Translational Aspects of Bone Quality – Vertebral Fractures, Cortical Shell, Microdamage and Glycation: A Tribute to Pierre Dominique Delmas

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Abstract

Among vertebral deformities, the prevalence of wedge fractures is about twice that of endplate (biconcave) deformities, both of which are greater than crush deformities. The anterior cortex is, therefore, a site of interest for understanding mechanisms of vertebral fracture. Despite its importance to vertebral mechanics, there are limited data describing the role of cortical shell, microdamage and bone matrix parameters in vertebral fragility. This review of literature emphasizes the translational aspects of bone quality and demonstrates that a greater understanding of bone fractures will be gained through bone quality parameters related to both cortical and cancellous compartments as well as from microdamage and bone matrix parameters. In context of vertebral fractures, measures of cortical shell and bone matrix parameters related to the organic matrix (Advanced Glycation Products and α/β CTX ratio) are independent of BMD measurements and can therefore provide an additional estimate of fracture risk in older patients.

Keywords: Vertebral fracture, cortical shell, microdamage, glycation, bone matrix

Introduction

Study Leave, Lyon 2007-2008

> "*It was the best of times, it was the worst of times, it was the age of wisdom, it was the age of foolishness, it was the epoch of belief, it was the epoch of incredulity, it was the season of Light, it was the season of Darkness, it was the spring of hope, it was the winter of despair, we had everything before us, we had nothing before us, we were all going direct to heaven, we were all going direct the other way…*" (Dickens, A Tale of Two Cities, 1859, pp 1).

Pierre Delmas named his research unit "Qualité Osseuse dans L'ostéoporose " or bone quality in osteoporosis. As a Professor of Medicine, and open advocate of translational research in public health, this gave an unexpected, but solid, endorsement to the believers of bone quality. Bone quality is regarded by many as the sum total of everything in bone that cannot be measured; and still remains somewhat of a mystery. However it is a mystery with unprecedented potential to revolutionize the diagnosis and treatment of osteoporotic and fragility fractures. Despite an impressive track record in classical endrocrinology, e.g. utilizing BMD as a surrogate marker for bone strength, Pierre recognized the importance of bone quality very early. Moreover, he dedicated much of his recent clinical and translational research to investigate the contribution of bone quality to osteoporosis. In doing so he exposed the tip of an iceberg. But that tip had clarity of purpose and an insight to elucidate the pathogenesis of fragility fracture. It was this aspect of Pierre's work and personality that drew two very different people, one an anatomist (Mark Forwood) and the other, a biomechanical engineer (Deepak Vashishth) from the opposite ends of the world (Australia and USA) to meet halfway in Lyon and work under his mentorship. We were fortunate to have had this opportunity and to have worked with him till his last days. He always inspired a sense of inquiry, scientific rigor and excellence; and remained restless till the last bits of a current skeletal puzzle fell in to place. In this review, we have attempted to present the contribution of different aspects of

bone quality to bone fragility using vertebral fractures as an example. Because excellent reviews on the role of trabecular architecture are already available, the emphasis of this review is on the role of cortical shell, microdamage and bone matrix on vertebral fragility. In instances where little data is available on vertebral bone, examples from other skeletal sites are given.

The Cortical Shell in Vertebral Fragility

> "*Well! I've often seen a cat without a grin; but a grin without a cat! It's the most curious thing I ever saw in my life!* " Alice's Adventures in Wonderland, Lewis Carrol

Resistance to vertebral fracture is provided by a bone structure that integrates the cortical shell (referred to, herein, as the cortex) with trabecular bone. Fracture of a vertebral body is evident by deformation of the vertebral body involving failure of trabeculae and of the surrounding cortex (1). It is acknowledged that the vertebral body behaves as a structure in which the cortex and trabeculae are inextricably linked (2, 3). Nonetheless, numerous attempts have been made to distinguish their relative contributions to vertebral strength. Such attempts have included physical separation of the cortical shell (4-6), and use of computational modeling to predict the contribution of the cortex to strength of the vertebral body (6-9). In addition, there is a small, but growing, body of evidence describing the structure of the cortex *per se*, or in relation to osteoporotic fracture.

The thickness of the cortex ranges from about 280 to 800 μm, the variation in which depends on the vertebral level (10-13), the anatomical location within the vertebra (10-12) and the method of determination, including histomorphometry (10-13), CT (6, 14, 15), and micro CT (8, 9). By histomorphometry, the thickness of the anterior cortex of lumbar vertebrae is about 450 microns. There is consensus that the posterior cortex is thinner, on average, than the anterior cortex, and has a higher degree of porosity (10-12). Although osteonal bone is observed in some sections, the dominant microstructure tends to be of lamellar bone (11, 16).

Interestingly, the distribution of cortical thickness throughout the thoracolumbar spine is similar to the distribution of trabecular BV/TV (12), but trabecular thickness in the adjacent cancellous bone remains constant (11). This morphological observation exemplifies the structural support provided by the cortex and suggests a distinct sensitivity of the cortex to the local loading history. Indeed, the pattern of cortical thickness in thoracolumbar vertebrae of patients with osteoporosis is similar to that in controls, but there is a highly significant loss of cortical thickness in each region (12). These patients were classified by clinical and histological criteria, and all had at least one vertebral fracture. The strength of vertebral bodies is also influenced by cortical BMD that shows a significant decline with age as assessed by QCT (12, 14, 15). This decline has been observed in both men and women (12, 15). Using single energy QCT, Andresen et al. observed an age-related decline in cancellous BMD of 68 men and 111 women (both aged between 30 and 84), but the decline in cortical BMD was only significant for the women (14). The implication suggesting a mechanism for the timing of vertebral fracture in women compared to men. Given the dimensions of the cortical shell, CT evaluation of cortical BMD is prone to error due to volume averaging effects, particularly when the cortical thickness is less than 2.0 mm (17). Thus, studies of cortical mineralisation in relation to age, or other variables, may be more accurately assessed using DEXA-Discovery (small animal software) or the degree of mineralization assessed by quantitative microradiography (18, 19), and the bone structure by micro-CT.

We collected a series of 2nd lumbar vertebrae (L2) in Lyon to determine the morphology of the cortex and to optimise conditions for microCT analysis. Samples of the anterior cortex and adjacent trabeculae were bulk-stained in xylenol orange for determination of *in vivo* microcracks (Mdx) and then embedded in plastic and scanned using a Skyscan 1076 at a resolution of 18 um, and sections cut for histology and Mdx analysis in the sagittal and transverse planes. These preliminary studies show that the concept of a cortical shell composed of a wall of dense bone, with osteons and a thickness of about 300-400 um, is misleading. Among a range of vertebrae collected, only a small proportion demonstrate this typical characteristic, and there is a wide range from classical osteonal

bone, to highly fenestrated structures in which the “cortical bone” elements are indistinguishable from the adjacent trabeculae (Fig 1).

Insert Fig 1 Near Here

Given this heterogeneity of cortical structure, it is no surprise that its contribution to load sharing and vertebral strength is a source of controversy. The disparate results for its role in load bearing can largely be attributed to the variety of methods employed to separate the cortex from the centrum, the choice of vertebral body, and the inclusion or dissection of the vertebral endplates. These methods give rise to estimates that range between 10 to 60% for the contribution of the cortex to vertebral body strength. The lowest of these estimates arises from manual removal of the cortex prior to mechanical testing of the remaining centrum (4), while the higher estimates are produced from virtual removal of the cortex using finite element analysis (FEA)(7-9). Among the FEA methods, the shell-force fraction has also been estimated as low as 10%, where the McBroom data were modeled using standard FEA meshes (6). If micro-mechanical FEA models were produced specifically for each specimen using microCT, the estimates range from 30 to 60% (7-9). In each of these latter cases, the contribution of the cortex is greatest towards the mid-transverse plane and diminishes towards the endplates.

The shell force fraction has also been demonstrated to be proportional to the cortical thickness, and inversely proportional to the apparent modulus of the cancellous bone (7). Inclusion of the radius of curvature of cortical bone in Cao’s model increased the contribution of the shell at the mid-transverse plane, but reduced its contribution to load sharing towards the endplates (7). Eswaran et al conclude that the contribution of the cortical shell to the load carrying capacity is significant because it represents a large proportion of the vertically oriented bone tissue within the vertebral body; and that it facilitates the load-carrying capacity of the peripheral trabeculae (because it provides a path for force transmission) (8). There is also evidence that the thickness of the endplates in L3 correlate with architectural parameters derived by uCT (BV/TV, DA, SMI, Tr.Th), but that

the anterior cortex does not (Roux JP, unpublished data). This too, suggests an independent contribution of the cortex to vertebral mechanics.

Microdamage and Vertebral Fragility:

The review of the literature, presented above, suggests that vertebral shell contributes to load sharing which is disproportionate to its relative bone volume fraction. Age- and disease- related alterations in cortical shell occurring in concert with decreased trabecular volume (25), reduced structural characteristics (25) and modified bone matrix (26) will inherently make human vertebrae more prone to microdamage and osteoporotic fractures.

Microdamage in human bone, occurring due to *in vivo* loading, occurs in distinct forms, i.e. linear microcracks and diffuse damage (Figure 2). Linear microcracks are intermediate in size (greater than a canaliculus but smaller than a vascular channel). Under bright-field microscopy, they appear as a sharply defined line with edges that are more deeply stained than the surrounding tissue (27-29). In contrast, diffuse damage appears as a focal but diffused area of pooled staining not corresponding to an entire microstructural feature (osteon or trabecular packet) (20). Under laser confocal microscope, areas of pooled staining reveal a meshwork of fine submicroscopic cracks (20). Whole bone and Micromechanical tests on bones containing linear microcracks and diffuse damage have revealed that the two forms of damage affect bone differently (29-30), with diffuse damage formation being more beneficial to bone's fracture resistance than linear microcracking (29). Consistent with in vitro mechanical assessment, in vivo incidence of these two forms of microdamage varies with chronological as well as tissue age (31). Diffuse damage and linear microcracks are more prevalent in 'younger' and 'older' tissues, respectively (31). Because tissue age is related to state of bone turnover, these data sets suggest that bone turnover may determine tissue age and consequently the extent and morphology of microdamage and fracture resistance of bone.

Insert Fig 2 Near Here

Human vertebrae are interesting models in which to investigate the role of microdamage in bone fragility. First they are rather heavily loaded up to two- to three- times body weight during daily activities (32). Second, canine studies show that vertebral bone is the site of highest bone turnover in the skeleton (33). And, third both the cortical shell and trabecular bone tissue composing the vertebrae contribute to load bearing, but their rates of turnover may be different, with one turning over faster than the other.

To our knowledge the incidence of microdamage in vertebral shell has never been reported. Preliminary studies conducted in Lyon on L2 vertebral body show that cortical shell is indeed susceptible to microdamage (Forwood and Follet, unpublished data). Representative images of linear microcracks and diffuse damage in human vertebral cortical shell are presented in Figure 3. Further work is currently in progress to investigate the in vivo incidence of microdamage in the cortical shell.

Insert Fig 3 near Here

Unlike the cortex, three different studies have investigated the incidence of in vivo microdamage in human vertebral trabecular bone. Wenzel et al. (21) and Vashishth et al. (20) reported the incidence of in vivo linear microcracks and diffuse damage in 66 L1 human vertebral trabecular bone (ages 23 to 91; males and females, Caucasian and blacks), respectively, but did not find any relationship of their accumulation with age. The numerical density of *in vivo* microcracks explained about 40% of the variance in ultimate failure load of adjacent tissue samples (22). In contrast to these results, the group in Lyon examined microdamage in cores of trabecular bone from 23 L2 vertebral bodies (age 54-93; 8 men and 15 women; Race: All caucasian) and found an exponential increase in trabecular crack density (Cr.Dn) and a linear increase in diffuse damage with age (Figure 4). Furthermore they found that microarchitecture (BV/TV, Tb. N, Tb. Sp. and structural model index (SMI)) influenced the accumulation of both linear microcracks and diffuse damage in bone and SMI was the best architectural variable explaining microdamage (34).

Insert Fig 4 Near Here

The discrepancies of Arlot et al's results with previous studies highlight some intriguing possibilities. In particular, because estrogen withdrawal in women is associated with high turnover and vertebral bone is a site of high skeletal turnover, the difference in microdamage accumulation may only be evident in older patients. Arlot et al. only examined the post-menopausal range (54-93) and their samples contained a greater proportion of older subjects, with 11/14 women and 6/9 men $\geq$ 70 yr. of age. Moreover, it seems that bone turnover may keep some parts of vertebral trabecular bone tissue in a 'young' state causing diffuse damage formation while the other parts of vertebral trabecular bone tissue do not turnover and accumulate linear microcracks. Because the rate of linear microcrack accumulation increases faster than that of diffuse damage accumulation with age (exponential vs linear), it is likely that the process of bone turnover becomes more selective and restricted to smaller regions with age.

Bone Matrix and vertebral fragility:

The unique associations between the superior resistance of bone against fracture, and its ability to form diffuse damage over linear cracking, has motivated a number of studies into the extent and the nature of modifications in bone's extracellular matrix quality. Furthermore, since tissue age is a key determinant of bone's damage morphology and is, in turn, related to bone turnover, a greater emphasis has been placed on the post-translational modifications of protein components and increase in the mean degree of tissue mineralization or DMB. Again through a number of studies over the last decade, the group in Lyon has demonstrated that reduced bone turnover increases DMB in a number of clinical situations including, but not limited to, bisphosphonate treatment (23) and cathepsin K deficiency (35). DMB content also increases with tissue age and is consequently higher in interstitial than in the osteonal bone compartment () but an association between DMB and microdamage morphology or between DMB and bone fracture properties has not been established.

In terms of post-translational modifications, similar to other collagenous tissues, crosslinking of the principal structural protein in bone, i.e. collagen, by non-enzymatic glycation (NEG) and by racemization and isomerization has gained increased significance. NEG occurs in the absence of an enzyme and involves reaction between an aldehyde of the open chain form of glucose and the ε-amino group of lysine or hydroxylysine on bone collagen. The resultant aldmine undergoes rearrangement and further reactions with other amino groups to form advanced glycation endproducts (AGEs) containing intermolecular crosslinks including pentosidine (36), vesperalysine (37) and other compounds (38). AGE products have characteristic fluorescence and can be measured by HPLC or calorimetrically.

Racemization occurs on aspartic (Asp) acid residues due to spontaneous conversion of L-enantiomeric form to D-form and accumulation of D-form is common in human tissues with low turnover including dentin, dermis and cartilage. β-isomerization causes a kink in the peptide backbone due to the transfer of Asp-residue α-carboxyl group to the side chain β- or γ- carboxyl group (39-40). In the case of bone, racemization and isomerization takes place on the α1-chain of type I collagen through Asp-residue localized in the CTX epitope of C-telopeptide (59). The degree of isomerization in type I collagen is measured by estimating the native (a) and isomerized (b) forms of CTX released through commercially available ELISA kits containing specific monoclonal antibodies raised against the α-CTX and β-CTX sequences (Nordic Biosciences, Denmark).

The accumulation of AGEs in bone alters the energy dissipation mechanisms leading to brittle fracture in both cortical (24) and cancellous bone (26). In vitro induced accumulation of AGEs to physiological levels increases and explains enhanced stiffness of the organic matrix and reduced measures of collagen deformation and microcracking (24, 42). Bone derives its resistance against fracture from collagen deformation (43) and from its ability to form microdamage (44). Collagen deformation and microcracking are therefore the primary mechanisms of toughening in bone (45) and NEG-induced alterations in these would alter bone toughness. Similar to NEG, the post-

translational modification of collagen in bone by β-isomerization has also been shown to reduce post-yield energy dissipation (46) but the mechanism of energy loss has not been determined.

In a recent study, Delmas and co-workers (47) analysed the contributions of the post-translational collagen modifications on vertebral fragility. Using 19 L3 vertebrae, collected after necropsy (Ages 26-93; 10 males, 9 females), they conducted compression tests to failure and analysed the association of resulting mechanical properties with DXA-estimated BMD, non-enzymatic crosslink-pentosidine and the extent of β-isomerization of bone collagen (α/β CTX ratio). Their results showed that BMD and pentosidine independently predicted vertebral failure load and work-to-fracture. Also BMD and α/β CTX ratio were independently related to vertebral stiffness. Bone matrix properties therefore have the potential to predict fracture properties at the whole bone level independent of BMD

Summary:

Among vertebral deformities, the prevalence of wedge fractures is about twice that of endplate (biconcave) deformities, both of which are greater than crush deformities (48-50). The anterior cortex is, therefore, a site of interest for understanding mechanisms of vertebral fracture. Despite its importance to vertebral mechanics, there are limited data describing the role of cortical shell, microdamage and bone matrix parameters in vertebral fragility. The review presented here emphasizes the translational aspects of bone quality and demonstrates that a greater understanding of bone fractures will be gained through bone quality parameters related to both cortical and cancellous compartments as well as from microdamage and bone matrix parameters. In context of vertebral fractures measures of cortical shell and bone matrix parameters related to the organic matrix (Advanced Glycation Products and α/β CTX ratio) are independent of BMD measurements and can therefore provide an additional estimate of fracture risk in older patients.

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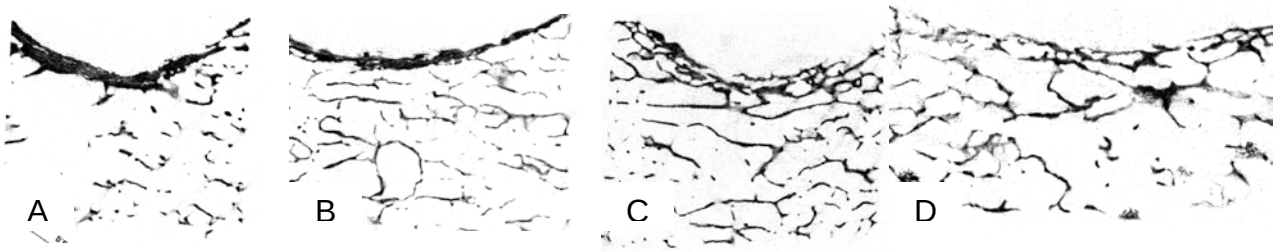
Figure Captions

Figure 1. Micro CT images of anterior cortical shell of L2 vertebrae, transverse plane (spatial resolution 18 um). A and B show typical cortical bone structures ranging from 200 to 400 um thickness, while C and D show porous and fenestrated cortical architecture comprised of trabeculae of similar size to adjacent cancellous bone (Roux JP, Arlot M, Delmas PD, Forwood MR, Unpublished Data).

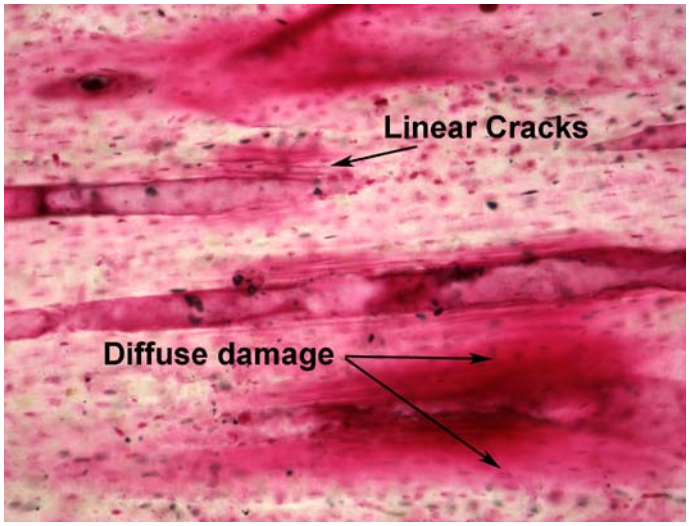
Figure 2: Micrographs showing representative linear microcracks and diffuse damage in a longitudinal section of human cortical bone (Forwood and Vashishth, unpublished data).

Figure 3: Micrograph illustrating the presence of *in vivo* microdamage in human vertebral cortical shell. L2 vertebrae were bulk stained in xylenol orange and imaged using fluorescence microscopy. This image is a transverse section from the superior antero-lateral cortex. EcS = endocortical surface PsS = periosteal surface. (Forwood M. and Follet H., unpublished data)

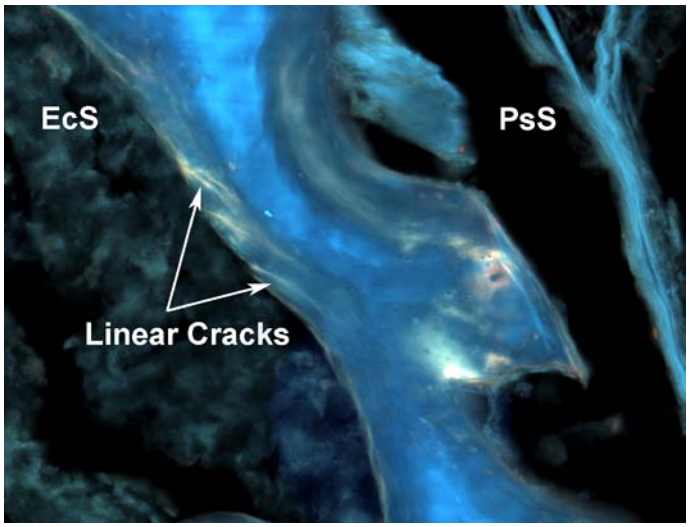
Figure 4. Age-related variation in numerical density of microcracks and diffuse damage in cancellous bone of the 2nd lumbar vertebrae (from Arlot et al (34), reproduced from J Bone Miner Res 2008:23:1613-1618, with permission of the American Society for Bone and Mineral Research).



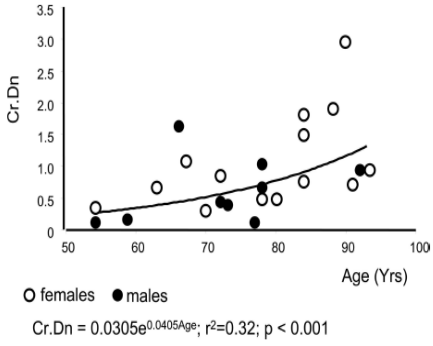
Forwood & Vashishth Fig 1



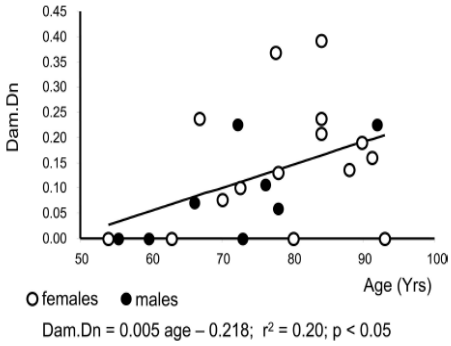
Forwood & Vashishth Fig 2



Forwood & Vashishth Fig 3



$Cr.Dn = 0.0305e^{0.0405Age}$; $r^2=0.32$; $p < 0.001$



$Dam.Dn = 0.005\ age - 0.218$; $r^2 = 0.20$; $p < 0.05$

Forwood & Vashishth Fig 4