COMMENTARY

JBMR®

Cortical Bone: A Challenging Geography

Roger Zebaze, and Ego Seeman

Departments of Endocrinology and Medicine, Austin Health, University of Melbourne, Melbourne, Australia

> My object is not to establish that I was right but to find out if I am. In fact we shall question everything all over again and we shall go forward not in seven-league boots but at a snail's pace. And whatever we wish to find we shall regard, once found, with particular mistrust.
>
> —Excerpt from Scene 9, Life of Galileo, Bertolt Brecht

Trabecular bone loss and fractures of the trabecular-rich vertebral bodies are the hallmarks of osteoporosis and have dominated thinking and research into the structural basis of bone fragility during the past 70 years.[1,2] Indeed, a reduction in vertebral fractures still forms the primary endpoint in almost all randomized trials. This trabeculo-centric view of bone fragility has diverted attention away from cortical bone despite evidence that: (1) ~80% of all fractures are nonvertebral; (2) these fractures occur at predominantly cortical sites; (3) ~70% of all age-related appendicular bone loss is cortical; and (4) most of this bone loss occurs by intracortical remodeling that cavitates the cortex producing porosity.[3,4] As age advances, intracortical void volume, which is formed mainly by the enlargement and coalescence of these canals, doubles, and so reciprocally halves the mineralized bone matrix volume—changes that compromise bone strength.

There are two challenges in the study of the effects of advancing age, menopause, disease, and therapy on cortical morphology. The first is to acquire high-resolution images of bone's material composition, microstructure, and macrostructure. The second is to then accurately, reproducibly, and unambiguously quantify these determinants of bone strength.

In this issue of the *Journal of Bone and Mineral Research*, three articles are published that used state-of-the-art methods of image acquisition and analysis to quantify the effects of therapy on cortical bone morphology.[5–7] Examples from these articles and several others are given to identify challenges that result from low image resolution and limitations in image processing and analyses. We first summarize the observations reported and then discuss the difficulties in making inferences concerning the effects of therapy that result from limitations in image resolution and analysis.

The Articles and the Challenges

Engelke and colleagues[5] report postmenopausal women randomized to odanacatib or placebo for 2 years who had proximal femur morphology measured using quantitative computed tomography (QCT) and analyzed using Medical Image Analysis Framework (MIAF). The authors report the following: (1) there were no increases in total bone cross sectional area (CSA) despite evidence in subhuman primates suggesting this treatment increases periosteal apposition[8]; (2) cortical thickness (and volume) increased by 1% to 2%, despite there being no evidence that antiresorptives deposit bone upon the endocortical surface; (3) trabecular and cortical volumetric BMD (vBMD) increased and about one-half of the increase in bone mineral content was cortical even though total CSA did not increase and medullary CSA did not decrease; and (4) cortical vBMD increased despite minimal or no change in periosteal or endocortical dimensions and there was no evidence for a reduction in porosity.

Tsai and colleagues[6] compared the effects of combined teriparatide and denosumab, and each treatment alone, on the microarchitecture of the distal tibia and distal radius measured using high-resolution peripheral QCT (HRpQCT) in postmenopausal women over 12 months. The authors report the following: (1) trabecular vBMD increased in the combined group with no change observed in the teriparatide group despite histomorphometric evidence of increases in trabecular bone volume[9,10]; (2) cortical vBMD increased in the combined group and decreased in the teriparatide group, despite evidence of antifracture efficacy of teriparatide; (3) cortical tissue mineral density increased in the combined group, decreased in the teriparatide group, and remained unchanged in the denosumab group despite evidence that antiresorptives increase tissue mineral density; (4) cortical thickness increased in the combined and denosumab group but not in the teriparatide group, despite histomorphometric evidence of periosteal and endocortical apposition with teriparatide[9,10]; (5) cortical thickness increased in the denosumab group even though antiresorptives do not have anabolic activity; (6) cortical porosity was unchanged in the combined group, but increased in the teriparatide group despite proven antifracture efficacy of teriparatide; and (7) porosity did

Address correspondence to: Ego Seeman, MD, Dept Endocrinology, Centaur Wing, Repatriation Campus, Waterdale Rd. Heidelberg 3081, Melbourne, Australia. E-mail: egos@unimelb.edu.au

This is a Commentary on Engelke et al. (J Bone Miner Res. 2015;30:30–38. DOI: 10.1002/jbmr.2292), Tsai et al. (J Bone Miner Res. 2015;30:39–45. DOI: 10.1002/jbmr.2315), and Poole et al. (J Bone Miner Res. 2015;30:46–54. DOI: 10.1002/jbmr.2325).

Journal of Bone and Mineral Research, Vol. 30, No. 1, January 2015, pp 24–29
DOI: 10.1002/jbmr.2419

not decrease in the denosumab group despite evidence to the contrary in subhuman primates and human subjects.[11,12]

Poole and colleagues[7] used 3D mapping of the proximal femoral cortex in postmenopausal women treated with denosumab or placebo. Cortical 3D thickness and mass surface density maps of each hip were created from CT scans annually for 3 years. Denosumab increased femoral cortical mass surface density and of the benefit achieved relative to controls, one-third was due to increasing density whereas two-thirds was due to increasing cortical thickness. These observations were reported despite lack of evidence that antiresorptives deposit bone upon the periosteal, trabecular, endocortical, or intracortical surfaces.

Using Photon Attenuation to Quantify the Heterogeneity of Cortical Morphology

Although the eye and brain seem to effortlessly distinguish an object from its surroundings, and distinguish the topology of facial features from each other, achieving this accuracy is a formidable task when differing photon attenuations of the mineralized matrix and void volumes of an image are acquired by radiation transmission (the eye) and processed by image analysis techniques (the brain).

The morphology of the cortex could be accurately quantified using the limited resolution of images acquired by current in vivo imaging devices if long bones were like drinking straws with distinctly definable and constant external and internal contours and a fixed cortical thickness three to four times the resolution of the image acquired using in vivo CT methods.[13,14]

However, long bones are heterogeneously designed structures. Cortical topology varies around the perimeter of any cross-section along the length of a tubular bone.[15,16] Differences in periosteal and endocortical diameters at each degree around a cross-section confer differences in the size and shape of the external cross-section and the medullary canal, and cortical thicknesses around the perimeter of each cross-section along the shaft—there is no single "thickness." The distribution of these thicknesses is not Gaussian; the median is a better predictor of cortical area and compressive strength than the commonly used mean.[17]

Similarly, the size, shape, and number of "pores" within a cross-section, also varies from cross-section to cross-section. Porosity could be readily quantified if the images of the pores acquired using HRpQCT in vivo (voxel size 82 μm) were at least 130 μm in diameter, or greater than 700 μm in images of the hip acquired using a CT scanner (voxel size ~500 μm).[14,15]

Quantifying this morphology presents several challenges. The first hurdle is to identify and define edges—the periosteal edge, pore edges, the endocortical edge, trabecular edges—and then to quantify the mineralized matrix they envelop. Images generated by radiation transmission form pixelated images (voxels in three dimensions).[18] The net attenuation of photon-produced by voxels is determined by the differing proportions of matrix volume and void volume (which may be a whole or part of a canal cross section or resorption cavity) within the voxel and the degree of completeness of the matrix mineralization.

The Periosteal Edge

Current image analysis algorithms identify the periosteal boundaries in cross-sectional studies with reasonable accuracy despite the low resolution of in vivo images. However, several technical difficulties still need to be addressed. Engelke and colleagues[5] report an increase in integral (total) hip volume by ~0.5% in controls and odanacatib-treated subjects after 24 months of therapy. As this was no greater in the treated group, the authors infer that this is not evidence that odanacatib produces periosteal apposition in humans, as reported in nonhuman primate studies.[8] They suggest the increase may be due to age-related periosteal apposition in both groups. However, periosteal apposition is 5 to 35 μm/year.[19] Given the limited resolution of hip images acquired in vivo, this change is unlikely to produce a detectable increase in external bone volume in 2 years. An alternative explanation may be an error produced by segmenting the periosteal boundary when the mean tissue mineralization of the subperiosteal matrix increases and so allows delineation of the edge in both groups, but more so in the odanacatib group.

A different challenge arises when images are analyzed using the HRpQCT software reported by Tsai and colleagues.[6] Whether periosteal apposition occurs with teriparatide cannot be established because the 2D region-matching software relies on the identification of the same total cross-sectional area. If periosteal apposition did occur, the algorithm would locate a different region to that used at baseline so that differences in cortical and trabecular morphology could not necessarily be ascribed to the therapy. Poole and colleagues[7] did not present changes in total cross-sectional area or hip volume. They report an increase in cortical thickness with denosumab, implying that periosteal or endocortical apposition occurred, but antiresorptives are not anabolic. Three alternative explanations include: (1) an increase in tissue mineralization density, (2) partial infilling of pores of cortex adjacent to the medullary canal, and (3) bone modeling that becomes detectable when remodeling is suppressed (see below).

The Endocortical Edge

Accurate identification of the endocortical surface of the cortical/medullary canal interface presents even more formidable challenges because the transition from cortical to trabecular bone is more gradual than the change from surrounding soft tissue to mineralized matrix at the periosteal edge. There is no single voxel that identifies the end of the cortex and the beginning of the medullary canal with its trabecular content. It is not possible to accurately identify an edge that corresponds to the endocortical surface when it is disrupted by endocortical resorption and intracortical remodeling which "trabecularizes" the inner cortex. This is why we and others suggest a transitional zone be recognized as a separate cortical compartment when bone morphology is quantified.[4,20] There are several reasons for this approach.

Quantifying the transitional zone avoids erroneously apportioning the fragmented cortex and the pores that fragmented it into the medullary (trabecular) compartment (Fig. 1). Failure to retain the fragments and porosity as part of the transitional zone underestimates the age-related and menopause-related increase in cortical porosity because porosity is erroneously "seen" as part of the medullary void volume. The age-related and menopause-related decline in trabecular number and thickness is also underestimated because cortical fragments in the transitional zone are "seen" as part of the medullary canal, which falsely elevates trabecular density in old age and so blunts

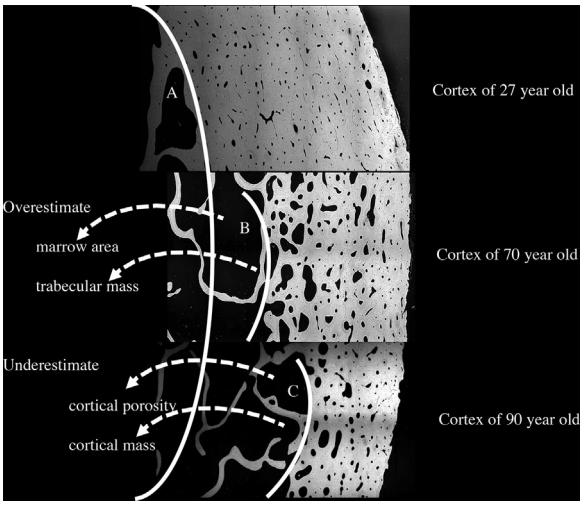

Fig. 1. Intracortical and endocortical remodeling erode the cortex. The endocortical surface (white line A of a specimen from a 27-year-old) denotes the true medullary cavity/cortical interface achieved at completion of growth. If the surface of the thinned but still compact-appearing cortex (white line B in a 70-year-old or C in a 90-year-old) is erroneously described as the endocortical surface, several errors occur by incorrectly apportioning in the cortical fragments and porosity that created them to the seemingly expanded medullary canal.

the diminution in trabecular bone across age and after menopause. Both of these errors underestimate fracture risk; therefore, persons in need of treatment will not be treated. The age-related and menopause-related loss of cortical mineralized bone matrix volume is overestimated because cortical fragments erroneously allocated to the "medullary" canal are not quantified as being part of the cortical bone in older persons. The blunting of the decline in trabecular bone and seemingly accelerated decline in cortical bone suggest that estrogen deficiency causes cortical, not trabecular bone loss.[21]

Cortical fragmentation produced by increased intracortical remodeling in primary hyperparathyroidism suggests this disease does not cause trabecular bone loss.[22] This view is erroneous because the cortical fragments measured as part of the medullary compartment obscure trabecular resorption and trabecular bone loss produced by parathyroid hormone excess. When the cortical fragments are correctly allocated to the transitional zone the reduction in trabecular density is observed.[23]

Similar errors may occur in randomized trials of an antiresorptive agent, but the error arises from the behavior of bone in the placebo group; cortical fragmentation continues and falsely increases "trabecular" density, thus underestimating trabecular bone loss. In the study by Farr and colleagues,[21] it appeared that trabecular density in estrogen-treated women was no higher than in the placebo group, leading to the inference that estrogen did not protect against trabecular bone loss. Cortical bone loss in the placebo group is overestimated (because cortical fragments are measured as "trabeculae"). This exaggerates the protective effect of estrogen against cortical bone loss.

The magnitude of this effect depends on the proportions of cortical and trabecular bone in the region measured. Cortical bone of the vertebral body is a thin shell of mineralized matrix so estrogen treatment appears to be beneficial against cortical *and* trabecular bone loss (because there is little cortical bone to trabecularize in controls). In the appendicular skeleton, the larger amount of cortical bone contributes cortical fragments that are "seen" as trabecular bone by the thresholding method of segmentation, making it appear that this antiresorptive is effective against cortical, not trabecular bone loss at this location.

Engelke and colleagues[5] recognize this transitional zone and used a constant 1.5-mm-thick region labeled "subcortical bone" to examine the effects of odanacatib. However, femoral neck cortical thickness varies around its perimeter, is thin in many locations, is well under 1.5 mm superiorly. Likewise, the compact-appearing cortex and transitional zones are thin and vary around the cortical perimeter. A consensus on how to segment this region into cortical, transitional, and trabecular compartments is not available. Neither Tsai and colleagues[6] nor Poole and colleagues[7] addressed this issue.

Cortical thickness and matrix volume

If neither the periosteal nor endocortical edges are accurately measured then quantifying cortical thicknesses and areas is also problematic. If the cortex is thick, about 3 mm, such as at the subtrochanteric region or inferior femoral neck, then images of low resolution may be sufficient. However, under these circumstances, a biologically important increase or decrease may be difficult to detect because the change in thickness is small relative to the baseline thickness. If cortices are thin, 300 μm, as is often the case at the superior femoral neck, parts of the trochanteric region, the distal tibia, and the radius, measurement is a challenge, but changes may produce detectable and biologically relevant effects on bone strength (Fig. 2).

The method reported by Poole and colleagues[7] and in more detail by Treece and colleagues[24] may be an advance. However, as acknowledged by the authors, the error in measuring ~500-μm-thick cortices is 25%, a biologically relevant value because ~25% of cortices at the hip are below 500 μm.[16,17]

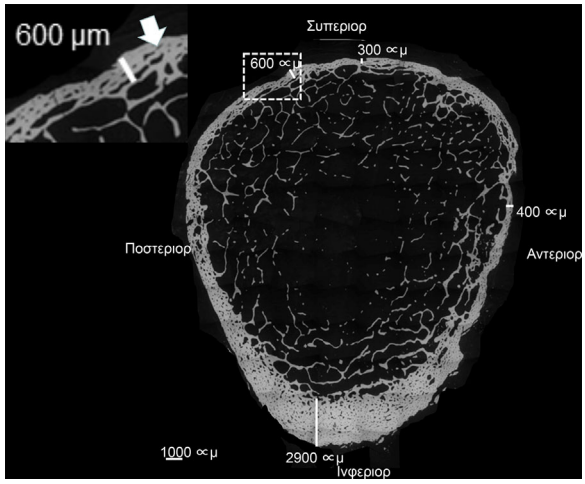

Fig. 2. There is no single cortical thickness. There is considerable heterogeneity in cortical thicknesses with both thinner and thicker regions. The magnified portion shows that it is difficult to separate cortical and trabecular bone as one transitions to another (thick arrow).

Tsai and colleagues[6] report *no* increase in cortical thickness with PTH, an anabolic agent, and an *increase* in cortical thickness with denosumab, an antiresorptive agent; two findings that illustrate the challenges in the validity and interpretation of findings produced by current imaging methods. These observations are likely to be partly the result of the use of the same threshold to segment (separate) cortical from trabecular bone and difficulties in correctly identifying edges when matrix mineralization is decreasing with an anabolic agent or increasing with an antiresorptive agent.

Dealing first with the effect of PTH, this anabolic agent deposits new osteoid upon quiescent surfaces, or commonly upon endocortical and trabecular surfaces actively participating in remodeling.[9,10] This osteoid will not attenuate photons until it is mineralized so any true periosteal apposition remains undetected. In addition, PTH-initiated remodeling upon intracortical, endocortical, and trabecular surfaces removes fully mineralized bone and replaces it with osteoid. This may reduce the net matrix mineral content of the region measured, leading to the fall in BMD as reported in this study by Poole and colleagues,[7] and despite an increase in matrix volume reported using histomorphometry.[9,10] There was also no detectable improvement in trabecular thickness, separation, or number with PTH despite histological evidence to the contrary in several studies, and the proven antifracture efficacy of PTH. The absence of detectable changes may also reflect the deposition of osteoid upon trabecular surfaces that attenuates photons below the threshold level that qualifies as bone.

Second, how can an antiresorptive agent like denosumab increase cortical thickness? Antiresorptives slow bone loss and so, at best, they should slow structural deterioration and maintain BMD. Yet these agents increase BMD, an observation that is interpreted as an increase in bone matrix mass which at least implies some repair of structural deterioration present prior to treatment. This is not necessarily so.

Antiresorptives reduce the rate of remodeling.[25,26] At the onset of treatment the great many excavated cavities generated by rapid remodeling *before* treatment enter their refilling phase, a process that takes ~3 months. Porosity decreases focally because of the slow refilling of these many excavated cavities upon intracortical canals. Partial refilling of excavated cavities upon the endocortical surface may restore cortical thickness *focally*. Likewise, partial refilling of excavated cavities upon trabecular surfaces may also partly restore trabecular thickness focally. Concurrently, fewer new BMUs appear because remodeling is suppressed. The net effect of concurrent refilling of the many more cavities that are now being excavated produces the early rapid rise in BMD. BMD also increases because primary and secondary mineralization occurs of the newly deposited matrix, secondary mineralization of bone no longer removed because remodeling is slow, and bone formed months to years before treatment begun (because secondary mineralization may take 12 to 36 months to complete).[25–27]

As pointed out by Tsai and colleagues,[6] the reported increase in cortical thickness with denosumab may be an error produced by this secondary mineralization. More voxels contain more matrix (from refilling cavities), more complete mineralization of this matrix, and reciprocally less void volume, leading to more photon attenuation produced by voxels at the periosteal or endocortical edge. There are now more voxels with photon attenuation crossing the threshold from a "non-bone" to a "bone" voxel, suggesting that bone size has increased.

There are two other possible mechanisms. Any real "increase" in cortical thickness with denosumab is likely to be mostly a result of infilling of focally excavated cavities upon intracortical canals traversing the cortex adjacent to the medullary canal. This infilling may partly *restore* focal thickness "from the inside" as the eroded cavity upon the endocortical surface partly refills; antiresorptives do not produce endocortical apposition, they are not anabolic. Another mechanism that may actually increase cortical thickness is continued periosteal and endocortical modeling no longer obscured by high remodeling.[28] The modeling may become detectable when denosumab reduces remodeling. Modeling-based bone formation has also been reported by Pennypacker and colleagues[29] using odanacatib. It is unclear whether this is caused by odanacatib, or is independent of this treatment but becomes detectable in the face of suppressed remodeling.

The reported increase in cortical thickness and cortical volume by Engelke and colleagues[5] and Poole and colleagues[7] is likely to also be partly due to changes in the degree of completeness of matrix mineralization. Engelke and colleagues[5] report an approximate 1000-mg difference in bone mineral content between the treated and control groups with about one-half arising from cortical bone. Although Poole and colleagues[7] use a method capable of accurately measuring thinner cortices in low-resolution images, the measured thickness is still a function of the material density. Furthermore, as stated by Poole and colleagues,[7] the method cannot distinguish an increase in thickness due to periosteal and/or endocortical bone formation producing a true increase in thickness versus infilling of intracortical pores. Again, any real increase in thickness using antiresorptives is likely to be a focal *restoration* in thickness.

Cortical Porosity

Tsai and colleagues[6] reported an increase in porosity in the PTH group and no reduction in porosity with denosumab. Dealing first with PTH administration, when PTH replaces older more fully mineralized bone with younger and perhaps a larger volume of osteoid, the attenuation of the voxel containing osteoid is reduced, producing a seeming increase in porosity. Another mechanism may be the result of new bone deposition upon surfaces of adjacent trabeculae abutting against the cortex at the corticomedullary junction. If deposition of bone upon surfaces of adjacent trabeculae is incomplete this may lead to incomplete coalescence of adjacent trabeculae resulting in higher 'porosity' in the transitional zone of a seemingly thickened cortex, much like delayed trabecular coalescence seen during rapid distal radius growth.[30]

The lack of reduction in porosity with denosumab reported by Tsai and colleagues[6] contrasts with studies in human subjects and nonhuman primates.[11,12] This null result might be explained by the methodological issues, but in addition, any real reduction in porosity is likely to be the result of partial refilling of cavities excavated upon smaller intracortical canal surfaces, a change that would remain undetected because HRpQCT will fail to detect increases or decreases in porosity unless the pores are over 130 μm in diameter.

Whatever the porosity measured by Tsai and colleagues,[6] the reported porosity values ranged from 2% to 9% at the distal radius and tibia. The best image resolution achievable in vivo using HRpQCT is ~130 μm. Although this resolution is now improved to 95 μm, it still precludes quantification of most

intracortical pores because over 60% of pores are less than 100 μm in diameter,[31–37] This leads to underestimation of porosity in most studies.[38–40] These low figures are widely accepted because the underestimates are widely reported.

Direct measurements of cortical bone water content across species using deuterium oxide or dehydration experiments report a void volume ranging from 15% to 40%.[31–37] The low porosities reported in this and most other studies[36–40] are incompatible with these direct measurements, and with the provision of a vascular supply. Bone receives 10% to 20% of total cardiac output essential for nutrient transport and waste removal.[41] We recently proposed a new non–threshold-based approach to quantify porosity which avoids exclusion of voxels that contain both matrix and void volumes; the presence of mineralized matrix increases photon attenuation leading to exclusion of that voxel with its void volume (a process called partial volume effects), leading to underestimation of porosity.[18]

Whatever the measured porosity, if this did occur, the effects on strength may vary depending on location. An increase in transitional zone porosity is likely to have a less deleterious effect on bending strength than subperiosteal porosity because the loss of a unit volume of subperiosteal cortical bone matrix reduces resistance to bending as a fourth power function of the radial distance from the neutral axis of a tubular bone.[42]

Man is unique among living things in that he measures his external and internal milieu. This is an extraordinary gift of evolution. Recognizing the limitations of the measurement methods and applying constraints accordingly is at the very heart of progress. Progress in quantification of bone's material composition, microstructure, and strength during growth, advancing age, disease, and therapy requires collaboration between biologists, biomechanical engineers, and physicists. This collaboration is likely to assist in defining bone's "qualities" and its "strengths," and will assist in the construction of appropriate methods of image acquisition and analyses essential to accurate measurement of this hierarchical structure in health and disease. Are we closer? Yes. Are we there yet? No.

Disclosures

R Zebaze and E Seeman are inventors of the StrAx1.0 software and Directors of Straxcorp.

References

1. Albright F, Osteoporosis. Ann Intern Med. 1947;27:861–882.
2. Riggs BL, Melton LJ III. Involutional osteoporosis. N Engl J Med. 1986;314:1676–1686.
3. Kanis JA, Borgstrom F, Zethraeus N, Johnell O, Oden A, Jonsson B. Intervention thresholds for osteoporosis in the UK. Bone. 2005; 36:22–32.
4. Zebaze RM, Ghasem-Zadeh A, Bohte A, et al. Intracortical remodeling and porosity in the distal radius and post-mortem femurs of women: a cross-sectional study. Lancet. 2010;375:1729–1736.
5. Engelke K, Fuerst T, Dardzinski B, et al. Odanacatib treatment affects trabecular and cortical bone in the femur of postmenopausal women: results of a 2-year placebo-controlled trial. J Bone Miner Res. 2015;30:30–38.
6. Tsai JN, Uihlein AV, Burnett-Bowie SM, et al. Comparative effects of teriparatide, denosumab, and combination therapy on peripheral compartmental bone density, microarchitecture, and estimated strength: the DATA-HRpQCT Study. J Bone Miner Res. 2015;30: 39–45.
7. Poole KE, Treece GM, Gee AH, et al. Denosumab rapidly increases cortical bone in key locations of the femur: a 3D bone mapping study in women with osteoporosis. J Bone Miner Res. 2015;30: 46–54.
8. Cusick T, Chen CM, Pennypacker BL, et al. Odanacatib treatment increases hip bone mass and cortical thickness by preserving endocortical bone formation and stimulating periosteal bone formation in the ovariectomized adult rhesus monkey. J Bone Miner Res. 2012;27:524–527.
9. Dempster DW, Cosman F, Parisien M, Shen V, Lindsay R. Anabolic actions of parathyroid hormone on bone. Endocr Rev. 1993;14:690–709.
10. Compston JE. Skeletal actions of intermittent parathyroid hormone: effects on bone remodelling and structure. Bone. 2007;40:1447–1452.
11. Ominsky MS, Jolette J, Smith SY, Vlasseros F, Samadfam R, Kostenuik PJ. Transition from alendronate to denosumab resulted in further reductions in local and systemic bone turnover parameters and reduced cortical porosity in ovariectomized cynomolgus monkeys. J Bone Miner Res. 2008;23:S61.
12. Zebaze RM, Libanati C, Austin M, et al. Differing effects of denosumab and alendronate on cortical and trabecular bone. Bone. 2014;59:173–179.
13. Dougherty G, Newman D. Measurement of thickness and density of thin structures by computed tomography. Med Phys. 1999;26:1341–1348.
14. Prevrhal S, Engelke K, Kalander WA. Accuracy limits for the determination of cortical width and density: the influence of object size and CT imaging parameters. Phys Med Biol. 1999;44:751–756.
15. MacNeil JA, Boyd SK. Accuracy of high-resolution peripheral quantitative computed tomography for measurement of bone quality. Med Eng Phys. 2007;29:1096–1105.
16. Zebaze RM, Jones A, Welsh F, Knackstedt M, Seeman E. Femoral neck shape and the spatial distribution of its mineral mass varies with its size: clinical and biomechanical implications. Bone. 2005;37:243–252.
17. Kersh ME, Pandy MG, Bui QM, et al. The heterogeneity in femoral neck structure and strength. J Bone Mineral Res. 2013;28:1022–1028.
18. Zebaze R, Ghasem-Zadeh A, M'bala A, Seeman E. A new method of segmentation of compact-appearing, transitional and trabecular compartments and quantification of cortical porosity from high resolution peripheral quantitative computed tomographic images. Bone. 2013;54:8–20.
19. Balena R. Shih M-S, Parfitt. Bone resorption and formation on the periosteal envelope of the ilium: a histomorphometric study in healthy women. J Bone Miner Res. 1992;7:1475–1482.
20. Keshawarz NM, Recker RR. Expansion of the medullary cavity at the expense of cortex in postmenopausal osteoporosis. Metab Bone Dis Relat Res. 1984;5:223–228.
21. Farr JN, Khosla S, Miyabara Y, Miller VM, Kearns AE. Effects of estrogen with micronized progesterone on cortical and trabecular bone mass and microstructure in recently postmenopausal women. J Clin Endocrinol Metab. 2013;98:E249–E257.
22. Dempster DW, Parisien M, Silverberg SJ, et al. On the mechanism of cancellous bone preservation in postmenopausal women with mild primary hyperparathyroidism. J Clin Endocrinol Metab. 1999;84: 1562–1566.
23. Vu TD, Wang XF, Wang Q, et al. New insights into the effects of primary hyperparathyroidism on the cortical and trabecular compartments of bone. Bone. 2013;55:57–63.
24. Treece GM, Gee AH, Mayhew PM, Poole KES. High resolution cortical bone thickness measurement from clinical CT data. Med Image Anal. 2010;14:276–290.
25. Seeman E. Bone morphology in response to alendronate as seen by high-resolution computed tomography: through a glass darkly. J Bone Miner Res. 2010;25:2277–2281.
26. Seeman E. Anabolic plus antiresorptive: Is one plus one more or less two? IBMS BoneKEy. 2011;8:221–228. DOI: 10.1138/20110510.
27. Akkus O, Polyakova-Akkus A, Adar F, Schaffler M. Aging of microstructural compartments in human compact bone. J Bone Miner Res. 2003;18:1012–1019.

28. Ominsky M, Libanati C, Boyce RW, et al. Continuous modeling-based bone formation: a novel mechanism that could explain the sustained increases in hip bone mineral density (BMD) with denosumab treatment. J Bone Miner Res. 2013;28(Suppl 1). [Presented at: Annual Meeting American Society for Bone and Mineral Research (ASBMR); 2013 Oct 7; Baltimore, MD, USA; Oral Poster Session, Presentation Number: LB-MO30]. Available from: http://www.asbmr.org/education/AbstractDetail?aid=89fd1f4a-ff73-4a62-accc-d9ae7dac6e22.
29. Pennypacker BL, Chen CM, Zheng H, et al. Inhibition of cathepsin K increases modeling-based bone formation, and improves cortical dimension and strength in adult ovariectomized monkeys. J Bone Miner Res. 2014;29:1847–1858.
30. Wang Q, Ghasem-Zadeh A, Wang X-F, Iuliano-Burns S, Seeman E. Trabecular bone of growth plate origin influences both trabecular and cortical morphology in adulthood. J Bone Miner Res. 2011; 26(7):1577–1583.
31. Techawiboonwong A, Song HK, Leonard MB, Wehrli FW. Cortical bone water: in vivo quantification with ultrashort echo-time MR imaging. Radiology. 2008;248:824–833.
32. Smith JW. Observations on the water content of bone. J Bone Joint Surg Br. 1964;46:553–562.
33. Cowin SC, Gailani G, Benalla M. Hierarchical poroelasticity: movement of interstitial fluid between porosity levels in bones. Philos Trans A Math Phys Eng Sci. 2009;367:3401–3444.
34. Mueller KH, Trias A, Ray RD. Bone density and composition. Age-related and pathological changes in water and mineral content. J Bone Joint Surg Am. 1966;48:140–148.
35. Biltz RM, Pe ED. The chemical anatomy of bone. I. A comparative study of bone composition in sixteen vertebrates. J Bone Joint Surg Am. 1969;51:456–466.
36. Chappard C, Bensalah S, Olivier C, et al. 3D characterization of pores in the cortical bone of human femur in the elderly at different locations as determined by synchrotron micro-computed tomography images. Osteoporos Int. 2013;24:1023–1033.
37. Martin RB. Porosity and specific surface of bone. CRC Critical Rev Biomed Eng. 1984;10:179–221.
38. Burghardt AJ, Kazakia GJ, Ramachandran S, Link TM, Majumdar S. Age- and gender-related differences in the geometric properties and biomechanical significance of intracortical porosity in the distal radius and tibia. J Bone Miner Res. 2010;25:983–993.
39. Nishiyama KK, Macdonald HM, Buie HR, Hanley DA, Boyd SK. Postmenopausal women with osteopenia have higher cortical porosity and thinner cortices at the distal radius and tibia than women with normal aBMD: an in vivo HR-pQCT study. J Bone Miner Res. 2010;25:882–890.
40. Nishiyama KK, Macdonald HM, Moore SA, Fung T, Boyd SK, McKay HA. Cortical porosity is higher in boys compared with girls at the distal radius and distal tibia during pubertal growth: an HR-pQCT study. J Bone Miner Res. 2012;27:273–282.
41. Brookes M, Revell JW. The blood supply of bone: scientific aspects. New York: Springer; 1998.
42. Ruff CB, Hayes WC. Subperiosteal expansion and cortical remodeling of the human femur and tibia with aging. Science. 1982;217: 945–948.