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Bone Histomorphometry -- The Renaissance?

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Bone histomorphometry enables the quantitative assessment of bone turnover and remodeling in histological sections of bone. It provides unique information about mechanisms of bone loss and gain in untreated and treated disease and about the safety of therapeutic interventions. Yet, bone histomorphometry remains poorly understood by a large part of the bone research community, and its value has not been fully exploited. The reasons for this lie in the widespread perception that histomorphometry is inaccessible to nonspecialists in the subject (a belief fostered by complicated and sometimes obscure terminology) and that bone biopsy is an invasive procedure, which in today's climate, is hard to justify for research purposes. In addition, the advent of techniques for measurement of bone mineral density (BMD) and of urine- and serum-based biochemical markers of bone turnover led many to believe mistakenly that bone histomorphometry had become redundant.

Although bone densitometry is a valuable tool for the diagnosis and risk assessment of osteoporosis, it provides no information about cellular activities in bone, but merely reflects the end result (at the time of measurement) of a lifetime's modeling and remodeling activities. Furthermore, bone densitometry does not distinguish between defective mineralization of bone (osteomalacia) and reduced mineralized bone mass (osteoporosis). Bone turnover markers reflect whole body bone turnover but do not provide any information about remodeling balance; the use by some of the term "uncoupling index" in the context of bone turnover markers to describe the balance between resorption and formation exemplifies the misconceptions that abound in this field. In addition, bone turnover markers provide a combined estimate of bone turnover in cortical and cancellous bone and are thus of limited use in dissecting out differential effects on either type of bone.

In contrast to bone densitometry, bone histomorphometry provides detailed information about both bone turnover and remodeling balance. Although bone histomorphometry has historically been applied mainly to cancellous bone, increasingly its use is being extended to include cortical bone. Its basic principles are simple and revolve around bone turnover (measured by tetracycline labelling), remodeling balance (assessed by wall width and resorption cavity depth), and mineralization (measured by osteoid seam width and mineralization lag time). A number of structural indices that reflect connectivity and trabecular shape can also be assessed in cancellous bone, using either two- or three-dimensional approaches. In cortical bone, changes in cortical porosity andwidth can be determined and the relative contributions of changes at the endosteal and periosteal borders to the latter ascertained.

Of course, bone histomorphometry has its limitations. In particular, measurement and sampling variance are high. There may be considerable heterogeneity in bone remodeling and turnover between skeletal sites, both in health and disease, so that changes in the iliac crest are not necessarily representative of other parts of the skeleton (*e.g.*, the spine) (1). Many of the measurements made in bone histomorphometry have a subjective

element, and this, together with differences in staining techniques, magnification, and measurement methods, contributes to significant inter- and intraobserver measurement variability (2-4). Even within a single biopsy, there may be considerable variation in remodeling and turnover, depending on the site chosen for sampling. Finally, current histomorphometric techniques are seriously limited by the lack of reliable markers for activation and active resorption (5). Dynamic indices related to these processes are calculated from bone formation rates, based on the assumptions that bone resorption and formation are coupled in time and space and that bone remodeling is in a steady state; these assumptions may not be tenable in disease states. Finally, bone biopsy cannot be repeated at frequent intervals, not only because of ethical considerations, but also because of the remodeling acceleratory phenomenon, which effectively limits the number of biopsies to two (one from each iliac crest). When planning prospective bone histomorphometric studies, therefore, a decision has to be made as to whether short-term or steady-state changes are of prime interest.

There are many examples where bone histomorphometry has enabled important advances in our understanding of mechanisms of disease and the changes induced by treatment. Preclinical studies have clearly documented the cellular changes and corresponding increase in bone turnover induced by estrogen deficiency and the subsequent reduction following estrogen repletion; these effects have also been shown in humans (6-8), together with the rapid microarchitectural changes that occur following estrogen withdrawal (9). Reductions in bone turnover with antiresorptive agents have been demonstrated in postmenopausal women with osteoporosis, varying from 30% to 50% with raloxifene and cyclic etidronate to 90% or more with the more potent bisphosphonates (6,7,10-15). This quantitative information may be of particular relevance to the theoretical concerns about prolonged suppression of bone turnover (although it should be noted that the degree of suppression, as assessed by histomorphometry, is generally greater than that indicated by biochemical markers of bone turnover). The role of increased bone turnover in the rapid early bone loss associated with glucocorticoid therapy and solid organ transplantation, which was not reliably predicted by biochemical markers of bone turnover, has been convincingly documented in histomorphometric studies (16,17).

Recently, the use of bone histomorphometry has led to major advances in our understanding of how anabolic skeletal effects can be achieved. The ingenious study of Hodsman *et al*. (18) demonstrated the early stimulation of *de novo* bone formation on cancellous bone surfaces in postmenopausal women treated with parathyroid hormone peptide, and evidence that this also occurs on cortical endosteal and periosteal surfaces has more recently been reported (19,20). These studies also demonstrated the importance of the combination of increased activation frequency and a positive remodeling balance (resulting from increased bone formation at the basic multicellular unit level) in achieving the anabolic effect of intermittent administration of parathyroid hormone.

Current European and North American regulatory requirements for bone histomorphometric studies have ensured not only that bone safety is addressed, but also that mechanisms of action of pharmacological interventions can be explored; furthermore, observations in placebo-treated individuals yield valuable information about the evolution of osteoporosis in postmenopausal women. Although biochemical markers and bone densitometry provide information about bone turnover and BMD, they cannot reveal mineralization defects or the presence of woven or lamellar bone, nor can they unravel in detail the mechanisms by which bone loss or gain occur. Finally, the application of three-dimensional measurements of microarchitecture (21) and qualitative aspects of bone mineral and matrix (22-24) to bone biopsy specimens is generating important new information about the effects of disease and its treatment that

cannot be obtained by other approaches. The unique and increasing value of bone histomorphometry as a clinical research tool merits wider recognition; it may be more difficult than measuring biochemical markers or BMD, but the extra effort is well justified.

References

1. Eventov I, Frisch B, Cohen Z, Hammel I. Osteopenia, hematopoiesis, and bone remodelling in iliac crest and femoral biopsies: a prospective study of 102 cases of femoral neck fractures. *Bone.* 1991;12(1):1-6.
2. de Vernejoul MC, Kuntz D, Miravet L, Goutallier D, Ryckewaert A. Bone histomorphometric reproducibility in normal patients. *Calcif Tissue Int.* 1981;33(4):369-74.
3. Chavassieux PM, Arlot ME, Meunier PJ. Intermethod variation in bone histomorphometry: comparison between manual and computerized methods applied to iliac bone biopsies. *Bone.* 1985;6(4):221-9.
4. Wright CD, Vedi S, Garrahan NJ, Stanton M, Duffy SW, Compston JE. Combined inter-observer and inter-method variation in bone histomorphometry. *Bone.* 1992;13(3):205-8.
5. Compston JE, Croucher PI. Histomorphometric assessment of trabecular bone remodelling in osteoporosis. *Bone Miner.* 1991 Aug;14(2):91-102.
6. Steiniche T, Hasling C, Charles P, Eriksen EF, Mosekilde L, Melsen F. A randomized study on the effects of estrogen/gestagen or high dose oral calcium on trabecular bone remodeling in postmenopausal osteoporosis. *Bone.* 1989;10(5):313-20.
7. Vedi S, Compston JE. The effects of long-term hormone replacement therapy on bone remodeling in postmenopausal women. *Bone.* 1996 Nov;19(5):535-9.
8. Eriksen EF, Langdahl B, Vesterby A, Rungby J, Kassem M. Hormone replacement therapy prevents osteoclastic hyperactivity: A histomorphometric study in early postmenopausal women. *J Bone Miner Res.* 1999 Jul;14(7):1217-21.
9. Compston JE, Yamaguchi K, Croucher PI, Garrahan NJ, Lindsay PC, Shaw RW. The effects of gonadotrophin-releasing hormone agonists on iliac crest cancellous bone structure in women with endometriosis. *Bone.* 1995 Feb;16(2):261-7.
10. Storm T, Steiniche T, Thamsborg G, Melsen F. Changes in bone histomorphometry after long-term treatment with intermittent, cyclic etidronate for postmenopausal osteoporosis. *J Bone Miner Res.* 1993 Feb;8(2):199-208.
11. Ott SM, Woodson GC, Huffer WE, Miller PD, Watts NB. Bone histomorphometric changes after cyclic therapy with phosphate and etidronate disodium in women with postmenopausal osteoporosis. *J Clin Endocrinol Metab.* 1994 Apr;78(4):968-72.
12. Chavassieux PM, Arlot ME, Roux JP, Portero N, Daifotis A, Yates AJ, Hamdy NA, Malice MP, Freedholm D, Meunier PJ. Effects of alendronate on bone quality and remodeling in glucocorticoid-induced osteoporosis: a histomorphometric analysis of transiliac biopsies. *J Bone Miner Res.* 2000 Apr;15(4):754-62.
13. Prestwood KM, Gunness M, Muchmore DB, Lu Y, Wong M, Raisz LG. A comparison of the effects of raloxifene and estrogen on bone in postmenopausal women. *J Clin Endocrinol Metab.* 2000 Jun;85(6):2197-202.
14. Reid IR, Brown JP, Burckhardt P, Horowitz Z, Richardson P, Trechsel U, Widmer A, Devogelaer JP, Kaufman JM, Jaeger P, Body JJ, Brandi ML, Broell J, Di Micco R, Genazzani AR, Felsenberg D, Happ

J, Hooper MJ, Ittner J, Leb G, Mallmin H, Murray T, Ortolani S, Rubinacci A, Saaf M, Samsioe G, Verbruggen L, Meunier PJ. Intravenous zoledronic acid in postmenopausal women with low bone mineral density. *N Engl J Med.* 2002 Feb 28;346(9):653-61.

15. Eriksen EF, Melsen F, Sod E, Barton I, Chines A. Effects of long-term risedronate on bone quality and bone turnover in women with postmenopausal osteoporosis. *Bone.* 2002 Nov;31(5):620-5.
16. Vedi S, Greer S, Skingle SJ, Garrahan NJ, Ninkovic M, Alexander GA, Compston JE. Mechanism of bone loss after liver transplantation: A histomorphometric analysis. *J Bone Miner Res.* 1999 Feb;14(2):281-7.
17. Dalle Carbonare L, Arlot ME, Chavassieux PM, Roux JP, Portero NR, Meunier PJ. Comparison of trabecular bone microarchitecture and remodeling in glucocorticoid-induced and postmenopausal osteoporosis. *J Bone Miner Res.* 2001 Jan;16(1):97-103.
18. 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 Mar;91(3):1138-48.
19. Dempster DW, Cosman F, Kurland ES, Zhou H, Nieves J, Woelfert L, Shane E, Plavetic K, Muller R, Bilezikian J, Lindsay R. Effects of daily treatment with parathyroid hormone on bone microarchitecture and turnover in patients with osteoporosis: a paired biopsy study. *J Bone Miner Res.* 2001 Oct;16(10):1846-53.
20. Dempster DW, Zhou H, Cosman F, Nieves J, Adachi JD, Fraher LJ, Watson PH, Linksay R. Hodsman AB. PTH treatment directly stimulates bone formation in cancellous and cortical bone in humans. *J Bone Miner Res.* 2001;16:S179.
21. Jiang Y, Zhao J, Genant HK. Macro and micro imaging of bone architecture. In: *Principles of Bone Biology.* 2nd edition ed. Bilizikian JP, Raisz LG, Rodan GA. San Diego: Academic Press; 2002 pp1599-1623.
22. Roschger P, Fratzl P, Eschberger J, Klaushofer K. Validation of quantitative backscattered electron imaging for the measurement of mineral density distribution in human bone biopsies. *Bone.* 1998 Oct;23(4):319-26.
23. Boivin GY, Chavassieux PM, Santora AC, Yates J, Meunier PJ. Alendronate increases bone strength by increasing the mean degree of mineralization of bone tissue in osteoporotic women. *Bone.* 2000 Nov;27(5):687-94.
24. Paschalis EP, Recker R, DiCarlo E, Doty SB, Atti E, Boskey AL. Distribution of collagen cross-links in normal human trabecular bone. *J Bone Miner Res.* 2003 Nov;18(11):1942-6.