related to the increased urinary excretion of calcium at night in women.

Such discrepancies between studies may be due to the fact that several factors affect rhythms of ionized calcium. These rhythms are different for males and females (5), are affected by age (8), and are altered by sleep shift or deprivation (17, 28). Changes in ionized calcium lag 4–5 h behind changes in PTH (20), but administration of prednisone at 2000 reverses the calcium rhythm while causing only minor fluctuations in the PTH rhythm (30). The mean daily level of calcium increases with increased calcium intake (5) and decreases with fasting (9), but the shape of the circadian rhythm does not change.

In the monkeys, ionized calcium adjusted to pH 7.4 was directly correlated with food consumption during the previous 4-h period. After ingestion of food, blood pH increased, in a phenomenon known as the “alkaline tide,” secondary to gastric acid secretion. In human studies in which ionized calcium was reported at blood pH, the alkaline tide caused a decrease in ionized calcium (5), due to increased binding of calcium to albumin. In these monkeys the lack of a decrease in ionized calcium at blood pH and increase in adjusted ionized calcium after eating is most likely due to a higher level of calcium in the diet.

Bone resorption markers. Urinary collagen cross-links increased in the early morning hours in these monkeys. Such a rhythm in bone resorption markers has been detected in many human studies (8, 12, 36). It had been postulated that nocturnal recumbency was responsible for increased bone resorption at night, because decreased activity and recumbency increase bone resorption and circadian rhythms of calcium excretion are absent in paraplegics (24). However, maintaining a vertical position even during sleep actually increases total bone resorption (1), and 5 days of bed rest did not alter the circadian rhythm of bone resorption, although total resorption was increased (38). This implies that the circadian rhythm is not driven by mechanical forces on the bones but is truly endogenous, not altered by bed rest (33), calcitonin (36, 39), or alterations in rhythms of PTH (16) or corticosteroids (37, 38).

Bone formation markers. No circadian rhythm was detected in osteocalcin in these monkeys. There is increasing evidence that the circadian rhythm of cortisol secretion, with its distinct morning peak, is responsible for the circadian rhythm in osteocalcin. Administration of prednisone at 2000 does not significantly alter the PTH rhythm but does reverse the osteocalcin rhythm (30). Elimination of the morning cortisol peak by administration of metapyrone or continuous administration of hydrocortisone eliminates the circadian rhythm of osteocalcin but does not affect bone resorption (27, 37). In these monkeys, cortisol increased following the second sample collection, and osteocalcin decreased after the third sample collection, apparently in response to the increase in cortisol (Fig. 3). The caveat in extrapolating this finding to bone formation as a whole is that cortisol directly downregulates osteocalcin expression (32), which may or may not represent decreased osteoblast activity.

Total alkaline phosphatase showed no effects of time or sampling, possibly because the bone isoform comprises <50% of the total in cynomolgus monkeys (unpublished data), so that any effect would be diluted by lack of change in other isoforms. On the other hand, PICP showed effects of both time and repeated sample collection. Serum for PICP was available for only two-thirds
of the 24-h samples, yet a circadian rhythm was detectable, in contrast to osteocalcin. There also appeared to be a decrease in PICP with repeated sampling, which was not significant in the 24-h study (Table 2), but did result in a significant decrease in the day/night study. Schlemmer et al. (37) demonstrated maintenance of such a rhythm following abolition of the cortisol rhythm in humans. In summary, it appears that physiological stress affects but does not abolish the circadian rhythm of bone formation.

Urinary electrolyte excretion. Whereas in the 24-h study circadian rhythms were not detected in the excretion of calcium or phosphorus, in the day/night study the urinary phosphorus-to-creatinine ratio was significantly lower during the night, consistent with findings in humans (7). Both urinary calcium concentration and the FE_{Ca} decreased with repeated sample collections, whereas urinary phosphorus concentration and FE_{P} increased with repeated sampling. Furthermore, increased calcium excretion and decreased phosphorus excretion correlated with food intake during the previous 4 h. Although it appears in this experiment that fasting decreases calcium excretion and increases phosphorus excretion, fasting of humans does not induce such a change (21).

Serum phosphorus. Although serum phosphorus was higher at night, it also increased after repeated sample collection in both monkeys. In humans, serum phosphorus is higher at night (5, 7, 9, 20, 34), but the rhythm can be altered by phosphorus intake (34) and can be abolished by fasting (9). We did not see a correlation between serum phosphorus and food consumption. The reason for the change with increased sample collection is not clear but could be related to blood loss or physiological stress.

PTH. The circadian rhythm of PTH itself in humans has an endogenous component that is not related to diet, posture, or sleep-wake-related events (10). A feedback loop could be involved in the generation of this rhythm (20), because an increase in PTH is followed by an increase in ionized calcium — 4 h later, which is in turn followed by a decrease in PTH ~ 2 h later. It has also been suggested that the circadian rhythm in bone turnover occurs in response to the rhythm of PTH, but suppression of PTH secretion by calcium infusion does not alter the circadian pattern of bone resorption (16). Prednisone does not significantly alter the circadian rhythm of PTH (30), so changes in cortisol are not likely to explain the absence of PTH rhythms in these monkeys.

The absence of such a rhythm may be explained in one of several ways. 1) The number of animals used in this study was small, and, because of volume limitations on blood collection, the number of collections per series may have been too few to detect a rhythm. 2) Blood samples were collected differently for each series. In the first series, heparin was used to maintain patency of the vascular access ports. In the second, 50% glucose was used, and in the third, blood was collected directly. Contamination of the samples or of the monkey with the anticoagulant could conceivably alter assay results. However, no difference in PTH was seen between samples collected at noon and midnight, even though both samples were collected directly without anticoagulant, making this possibility less likely. 3) Blood loss has been shown to alter bone metabolism (18) and could theoretically affect the circadian rhythm of PTH. 4) Monkeys may not possess a circadian rhythm of PTH similar to that of humans. It has been shown using a tether system that cynomolgus monkeys do not possess the circadian rhythm of growth hormone that humans do (4); some species variation must be expected.

This experiment has provided valuable information concerning the ways in which monkeys are similar to, and different from, humans. Although the number of animals used in this study was small, certain conclusions can be drawn. 1) Bone turnover, as evidenced by urinary collagen cross-link excretion and serum procollagen, increased in the early morning hours in these cynomolgus monkeys, as it does in humans. 2) Serum osteocalcin, representing bone formation, was reduced by experimental procedures, probably due to physiological stress-related increases in cortisol. 3) Urinary excretion of calcium and phosphorus were affected by the experimental procedure, perhaps through changes in food consumption. 4) Modifications would have to be made to the model, such as the use of a tethering system or highly conditioned, colony-reared animals, to make it useful as a model for humans.

Perspectives

Although the cynomolgus monkey may not be a useful model for studying circadian rhythms of bone metabolism, this study raises some intriguing questions. It appears that circadian rhythms can be altered by experimental procedures. How does this relate to the human condition? Are controlled circadian studies in humans reflective of the natural situation? If circadian rhythms are important in the maintenance of bone mass, as has been suggested in the literature (7), does the disruption of these rhythms by physiological stress or changes in eating and sleeping habits play a role in the development of osteoporosis?

The authors acknowledge the Yerkes Regional Primate Research Center Assay Laboratory for performance of some of the osteocalcin, cortisol, and PTH assays.

This work was supported in part by the Forsyth County United Way and by a Venture Grant from the Bowman Gray School of Medicine of Wake Forest University.

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Received 14 November 1997; accepted in final form 29 April 1998.

REFERENCES


