J Bone Miner Metab (1999) 17:283–288

Journal of
Bone and
Mineral
Metabolism

Microstructure of the trabecula and cortex of iliac bone in primary hyperparathyroidism patients determined using histomorphometry and node–strut analysis

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Abstract: The purpose of this study was to use histomorphometry to compare the microstructure of trabecular and cortical bone in patients with primary hyperparathyroidism (PH) with that seen in osteoporosis. Histomorphometric and node–strut analyses of iliac bones were performed on 11 female patients with PH (61.3 ± 8.0 years old) and 61 age-matched female patients with involutional osteoporosis (OP) (63.6 ± 5.6 years old). Cancellous bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), and wall thickness (W.Th) were not significantly different in these two groups. The bone formation rate (BFR) tended to be higher in the PH group than in the OP group. The number of nodes (N.Nd/TV) and node-to-node strut length (Nd.Nd/TV) were significantly higher in the PH group than in the OP group. The number of termini (N.Tm/TV) and terminus-to-terminus strut length/total strut length (Tm.Tm/TSL) were significantly lower in the PH group; cortical porosity was significantly higher in the PH group than in the OP group. No correlation was found between age and N.Nd in the PH group, but there was a negative correlation between age and N.Nd in the OP group. Our results show that trabecular connectivity was maintained while cortical porosity deteriorated in patients with PH compared with OP. These results suggest that there are microstructural differences between PH and OP in cancellous and cortical bone that result from the bone remodeling sequence in humans.

Key words: primary hyperparathyroidism, trabecular connectivity, cortical porosity, histomorphometry

Introduction

Primary hyperparathyroidism (PH) is a disorder of the calcium endocrine system caused by increased and incompletely regulated secretion of parathyroid hormone (PTH) [1]. It predominantly affects women in the first decade after menopause [2]. Both PH and osteoporosis (OP) generally produce bone loss. However, the pattern of bone loss in patients with PH is generally different from that seen in osteoporosis. In PH, densitometric studies indicate that bone mineral density (BMD) is preserved at sites containing a high percentage of cancellous bone, whereas BMD is reduced in regions dominated by cortical bone [3–5]. Histomorphometric studies have also shown the relative preservation of cancellous bone in patients with PH [6,7].

It is generally accepted that bone strength depends on both bone mass and bone microarchitecture [8,9]. Therefore, microstructural analysis of bone is important in a discussion of bone strength and fracture risk. As for trabecular bone, some investigators have reported that trabecular connectivity is maintained in patients with PH [6–8,10–12]. Because bone is composed of both trabecular and cortical bone, the cortical bone structure is also important to determine bone strength [13]. In this regard, cortical porosity deteriorates in patients with PH [14–16]. However, there have been few simultaneous studies of trabecular and cortical microstructure in PH patients. The purpose of this study was to evaluate trabecular microstructure using node–strut analysis, which permits quantitative evaluation of two-dimensional trabecular connectivity, and simultaneously to evaluate cortical porosity in patients with mild primary hyperparathyroidism.

Patients and methods

The subjects consisted of women with PH and age-matched controls with involutional osteoporosis. The PH group included 11 women from 50 to 77 years in age (mean, 61.8 years). The majority of the patients

Offprint requests to: T. Uchiyama
Received: Nov. 20, 1998 / Accepted: March 12, 1999

presented with clinical and biochemical evidence of mild disease. None had nephrolithiasis, classical osteitis fibrosa, or bone pain. Three had one or more vertebral compression fractures, but none had any fractures at other sites. The mean serum calcium, phosphorus, and alkaline phosphatase levels of our patients were 11.3 mg/dl (normal, 8.7–10.0 mg/dl), 2.48 mg/dl (normal, 2.5–4.6), and 505 IU/l (normal, 80–180), respectively. The PTH levels were increased in all patients, but these were measured by different methods, which are not comparable (Table 1). The involutional osteoporosis group consisted of 61 women from 50 to 72 years in age (mean, 63.6 years), whose samples were biopsied in our department or at other hospitals and referred to our department for bone histomorphometry. The clinical diagnosis was made according to the diagnostic criterion proposed by the Silver Science Research Group of the Japanese Ministry of Health and Welfare [17].

The iliac bone biopsies were taken from the standard site, 2 cm behind and below the anterosuperior iliac spine, following double tetracycline labeling (oral tetracycline hydrochloride, 250 mg four times a day) on a schedule of 2 days on, 7 days off—2 days on, 7 days off. The bone specimens were fixed in ethanol, stained with Villanueva bone stain, and embedded in methylmethacrylate. Sections 5 μm thick were cut from the embedded bones with a Jung Model K microtome (Carl Zeiss, Heidelberg, Germany) [18].

The following histomorphometric variables were measured or calculated for an area of cancellous bone in each section: bone volume (BV/TV), trabecular thickness (Tb.Th), trabecular number (Tb.N), trabecular separation (Tb.Sp), wall thickness (W.Th), and bone formation rate-volume referent (BFR/BV). Tb.Th was obtained using the equation for the parallel plate model [19].

The trabecular microstructure was measured using node–strut analysis as described by Garrahan et al. [20]. Nodes (Nd) and Termini (Tm) were identified and the numbers of nodes (N.Nd) and termini (N.Tm) were counted per square millimeter of cancellous space (N.Nd/TV, N.Tm/TV). The strut length was measured and the node-to-node strut length (Nd.Nd) was expressed per square millimeter of cancellous space (Nd.Nd/TV); the terminus-to-terminus strut length was expressed relative to the total strut length (Tm.Tm/TSL). In cortical bone, cortical porosity was defined as the sum of the Haversian canal area and resorption area as a percentage of the total cortical area ((CtHCa + CtRsAr)/CtAr). Histomorphometric parameters, cortical porosity, and strut analysis parameters were measured in the bone specimens using a semiautomatic image analyzer system connected to a personal computer (System Supply, Nagano, Japan). All the parameters were designated using nomenclature approved by the American Society for Bone and Mineral Research [19].

Table 1. Biochemical data of 11 patients with primary hyperparathyroidism

Parameter	Mean ± SD	Range	Reference value
Calcium	11.39 ± 0.79	10.3–12.6	8.7–10.0 mg/dl
Phosphate	2.48 ± 0.62	1.7–4.0	2.5–4.6 mg/dl
Alkaline phosphatase	505.3 ± 530	134–1815	80–180 IU/l
Creatinine	0.81 ± 0.23	0.5–1.2	0.5–0.8 mg/dl

Statistical analysis

Differences between groups were assessed using two-tailed unpaired Student's *t*-tests; Welch's corrections were used when the variance between groups was significantly different. All the correlations were performed by linear regression analysis. P values less than 0.05 were considered significant.

Results

Table 2 shows all the histomorphometric values for the PH and OP groups. Histomorphometry (BV/TV, Tb.Th, Tb.N, Tb.Sp, and W.Th) was not significantly different in the PH and OP groups. The bone formation rate (volume referent) tended to be higher in the PH group than in the OP group, but the difference was not significant ($P = 0.14$) (Fig. 1).

For node–strut analysis, Nd-related variables such as N.Nd/TV and Nd.Nd/TV indicate trabecular connectivity. These were significantly higher in the PH group than in the OP group (N.Nd/TV, $P < 0.0001$; Nd.Nd/TV, $P < 0.01$). Tm-related variables such as N.Tm/TV and Tm.Tm/TSL indicate trabecular disconnectivity, and these were significantly lower in the PH group than in the OP group (N.Tm/TV, $P < 0.05$; Tm.Tm/TSL, $P < 0.05$) (Fig. 2). The cortical porosity was more than three times higher in the PH group than it was in the OP group ($P < 0.0005$) (Fig. 3).

In the OP group, there was a negative correlation between age and the Nd-related variables, while there was a positive correlation between age and the Tm-related variables. There was no correlation between the structural variables and age in the PH group (Table 3).

Discussion

Conventional histomorphometry is not always appropriate for evaluating trabecular microstructure.

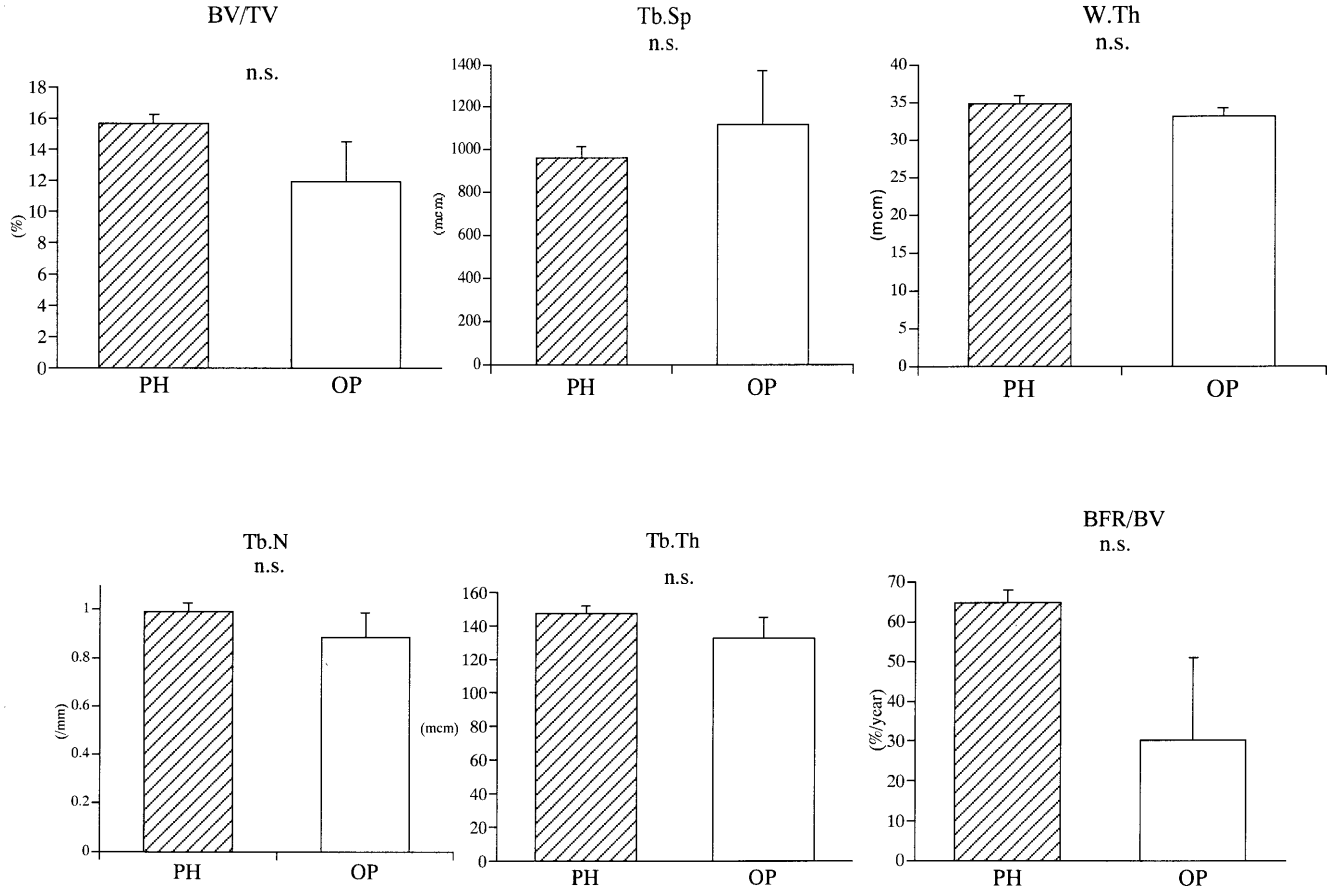

Fig. 1. Histomorphometric parameters in patients with PH (primary hyperparathyroidism; *shaded bars*) and OP (involutional osteoporosis; *open bars*) (mean ± SEM). For explanation of abbreviations, see Table 2

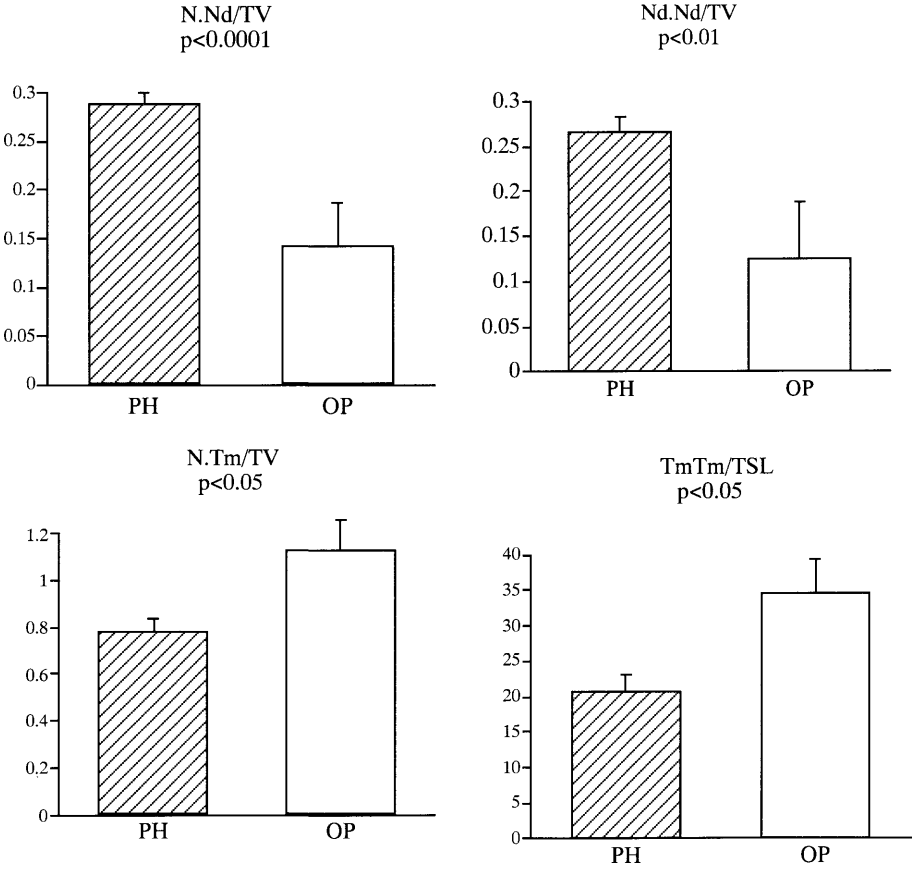

Fig. 2. Structural parameters were determined using node–strut analysis in patients with PH (primary hyperparathyroidism) and OP (involutional osteoporosis) (mean ± SEM)

Table 2. Histomorphometrical indices in patients with primary hyperparathyroidism (PH) and involutional osteoporosis (OP)

Index	PH (n = 11)	OP (n = 61)	P-value	Unit
Age	61.2 ± 8.0	63.6 ± 5.6	n.s.	
BV/TV	15.67 ± 8.6	11.86 ± 4.8	n.s.[a]	%
Tb.Th	147.6 ± 42.2	132.7 ± 33.3	n.s.	μm
W.Th	33.0 ± 8.4[b]	34.8 ± 3.8	n.s.	μm
Tb.N	0.884 ± 0.268	0.994 ± 0.338	n.s.[a]	/mm
Tb.Sp	1117 ± 431	962 ± 835	n.s.[a]	μm
N.Nd/TV	0.29 ± 0.15	0.14 ± 0.10	<0.0001	#/mm^2
Nd.Nd/TV	0.27 ± 0.21	0.13 ± 0.14	<0.01	μm
N.Tm/TV	0.78 ± 0.40	1.14 ± 0.42	<0.05	#/mm^2
Tm.Tm/TSL	0.16 ± 0.12	0.24 ± 0.12	<0.05	μm
Cortical porosity	18.4 ± 7.9	5.1 ± 3.0	<0.00005[a]	%

BV/TV, cancellous bone volume; Tb.Th, trabecular thickness; W.Th, wall thickness; Tb.N, trabecular number; Tb.Sp, trabecular separation; N.Nd/TV, number of nodes; Nd.Nd/TV, node-to-node strut length; N.Tm/TV, number of termini; Tm.Tm/TSL, terminus-to-terminus/total strut length
Values are expressed as mean ± SD
[a] Welch's correction was performed
[b] n = 10; wall thickness in 1 case of PH could not be measured because of lack of resting surface

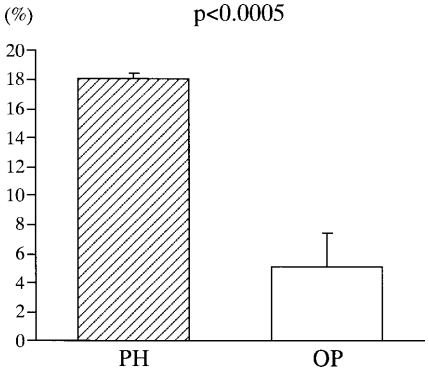

Fig. 3. Comparison of cortical porosity in patients with PH (primary hyperparathyroidism) and OP (involutional osteoporosis) (mean ± SEM)

Table 3. Correlation between age and structural parameters

	r value	P-value
PH:		
N.Nd/TV	−0.19	n.s.
Nd.Nd/TV	−0.052	n.s.
Tm.Tm/TSL	0.391	n.s.
OP:		
N.Nd/TV	−0.567	<0.0001
Nd.Nd/TV	−0.545	<0.0001
Tm.Tm/TSL	0.457	<0.001

We previously reported a typical example of two cases with almost identical conventional histomorphometric parameters, including BV/TV, Tb.Th, Tb.N, and Tb.Sp, but with two- to fourfold differences by node–strut analysis [21].

In this regard, new methods were developed to quantify trabecular microstructure. Node–strut analysis is one of the methods used to evaluate trabecular structure more directly. Mellish et al. [22] reported the usefulness of this method by showing a high correlation between ultimate compressive strength and the number of nodes (N.Nd).

Our node-strut analysis shows that trabecular connectivity was maintained in patients with PH compared with OP patients. This finding is consistent with previous reports [10,11]. Vogel et al. [12] also showed that trabecular microstructure was maintained in PH patients using node–strut analysis and another structural parameter, trabecular bone pattern factor (TBPf). The mechanism that maintains higher trabecular connectivity in PH is not well understood. Eriksen et al. [23] reported the decreased resorption depth of trabeculae in a case of PH by counting the eroded lamellae. This result may explain why trabecular microstructure is maintained against trabecular perforation in PH despite the increased activation frequency and erosion of a large proportion of the trabecular surface [10]. This mechanism appears to clearly contrast with the pattern of bone loss in patients with osteoporosis, which is caused by the loss of entire trabeculae resulting from trabecular perforation caused by osteoclastic bone resorption [24]. Parfitt et al. [25] advocated another mechanism in PH, in which

osteoclasts seem to change direction and erode the interior of the trabeculae longitudinally rather than penetrate their full thickness.

Christiansen et al. [15] suggested that the reduction of the appendicular bone mineral density in PH might be the result of three mechanisms: (1) increased cortical porosity, (2) reduced cortical width, and (3) an increased proportion of new, lightly mineralized bone. Although we did not investigate the latter two factors, our results show that the marked cortical porosity seen in PH compared with OP may cause bone fragility in predominantly cortical bone. This result is in accordance with previous studies [14–16]. Brockstedt et al. [16] argued that cortical porosity depends on the size of the remodeling space because of the enhanced activation frequency in PH.

The risk of fracture in mild PH is controversial. A low incidence of spinal fracture has been reported in osteoporotic patients with high PTH [26], which suggests that PTH has some beneficial effects at sites that contain cancellous bone predominantly. Combining previous reports [10–12,14–16] with our results, which show that PTH maintains the mass and microstructure of trabecular bone while enhancing cortical porosity, we hypothesize that in mild PH the incidence of trabecular fractures such as vertebral fractures should be expected to decrease, whereas that of cortical fractures should increase. Wilson et al. [27] reported that the risk of vertebral fractures does not increase in patients with mild primary hyperparathyroidism. Larsson et al. [28] reported an increase in the frequency of distal radius fractures in women with mild hypercalcemia caused by PH. These two reports support our hypothesis. On the other hand, Kochersberger et al. [29] reported a greater prevalence of vertebral fractures in patients operated on for PH. A prospective study on this is necessary before recommending surgical intervention in asymptomatic PH patients.

In conclusion, an analysis of biopsied iliac bone specimens showed that in mild hyperparathyroidism bone loss occurred mainly in cortical bone and was reflected by increased porosity, while trabecular connectivity was maintained. This finding is in contrast to osteoporosis, in which connectivity is presumed to be lost as a result of trabecular perforation.

Acknowledgment. We greatly appreciate Mr. Akazawa's work preparing the tissue specimens.

References

1. Lobaugh B, Drezner MK (1992) Evaluation of hypercalcemia and hypocalcemia. In: Kelley WN (ed) Textbook of internal medicine. Lippincott, Philadelphia, pp 2115–2121
2. Silverberg SJ, Bilezikian JP (1997) Primary hyperparathyroidism: still evolving? J Bone Miner Res 12:856–862
3. Silverberg SJ, Shane E, De La Cruz L, Dempster DW, Feldman F, Seldin D, Jacobs TP, Siris ES, Cafferty M, Parisien MV, Lindsay R, Clemens T, Bilezikian JP (1989) Skeletal disease in primary hyperparathyroidism. J Bone Miner Res 4:283–291
4. Guo CY, Thomas WE, al-Dehaimi AW, Assiri AM, Eastell R (1996) Longitudinal changes in bone mineral density and bone turnover in postmenopausal women with primary hyperparathyroidism. J Clin Endocrinol Metab 81:3487–3491
5. Christiansen P, Steiniche T, Brixen K, Hessov I, Melsen F, Chales P, Mosekilde L (1997) Primary hyperparathyroidism: biochemical markers and bone mineral density at multiple skeletal sites in Danish patients. Bone (NY) 21:93–99
6. Christiansen P, Steiniche T, Vesterby A, Mosekilde L, Hessov I, Melsen F (1992) Primary hyperparathyroidism: iliac crest trabecular bone volume, structure, remodeling, and balance evaluated by histomorphometric methods. Bone (NY) 13:41–49
7. Parisien M, Silverberg SJ, Shane E, De La Cruz L, Lindsay R, Bilezikian JP, Dempster DW (1990) The histomorphometry of bone in primary hyperparathyroidism: preservation of cancellous bone structure. J Clin Endocrinol Metab 70:930–938
8. Kleerekoper M, Villanueva AR, Stanciu J, Sudhaker Rao D, Parfitt AM (1985) The role of three-dimensional trabecular microarchitecture in the pathogenesis of vertebral compression fractures. Calcif Tissue Int 37:594–597
9. Mosekilde L (1993) Vertebral structure and strength in vivo and in vitro. Calcif Tissue Int 53:S121–S126
10. Parisien M, Mellish RWE, Silverberg SJ, Shane E, Lindsay R, Bilezikian JP, Dempster DW (1992) Maintenance of cancellous bone connectivity in primary hyperparathyroidism: trabecular strut analysis. J Bone Miner Res 7:913–919
11. Parisien M, Cosman F, Mellish RWE, Schnitzer M, Neives J, Silverberg SJ, Shane E, Kimmel D, Recker RR, Bilezikian JP, Lindsay R, Dempster DW (1995) Bone structure in postmenopausal hyperparathyroid, osteoporotic, and normal women. J Bone Miner Res 10:1393–1399
12. Vogel M, Hahn M, Delling G (1995) Trabecular structure in patients with primary hyperparathyroidism. Virchows Arch 426:127–134
13. Mazess RB (1990) Fracture risk: a role for compact bone. Calcif Tissue Int 47:191–193
14. van Doorn L, Lips P, Netelenbos JC, Hackeng WHL (1993) Bone histomorphometry and serum concentrations of intact parathyroid hormone (PTH (1–84)) in patients with primary hyperparathyroidism. Bone Miner 23:233–242
15. Christiansen P, Steiniche T, Brockstedt H, Mosekilde L, Hessov I, Melsen F (1993) Primary hyperparathyroidism: iliac crest cortical thickness, structure, and remodeling evaluated by histomorphometric methods. Bone (NY) 14:755–762
16. Brockstedt H, Christiansen P, Mosekilde L, Melsen F (1995) Reconstruction of cortical bone remodeling in untreated primary hyperparathyroidism and following surgery. Bone (NY) 16:109–117
17. Orimo H (1989) General research reports concerning the prevention and the treatment of osteoporosis. Research Report of Silver Research of the Ministry of Health and Welfare, Tokyo, p 81
18. Konno T, Takahashi H (1997) Preparation of undecalcified bone sections. In: Takahashi H (ed) Handbook of bone morphometry, 2nd edn. Nishimura, Niigata, pp 11–16
19. Parfitt AM, Drezner MK, Clorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR (1987) Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR histomorphometry nomenclature committee. J Bone Miner Res 2:595–610

20. Garrahan NJ, Mellish RWE, Compston JE (1986) A new method for the two-dimensional analysis of bone structure in human iliac crest biopsies. J Microsc (Oxf) 142:341–349
21. Uchiyama Y, Tanizawa T, Takahashi E (1994) Evaluation of trabecular structure in bone histomorphometry (in Japanese). J Jpn Soc Bone Morphom 4:83–89
22. Mellish RWE, Ferguson-Pell MW, Cochran GVB, Lindsay R, Dempster DW (1991) A new manual method for assessing two-dimensional cancellous bone structure: comparison between iliac crest and lumbar vertebra. J Bone Miner Res 6:689–696
23. Eriksen EF, Mosekilde L, Melsen F (1986) Trabecular bone remodeling and balance in primary hyperparathyroidism. Bone (NY) 7:213–221
24. Parfitt AM, Matthews CHE, Villanueva AR, Kleerekoper M, Frame B, Rao DS (1983) Relationship between surface, volume, and thickness of iliac trabecular bone in aging and in osteoporosis. J Clin Invest 72:1396–1409
25. Parfitt AM (1988) Bone remodeling: relationship to the amount and structure. In: Riggs BL, Melton LJ III (eds) Osteoporosis. Etiology, diagnosis and management, 1st edn. Raven Press, New York, pp 45–93
26. Saphier PW, Stamp TCB, Kelsey CR, Loveridge N (1987) PTH bioactivity in osteoporosis. Bone Miner 3:75–83
27. Wilson RJ, Rao S, Ellis B, Kleerekoper M, Parfitt AM (1988) Mild asymptomatic primary hyperparathyroidism is not a risk factor for vertebral fractures. Ann Intern Med 109:959–962
28. Larsson K, Lindh E, Lind L, Persson I, Ljunghall S (1989) Increased fracture risk in hypercalcemia. Acta Orthop Scand 60:268–270
29. Kochersberger G, Buckley NJ, Leigh GS, Martinez S, Studenski S, Bogler J, Lyles KW (1987) What is the clinical significance of bone loss in primary hyperparathyroidism? Arch Intern Med 147:1951–1953