Osteoporos Int (1998) 8:121–126

Osteoporosis International

Original Article

Alteration of the Circadian Rhythm of Intact Parathyroid Hormone and Serum Phosphate in Women with Established Postmenopausal Osteoporosis

W. D. Fraser[1], F. C. Logue[2], J. P. Christie[2], S. J. Gallacher[3], D. Cameron[2], D. St. J. O'Reilly[2], G. H. Beastall[2] and I. T. Boyle[3]

[1]Department of Clinical Chemistry, Royal Liverpool University Hospital, Liverpool; [2]Institute of Biochemistry, Glasgow Royal Infirmary, Glasgow; and [3]Department of Medicine, Glasgow Royal Infirmary, Glasgow, UK

Abstract. Several studies have established that the circulating concentration of intact parathyroid hormone, PTH (1–84), over 24 h follows a circadian rhythm. The importance of this circadian rhythm is not known although some authors have detected alterations in the rhythm in metabolic bone disease and following dietary manipulation. We have studied the circadian rhythm of PTH (1–84) in 8 premenopausal women, 8 postmenopausal women with established osteoporosis and 8 postmenopausal women with no evidence of osteoporosis. Blood samples were obtained at 30-min intervals over a 24-h period and significant differences were found in the profiles of PTH (1–84) and serum phosphate in the three groups studied. Premenopausal women possessed a nocturnal/early morning increase in PTH (1–84) and phosphate (between 2200 and 0700 hours), as did postmenopausal women without osteoporosis. In postmenopausal women with osteoporosis the nocturnal increase in PTH (1–84) and serum phosphate was absent and PTH (1–84) decreased during the period 2200–0700 hours. A shift in acrophase is observed between premenopausal and postmenopausal women without osteoporosis. No acrophase was found in postmenopausal women with osteoporosis for either PTH (1–84) or serum phosphate. No circadian rhythm, acrophase or significant amplitude was observed in serum adjusted calcium or ionized calcium in any group studied. Alterations in the circadian rhythms for PTH (1–84) and serum phosphate occur in patients with postmenopausal osteoporosis that suggest the normal dynamics of PTH (1–84) secretion may play a role in both calcium and phosphate metabolism and the bone remodelling process. Whether these changes are causative or a response to the pathology will require further investigation.

Keywords: Circadian rhythm; Osteoporosis; Parathyroid hormone; Phosphate

Correspondence and offprint requests to: Dr William D. Fraser, Department of Clinical Chemistry, Royal Liverpool University Hospital, Prescot Street, PO Box 147, Liverpool L69 3BX, UK. Fax: (+44) 0151 706 5813.

Introduction

The development of sensitive and specific assays for intact parathyroid hormone, PTH (1–84), has improved the clinical utility of PTH (1–84) measurements and their diagnostic value [1–3]. PTH (1–84) is detectable in all normal subjects and the normal physiology of secretion has been established [4–6]. The existence of a circadian rhythm for PTH (1–84) has been confirmed in normal male subjects and in premenopausal women [5,7], with different authors noting only minor variations in the profiles obtained [4,5,6,8]. This rhythm is lost in primary hyperparathyroidism [9,10] and pseudohypoparathyroidism [11], and can be altered by dietary manipulation [12] or a prolonged fast [13]. A previous study in postmenopausal women has identified abnormalities in circadian patterns of bone resorption and renal calcium conservation in type I osteoporosis that were associated with a trend towards blunting of the nocturnal increase in PTH (1–84) [14].

Controversy exists over basal levels of PTH in postmenopausal osteoporosis. Some authors report increases in PTH in osteoporosis [15,16], others no difference [17,18] and yet others decreases in PTH [19–21] relative to age-matched controls. When basal PTH (1–84) has been measured no difference [22,23] or a reduction [2,22,24] has been observed in osteoporotic patients relative to normals. Single time-point estimates of PTH (1–84) may be misleading and it is important to recognize that dynamic changes over a 24-h period could influence end-organ function. Evidence exists in both man [25,26] and animals [27,28] that regular bolus injections of PTH or active fragments of PTH have the ability to increase bone mass whereas continuous infusions of PTH lack this anabolic effect. This gradual accumulation of evidence on the importance of dynamic changes in PTH led us to investigate whether the circadian rhythm of PTH (1–84) was altered in patients with osteoporosis and the changes in calcium and phosphate metabolism that may accompany the PTH (1–84) rhythm.

Materials and Methods

Subjects were recruited from the wives of hospital personnel, a local advertising campaign through Women's Institutes and from a hospital specialist clinic in bone disease. None of the subjects studied was taking tablets or medicine known to interfere with calcium metabolism and none of the subjects had received treatment for osteoporosis prior to the study.

Eight premenopausal women, 8 postmenopausal women with no evidence of established osteoporosis and 8 postmenopausal women with osteoporosis were studied. Bone densitometry was performed by dual-energy X-ray absorptiometry (DXA) using a Lunar DPX, measurement being made at both lumbar spine (L2–4) and neck of femur and compared with the United Kingdom Lunar database. Typical precision expressed as the coefficient of variation with this method in normal subjects is 0.5% at L2–4 and 1.05% at the dominant femoral neck [29]. Plain radiographs of dorso-lumbar spine were obtained on all postmenopausal subjects. Osteoporotic subjects all had at least two vertebral compression fractures of either the thoracic or lumbar vertebrae other than L2–4 on plain radiographs of the spine. There were no fractures present in postmenopausal women with no evidence of osteoporosis.

All subjects were hospitalized for sampling and blood samples were obtained via indwelling cannulae every 30 min for 24 h. Patients were sampled in groups of 2, 3 or 4 at the same time of year over a 3-year period. At each sampling patients from different groups were included randomly. During the study period subjects ate the hospital diet at the same time (0800, 1200, 1800 and 2100 hours) and were ambulant but avoided exercise. The subjects lay down to sleep from 2300 to 0800 hours. Whole blood was allowed to clot, the serum was separated and snap-frozen within 30 min and stored at −50 °C before analysis.

PTH was measured using an in-house immunoradiometric assay [3] with a detection limit of 0.5 pmol/l, a range of 1.5–250 pmol/l and an intra-assay coefficient of variation of less than 10% across this range. Serum calcium, phosphate, creatinine, urea and albumin were measured by a standard autoanalyser method (Technicon, Tarrytown, NY). Serum calcium was adjusted for albumin as described previously [30]. Ionized calcium (with and without pH correction) was measured on a subgroup of women (4 per group) using a Radiometer ICA1 analyser (Radiometer Copenhagen, Copenhagen, Denmark) using sealed heparinized blood samples.

Group comparison of data was performed using a Mann–Whitney *U*-test. Statistical analysis of circadian rhythm parameters was carried out using the Cosinor technique [31]. In this analysis a cosine curve with a period of 16 h was fitted to the data for each individual using the method of least squares. An *F* statistic was then used to test the zero amplitude hypothesis for the circadian rhythm. The following parameters were calculated: the mesor (rhythm adjusted mean), the amplitude (half the total extent of the predictable change) and the acrophase (crest time of the best-fit cosine function, in relation to local midnight). The mean-cosinor data were then plotted on a polar coordinate graph. The mean amplitude and acrophase of the rhythm were displayed by the length and angle of the vector. The envelope representing the bivariate statistic and confidence region was also derived for each measurement.

The study was appproved by the hospital ethics committee and all subjects gave written informed consent prior to inclusion in the study.

Results

Premenopausal women had a mean age of 36 years (range 27–45 years). The postmenopausal women had a mean age of 64 years (range 59–78 years) and were a mean of 20 years from menopause (range 10–32 years). Their lumbar spine mean bone density was 95% (range 90–104%) that of their age-matched reference population. The osteoporotic patients were matched to the control postmenopausal group and had a mean age of 65 years (range 60–79 years) and were a mean 21 years from the menopause (range 12–34 years), which was not significantly different from the postmenopausal group (Mann–Whitney *U*-test). Mean lumbar spine bone density in these patients was 71% (range 62–82%) that of their age-matched reference population.

Each subject's individual and the mean 24 h profiles of PTH (1–84) and serum phosphate are shown in Fig. 1 and 2.

PTH (1–84) increases and decreases in a broad peak through the night from 2200 to 0700 hours in premenopausal and non-osteoporotic subjects. In patients

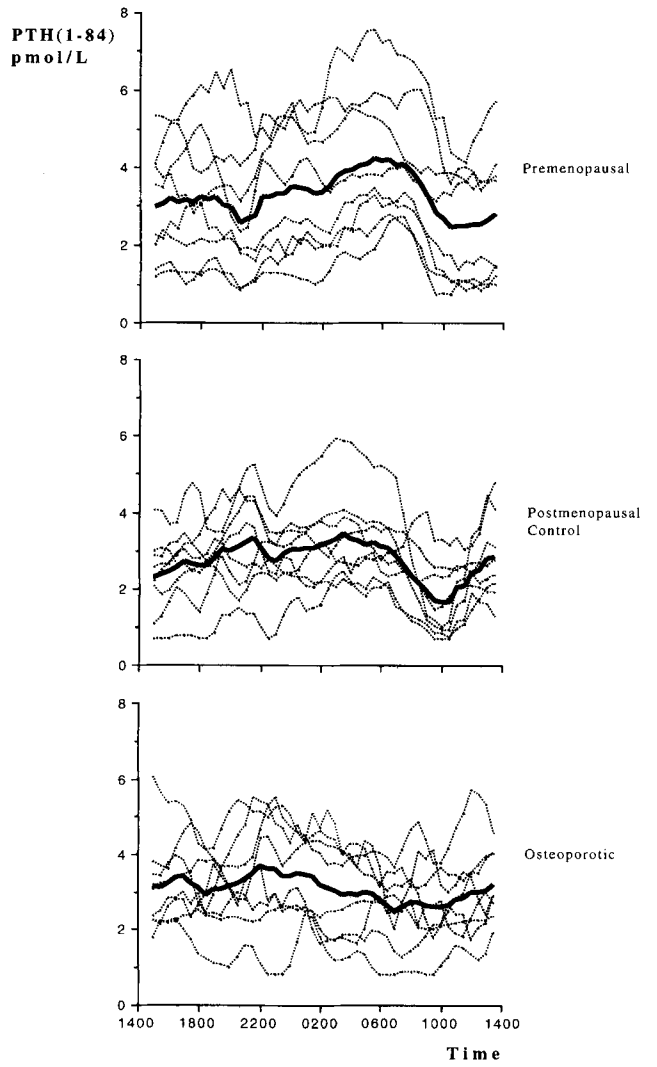

Fig. 1. Individual (*dotted lines*) and mean (*bold line*) PTH (1–84) concentrations during a 24-h period in premenopausal women, postmenopausal women with no osteoporosis (postmenopausal control) and postmenopausal women with osteoporosis.

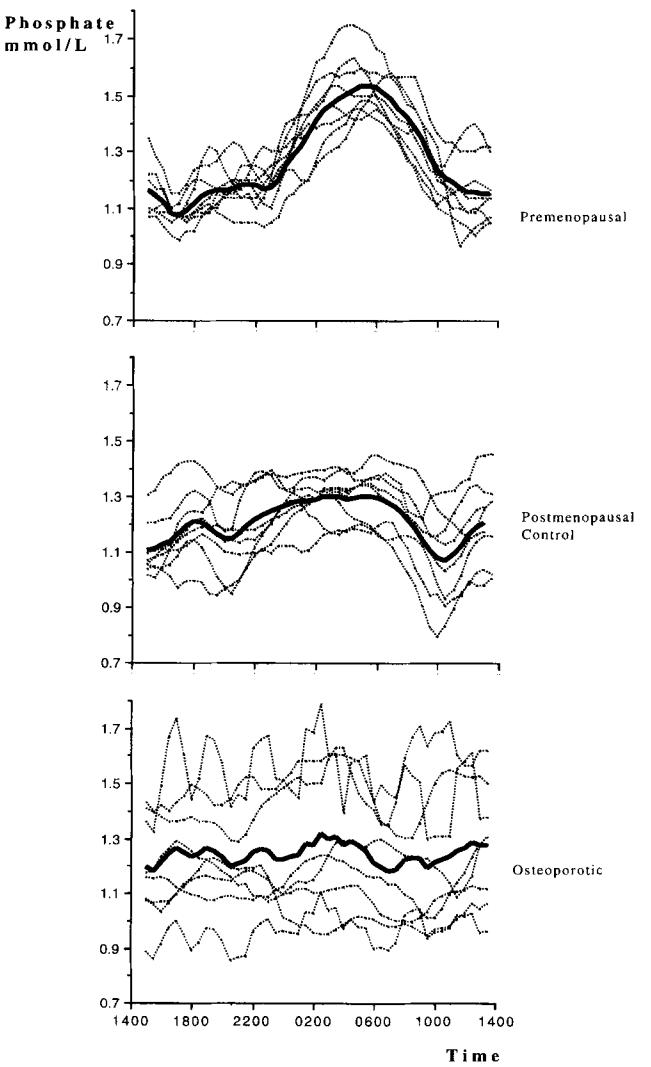

Fig. 2. Individual (*dotted lines*) and mean (*bold line*) serum phosphate concentrations during a 24-h period in premenopausal women, postmenopausal women with no osteoporosis (postmenopausal control) and postmenopausal women with osteoporosis.

with postmenopausal osteoporosis this nocturnal rise in PTH (1–84) is absent and PTH (1–84) instead decreases steadily throughout the night from 2200 to 0700 hours. In both premenopausal and non-osteoporotic postmenopausal women a rapid decrease in PTH (1–84) occurs from 0700 to 0900 hours. In all subjects the lowest PTH (1–84) concentration was observed between 0930 and 1030 hours.

A pronounced rise in serum phosphate is observed in premenopausal women throughout the night from 2200 to 0800 hours (Fig. 2). A rise in serum phosphate is also seen in non-osteoporotic postmenopausal women over the same time period but with a markedly attenuated serum phosphate peak. In osteoporotic women there is very little rhythm observed in serum phosphate and complete absence of a nocturnal rise in the majority of subjects studied.

Adjusted calcium transiently decreases overnight in premenopausal women but fails to demonstrate an acrophase or significant amplitude. No rhythm exists for ionized calcium in premenopausal women. There was no rhythm detected in adjusted calcium or ionized calcium in either group of postmenopausal women (data not shown).

Examination of the individual profiles of premenopausal and non-osteoporotic postmenopausal women indicates that a biphasic rhythm exists for PTH (1–84) and phosphate with a smaller increase in PTH (1–84) and phosphate occurring between 1400 and 2200 hours. Cosinor analysis has, therefore, been applied to the 16-h period between 2200 and 1400 hours.

Table 1 gives the acrophase mesor and amplitude for PTH (1–84), serum adjusted calcium and serum phosphate in the study groups. The polar coordinate

Table 1. Cosinor analysis of profiles

	Acrophase	Mesor	Amplitude	Significance value
Normal premenopausal women				
PTH (1–84)	0612	3.3 pmol/l	0.45 pmol/l	$p<0.05$
Adjusted Ca	NS	2.4 mmol/l	NS	
Ionised Ca	NS	1.1 mmol/l	NS	
Serum phosphate	0528	1.3 mmol/l	0.23 mmol/l	$p<0.01$
Normal postmenopausal women				
PTH (1–84)	0310	2.8	0.47	$p<0.05$
Adjusted Ca	NS	2.3	NS	
Ionized Ca	NS	1.0	NS	
Serum phosphate	0215	1.2	0.09	$p<0.01$
Postmenopausal osteoporotic women				
PTH (1–84)	NS	3.0	NS	
Adjusted Ca	NS	2.4	NS	
Ionized Ca	NS	1.1	NS	
Serum phosphate	NS	1.2	NS	

NS, not significant.

plots indicated a tight phase and frequency synchronization in PTH (1–84) for premenopausal and non-osteoporotic postmenopausal women. Wide scatter is observed in these plots in postmenopausal osteoporotic women. A shift in the acrophase is seen between premenopausal and postmenopausal women. Osteoporotic women show no significant amplitude in PTH (1–84) or serum phosphate. There was no significant amplitude and no acrophase detected in serum adjusted calcium in any of the study groups.

Discussion

Bone loss begins in midlife and continues to occur in both sexes into old age. The importance of oestrogen withdrawal at the time of the menopause in increasing the rate of bone loss in women is well established [32,33] but other factors are also likely to be involved. In particular the role of secondary hyperparathyroidism and deficiency of vitamin D have been a matter of debate in 'senile' osteoporosis [32,34]. In this study we have shown that significant differences exist in the circadian rhythm of PTH (1–84) in patients with proven 'senile' osteoporosis when compared with postmenopausal women without osteoporosis and healthy premenopausal women.

The role of the circadian rhythm of PTH (1–84) in bone metabolism has not been established but there is increasing evidence of the importance of these fluctuations in PTH in health and disease. Loss of the circadian rhythm is observed in primary hyperparathyroidism and the rhythm returns following parathyroid surgery [9,10]. A prolonged period of fasting will alter the nocturnal rise in PTH (1–84) and produce a flat rhythm with a pattern similar to that observed in this study in women with osteoporosis [13]. Studies in both animals and man have shown the importance of intermittent injections of PTH in increasing trabecular bone mass and the relatively poor or detrimental, effect of continuous infusions of PTH [25–28]. It would appear, therefore, that the dynamics of PTH secretion have an important role to play in both calcium metabolism and the bone remodelling process.

Our data indicate that the circadian rhythm for PTH (1–84) may change throughout life. Cosinor analysis of the data identifies a gradual shift in acrophase and amplitude when comparing premenopausal women with postmenopausal women without osteoporosis and with women with established osteoporosis. Our results are in keeping with a previous study that compared postmenopausal women using a 2-h blood sampling frequency over a 24-h period [14]. The clearer differences between subjects we have observed may be a reflection of the increased sampling frequency we adopted. From current data it is not possible to establish when during life the rhythm is flattened in patients with osteoporosis, and whether an alteration in the rhythm is caused by osteoporosis and fractures or is a causative factor in the development of osteoporosis.

Detailed study of the PTH (1–84) rhythms suggests that both the rise to a peak and the subsequent fall to a low level are equally important. There is evidence of circadian rhythms in urinary pyridinoline (PYR) and deoxypyridinoline (D-PYR) secretion and in serum osteocalcin [14,35]. The published data indicate that an increase in bone resorption at night is associated with an increased secretion of PYR and D-PYR. Bone formation assessed by a rise in osteocalcin is also predicted to occur during the night-time [14]. This would seem to associate a rise in PTH (1–84) with both bone resorption as estimated by PYR and D-PYR secretion and bone formation as assessed by osteocalcin. Recent work [26] has demonstrated that PTH infusion is associated with a significant decrease in the bone formation markers alkaline phosphatase, osteocalcin and the carboxy-terminal extension peptide of procollagen 1, whilst urinary calcium and hydroxyproline increased. By

contrast, following intermittent PTH injections, the circulating markers of bone formation increased rapidly, with modest increases of bone resorption markers at the end of 28 days of injections [26]. Evidence at a cellular level indicates that indirect stimulation of osteoclasts by PTH occurs, but factors controlling osteoclast stimulation are still a matter of some debate [26,37]. Mature osteoblasts show multiple responses to PTH but the major effects of increasing PTH in the short term in vitro are inhibitory [38].

The factor(s) that initiate and regulate the circadian rhythm of PTH (1–84) are as yet unknown. A strong correlation with the circadian rhythm of prolactin [7] suggested an element of neuroendocrine control and there is also some evidence of a temporal correlation of PTH with sleep cycles [39]. Nocturnal PTH concentrations rise so that they are temporally correlated with cycles of stage 3 and 4 sleep [39]. An acute sleep shift does not alter the circadian pattern of PTH (1–84) substantially but does blunt the initial rise from 0200 to 0300 hours [40], consistent with the postulate that the control is primarily circadian but may be modified by sleep. An obvious candidate for control of the rhythm is calcium, but in our study there was no consistent decrease in adjusted calcium and no decrease in ionized calcium (although we have studied ionized calcium in only 4 patients in each group) that correlated with the changes in PTH (1–84).

Previous studies have produced inconsistent relationships between ionized calcium and the circadian rhythm for PTH [14,41–43]. In particular the PTH circadian rhythm, measured by radioimmunoassay, persists in subjects infused with calcium to maintain a steady serum calcium level [44]. Phosphate is known to regulate PTH secretion [44,45] and both acute [45–47] and chronic [48] oral phosphate intake can increase circulating serum phosphate and PTH. Although in such circumstances transient decreases in ionized calcium may contribute to PTH release, phosphate can stimulate PTH secretion independent of the concentration of ionized calcium [49,50]. Our data show that the circadian rhythms for phosphate and PTH (1–84) are very similar in pattern and in the changes observed between the groups studied. The acrophase for serum phosphate precedes that for PTH (1–84). This may imply an important role for fluctuations in serum phosphate in the generation of the circadian rhythm of PTH (1–84). Blunting of the phosphate rhythm in postmenopausal women has been noted previously [14,43] and fasting results in a loss of PTH (1–84) and serum phosphate rhythm in the presence of a lower, flatter calcium profile [13]. Studies in teenage girls have also shown the importance of dietary phosphate in influencing the pattern of PTH (1–84) secretion, with high-phosphate diets resulting in loss of the circadian pattern and the generation of a high mean PTH (1–84) throughout the day [48].

The results obtained in our study suggest that an important association exists between the circadian rhythm of PTH (1–84) and osteoporosis. Further detailed work is required to establish the exact nature of this relationship. These preliminary observations may help our understanding of this complex condition and may also suggest new and novel therapeutic options which could manipulate these rhythms to obtain beneficial effects on bone metabolism.

Acknowledgements. This work was supported by Scottish Home and Health Department Award K/MRS/50/C1264.

References

1. Nussbaum SR, Zahradrick RJ, Lavigne JR, et al. Highly sensitive two-site immunoradiometric assay of parathyrin and its clinical utility in evaluating patients with hypercalcaemia. Clin Chem 1987;33:1364–7.
2. Brown RC, Aston JP, Weeks I, Woodhead JS. Circulating intact parathyroid hormone measured by a two-site immunochemiluminometric assay. J Clin Endocrinol Metab 1987;65:407–14.
3. Logue FC, Perry B, Chapman RS, Milne I, James K, Beastall GH. A two-site immunoradiometric assay for PTH (1–84) using N and C terminal specific monoclonal antibodies. Ann Clin Biochem 1991;28:160–6.
4. Logue FC, Fraser WD, O'Reilly DStJ, Beastall GH. The circadian rhythm of intact parathyroid hormone and nephrogenous cyclic adenosine monophosphate in normal men. J Endocrinol 1989;121:R1–3.
5. Calvo MS, Eastell R, Offord KP, Bergstralh EJ, Burritt MF. Circadian variation in ionized calcium and intact parathyroid hormone: evidence for sex differences in calcium homeostasis. J Clin Endocrinol Metab 1991;72:69–76.
6. Harms HM, Kaptaina V, Kulpmann WR, Brabant G, Hesch RD. Pulse amplitude and frequency modulation of parathyroid hormone in plasma. J Clin Endocrinol Metab 1989;69:843–51.
7. Logue FC, Fraser WD, O'Reilly DStJ, Cameron DA, Kelly AJ, Beastall GH. The circadian rhythm of intact parathyroid hormone (1–84): temporal correlation with prolactin secretion in normal men. J Clin Endocrinol Metab 1991;71:1556–60.
8. Nielsen HK, Laurberg P, Brixen K, Mosekilde L. Relations between diurnal variations in serum osteocalcin, cortisol, parathyroid hormone and ionised calcium in normal individuals. Acta Endocrinol 1991;124:391–8.
9. Logue FC, Fraser WD, Gallacher SJ, et al. The loss of circadian rhythm for intact parathyroid hormone and nephrogenous cyclic AMP in patients with primary hyperparathyroidism. Clin Endocrinol (Oxf) 1990;32:475–83.
10. Lobaugh B, Neelon FA, Oyama H, et al. Circadian rhythms for calcium, inorganic phosphorus and parathyroid hormone in primary hyperparathyroidism: functional and practical considerations. Surgery 1989;106:1009–17.
11. Gallacher SJ, Fraser WD, Logue FC, et al. The loss of diurnal rhythm of PTH secretion in untreated psuedohypoparathyroidism. Bone 1991;12:297.
12. Calvo MS, Kumar R, Heath H III. 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.
13. Fraser WD, Logue FC, Christie JP, et al. Alteration of the circadian rhythm of intact parathyroid hormone following a 96-hour fast. Clin Endocrinol 1994;40:523–8.
14. Eastall R, Calvo MS, Burritt MF, Offord KP, Russell GG, Riggs BL. Abnormalities in circadian patterns of bone resorption and renal calcium conservation in type I osteoporosis. J Clin Endocrinol Metab 1992;74:487–94.
15. Gallagher J, Riggs B, Jerpbak C, Arnaud C. The effect of age on serum immunoreactive parathyroid hormone in normal and osteoporotic women. J Lab Clin Med 1980;95:373–85.
16. Teitelbaum S, Rosenberg E, Richardson C, Avioli L. Histologic studies of bone from normocalcemic postmenopausal women with increased circulating parathyroid hormone levels. J Clin Endocrinol Metab 1976;42:537–43.

17. Civitelli R, Agnusdei D, Nardi P, et al. Effect of one year's treatment with estrogens on bone mass, intestinal calcium absorption and 25-hydroxyvitamin D 1α-hydroxylase reserve in postmenopausal osteoporosis. Calcif Tissue Int 1988;42:76–86.
18. Bouillon R, Geusens P, Dequeker J, DeMoor P. Parathyroid function in primary osteoporosis. Clin Sci 1979;578:167–71.
19. Riggs B, Ryan R, Wahner H, et al. Serum concentration of estrogen, testosterone and gonadotropins in osteoporotic and nonosteoporotic postmenopausal women. J Clin Endocrinol Metab 1973;36:1097–9
20. Gallagher J, Riggs B, Eisman J, Hamstra A, Arnaud S, DeLuca H. Intestinal calcium absorption and serum vitamin D metabolites in normal subjects and osteoporotic patients. J Clin Invest 1979;66:729.
21. Sorenson O, Lumholtz B, Lund B, et al. Acute effects of parathyroid hormone on vitamin D metabolism in patients with the bone loss of aging. J Clin Endocrinol Metab 1981;54:1258–61.
22. Kotowicz M, Klee G, Kao P, et al. Relationship between serum intact PTH and bone remodelling in type I osteoporosis: evidence that skeletal sensitivity is increased. Osteoporos Int 1990;1:14–20.
23. Cosman F, Shen V, Herrington B, Lindsay R. Responses of the parathyroid gland to infusion of human parathyroid hormone (1–34). J Clin Endocrinol Metab 1991;73:1345–51.
24. Ebeling P, Jones J, Burritt M, et al. Skeletal responsiveness to endogenous parathyroid hormone in postmenopausal osteoporosis. J Clin Endocrinol Metab 1992;75:1033–8.
25. Reeve J, Davies UM, Hesp R, McNally E, Katz D. Treatment of osteoporosis with human parathyroid peptide and observations on effect of sodium fluoride. BMJ 1990;301:314–8.
26. Hodsman AB, Fraher LJ, Ostbye T, Adachi JD, Steer BM. An evaluation of several biochemical markers for bone formation and resorption in a protocol utilizing cyclical parathyroid hormone and calcitonin therapy for osteoporosis. J Clin Invest 1993;91:1138–48.
27. Podbesek R, Edonard L, Meunier P, et al. Effects of two treatment regimens with synthetic human parathyroid hormone fragment on bone formation and the tissue balance of trabecular bone in greyhounds. Endocrinology 1983;112:1000–6.
28. Malluche H, Sherman D, Meyer W, Ritz E, Norman A, Massry S. Effects of long-term infusion of physiological doses of 1–34 PTH on bone. Am J Physiol 1982;242:197–201.
29. Gallacher SJ, Fenner JAK, Anderson K, et al. Intravenous pamidronate in the treatment of corticosteroid dependent lung disease: an open pilot study. Thorax 1992;47:932–6.
30. Gardner MD, Dryburgh FJ, Fyffe JA, Jenkins AS. Predictive value of derived figures based on the measurement of ionised calcium. Ann Clin Biochem 1981;81:106–9.
31. Nelson W, Tong YL, Lee JK, Halberg F. Methods for cosinor rhythmometry. Chrononbiological 1979;6:305–23.
32. Riggs BL, Melton LJ III. Involutional osteoporosis. N Engl J Med 1986;314:1676–86.
33. McKenna MJ, Frame B. Hormonal influences on osteoporosis. Am J Med 1987;82(Suppl 1B):61–7.
34. Scherman SS, Hollis BW, Tobin JD. Vitamin D status and related parameters in a healthy population: the effects of age, sex and season. J Clin Endocrinol Metab 1990;71:405–13.
35. Eastall R, Simmons PS, Colwell A, et al. Nyctohemeral changes in bone turnover assessed by serum bone Gla-protein concentration and urinary deoxypyridinoline excretion: effects of growth and ageing. Clin Sci 1992;83:375–82.
36. Raisz LG. Bone resorption in tissue culture: factors influencing the response to parathyroid hormone. J Clin Invest 1965;44:103–16.
37. Ibbotson KJ, Roodman GD, McManus LM, Mundy GR. Identification and characterisation of osteoclast-like cells and their progenitors in cultures of feline marrow mononuclear cells. J Cell Biol 1984;99:471–80.
38. Dietrech HW, Canalis EM, Maina DM, Raisz LG. Hormonal control of bone collagen synthesis in vitro: effects of parathyroid hormone and calcitonin. Endocrinology 1976;98:943–9.
39. Kripke DF, Lavie P, Parker D, Huey L, Deftos LJ. Plasma parathyroid hormone and calcium are related to sleep cycles. J Clin Endocrinol Metab 1978;47:1021–7.
40. Logue FC, Fraser WD, O'Reilly DStJ, et al. Sleep shift dissociates the nocturnal peaks of parathyroid hormone (1–84), nephrogenous cyclic adenosine monophosphate and prolactin in normal men. J Clin Endocrinol Metab 1992;75:25–9.
41. Markowitz ME, Arnaud S, Rosen JF, Thorpy M, Laximinarajan S. Temporal interrelationships between circadian rhythms of serum parathyroid hormone and calcium concentrations. J Clin Endocrinol Metab 1988;67:1068–73.
42. Mallete LE, Kirkland JL. Fine regulation of serum calcium: acute midday decreases in calcium ion concentration trigger a parathyroid response. In: Cohn DV, Fujita T, Potts JT Jr, editors. Endocrine control of bone and calcium metabolism, vol 8A. New York: Elsevier, 1984:71–88.
43. Perry 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.
44. Jubiz W, Canterbury JM, Reiss JM, Tyler FM. Circadian rhythm in serum parathyroid hormone concentrations in human subjects: correlation with serum calcium, phosphate, albumin and growth hormone levels. J Clin Invest 1972;51:2040–6.
45. Reiss E, Canterbury JM, Bercovitz MA, Kaplan EL. The role of phosphate in the secretion of parathyroid hormone in man. J Clin Invest 1970;49:2146–9.
46. Silverberg SJ, Shane E, Clemens TL, et al. The effect of oral phosphate administration on major indices of skeletal metabolism in normal subjects. J Bone Miner Res 1986;1:383–8.
47. Karkkainen M, Lamberg-Allardt C. An acute intake of phosphate increases parathyroid hormone secretion and inhibits bone formation in young women. J Bone Miner Res 1996;11:1905–12.
48. Calvo MS, Kuman R, Heath H III. Persistently elevated parathyroid hormone secretion and action in young women after four weeks of ingesting high phosphorus, low calcium diets. J Clin Endocrinol Metab 1990;70:1334–40.
49. Fine A, Cox D, Fontaine B. Elevation of serum phosphate affects parathyroid hormone levels in only 50% of hemodialysis patients, which is unrelated to changes in serum calcium. J Am Soc Nephrol 1993;3:1947–53.
50. Marks KH, Kilav R, Naveh-Many T, Silver J. Calcium, phosphate, vitamin D and the parathyroid. Pediatr Nephrol 1996;10:364–7.

Received for publication 11 March 1997
Accepted in revised form 13 August 1997