Combining High-resolution Micro–computed Tomography with Material Composition to Define the Quality of Bone Tissue

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Atraumatic fractures of the skeleton in osteoporotic patients are directly related to a deterioration of bone strength. However, the failure of the bone tissue to withstand functional load bearing cannot be explained as a simple decrease in bone mineral density (quantity); strength is also significantly dependent upon bone quality. While a formal definition of bone quality is somewhat elusive, at the very least, it incorporates architectural, physical, and biologic factors that are critical to bone strength. Such factors include bone morphology (*ie*, trabecular connectivity, cross-sectional geometry, longitudinal curvature); the tissue's material properties (*eg*, stiffness, strength); its chemical composition and architecture (*eg*, ratio of calcium to other components of the organic and/or inorganic phase, collagen orientation, porosity, permeability); and the viability of the tissue (*eg*, responsivity of the bone cell population). Combining high-resolution structural indices of bone, as determined by micro–computed tomography; material properties determined by nanoindentation; and the chemical make-up of bone, as determined by infrared spectroscopy, helps to provide critical information toward a more comprehensive assessment of the interdependence of bone quality, quantity, and fracture risk.

Introduction

The principal responsibility of the skeleton is to withstand the loads and moments that are placed on it. When these loads exceed the bone tissue's ability to support them, fractures inevitably will occur. In the case of osteoporosis, fractures can occur without a singular traumatic event, and thus early detection of bone loss, or a skeleton at risk, will allow a more prompt and appropriate treatment, ultimately improving the ability to prevent fractures. Unfortunately, a skeleton at risk of fracture cannot simply be determined by the amount of bone that exists. To a large degree, the quality of the bone is just as important. This becomes apparent when considering that interventions of low bone mass may deteriorate mechanical properties of the skeleton. For example, fluorides never became an effective treatment of osteoporosis despite their ability to effectively promote bone formation and quantity [1]. Their incorporation into the bone matrix appears to weaken bone architecture, decrease bone strength, and increase the risk for hip fractures [2]. More recently, bisphosphonates have become the most commonly prescribed pharmaceutical treatment for osteoporosis because of their powerful antiresorptive abilities that may lead to increased bone quantity and decreased short-term fracture rates [3]. Unfortunately, treatment with bisphosphonates can also inhibit bone cell activity, and thus bone turnover, increasing the incidence of microdamage within the bone matrix [4], which in the long term, has the potential to increase fracture rates. It is apparent from these two examples that bone quantity is not sufficient to fully evaluate risk of fracture.

Although these examples demonstrate the necessity to assess the quality of bone, it is difficult to define what exactly "quality" means in this context. Certainly, quality is greatly influenced by morphologic features such as a bone's cross-sectional geometry, trabecular connectivity, architecture of the trabecular elements, presence (or absence) of microdamage, physical properties of the bone material, and the chemical composition of the matrix. Less direct influences on bone quality also exist, such as a genetically determined viability and responsiveness of the bone cell population to anabolic (or resistance to catabolic) factors. The goal of this paper is to discuss these factors in the context of how they ultimately contribute to

the structural success of the skeleton, and describe some of the techniques that can be used to assess them.

Limitations of Measuring Bone Quantity

Dual-energy x-ray absorptiometry (DXA) provides an effective measure of bone mineral density (BMD), reflecting the bone mineral content averaged across a specific region of interest. This two-dimensional approximation of BMD is the most widely used and accepted means of diagnosing osteopenia and osteoporosis [5]. The precision of DXA is very high (1% for the lumbar spine and 1.5% for the hip) [5], but its accuracy in assessing bone volumetric density is severely limited due to the two-dimensional nature of the projection measurement. For example, bones from larger subjects invariably appear to have greater apparent bone densities, and thus, when considered in the pool of age-matched means, predisposes those with a more petite habitus to a diagnosis of osteoporosis. Further, as a scalar indication of bone quantity, the poor resolution of DXA confounds a detailed analysis of bone architecture, omitting information on structural morphology (*eg*, contribution of trabecular architecture), misrepresenting material properties (*eg*, insensitive to microdamage), and potentially missing key site-specific changes within a given region of interest. In essence, DXA provides an apparent estimate of bone quantity, and is essentially insensitive to bone quality.

The limitations of DXA have become even more apparent in the diagnosis of osteoporosis. Osteoporosis is a disease characterized by a reduction in bone mass and a skeleton that is more susceptible to fracture. However, there is a considerable overlap in bone densities among normal individuals and those that sustain fractures, indicating that low bone mass cannot solely explain the increased fracture rate in the postmenopausal and aging population [6]. Of course, this becomes even more clear when considering that teenagers have essentially the same BMD as the elderly, but they are in no way considered osteoporotic, and are certainly at little risk of fracture given the greater physical demands they place on the skeleton. While fracture rates are most closely associated with bone strength, this critical parameter is only inferred by DXA, and some means of determining the contribution of bone quality must be developed.

Bone Morphology and Bone Quality

The ideal imaging modality for the detection of osteoporotic fractures in patients at risk would be able to determine bone strength at the organ as well as at the tissue level. Certainly, there is great promise in diagnostics such as ultrasound [7], where the velocity and attenuation of a transcutaneous ultrasound signal is directly proportional to bone's physical properties, but a full characterization of bone by this mode has not yet been realized. Another promising noninvasive diagnostic tool is high-resolution micro–computed tomography (micro-CT), which has begun to provide insights into the contributions of bone's three-dimensional morphology to bone quality. Combining the architectural intricacies of trabecular bone as defined by micro-CT with nanotechnology and infrared microscopy to determine bone's physical and chemical properties may ultimately advance our understanding of bone quality and bone strength. Being developed primarily to improve research methodologies, these technologies and applications will eventually find their way into the clinic for use in early detection and monitoring of osteoporosis.

Quantification of bone morphology by micro-CT

Gross morphologic skeletal features, such as cross-sectional diaphyseal geometry, cortical second moments of inertia, and the longitudinal curvature of a bone, have been intimately linked with traditional engineering methodology (*eg,* beam theory) [8], and can be determined in a relatively straightforward way with low-resolution three-dimensional imaging (*eg,* quantitative CT). Bone, however, is comprised of both cortical and trabecular bone, and changes in trabecular bone architecture can make a significant contribution to the quality of the bone despite the smaller relative volume of trabecular bone.

The development of micro-CT was first driven by the need for having a highly precise and accurate means of reconstructing the complex architecture of bone tissue at a high resolution [9–12,13••], and is becoming a critical parameter in the evaluation of disease pathogenesis [14] and efficacy of interventions [15]. Scanning with micro-CT can be achieved at resolutions as low as 5 µm, allowing the determination of porosities and subtle remodeling events such as the scalloped edges of resorption fronts. Further, true three-dimensional image reconstructions permit the assessment of bone microarchitecture as a three-dimensional structure, adding critical information to images collected through histomorphometry, which represents only bone in unconnected slices [12], a limitation not unlike inferring bone quality from the two-dimensional estimates provided by DXA. An overall characterization of both bone quantity (*eg*, percent bone volume per total volume) and quality (*eg*, trabecular connectivity and anisotropy) will certainly lead to a more accurate determination of the interdependence of these factors in defining resilience and tendency to fracture.

Parameters to assess bone quantity and microarchitecture

The most common parameters used by micro-CT to describe bone quantity are similar to those used in static histomorphometry, such as bone volume, tissue volume, and bone surface. Metric architectural parameters of trabecular bone can be determined, in contrast to conventional stereology, from three-dimensional images without assumptions

of the underlying model type [9]. For instance, mean trabecular thickness (Tb.Th) can be calculated directly by determining a local thickness at each voxel representing bone, while mean trabecular separation (Tb.Sp) is the "thickness" of the marrow cavities. Trabecular number (Tb.N) is the inverse distance between the midsection of the plates. Of course, determining these parameters computationally, and in full three-dimensions within the region of interest, minimizes subjective errors inherent in manual input, and does not require assumptions about converting two-dimensional slices (histology sections) into three-dimensional structures.

In addition to the quantification of direct metric parameters of bone quality, a number of nonmetric parameters can be calculated to describe the three-dimensional nature of bone structures and architectural features indicative of bone quality. For example, an estimation of the plate-rod characteristic of the structure can be achieved using the structure model index (SMI) [16]. For an ideal plate and rod structure, the SMI value is zero and three, respectively, with intermediate values depending on the volume ratio between rods and plates. Connectivity density (Conn.D), as defined over the Euler characteristics [17], and the trabecular bone pattern factor are measures for the connectivity in a three-dimensional architecture. These nonmetric indices are critical toward the assessment of bone quality as they, in a quantitative sense, influence the strength of a bone similar to the effect of a strategic placement of beams and rafters for building a house. They also provide insight into the severity of the disease. For example, a change in connectivity (*ie*, lost trabeculae) in osteoporosis may represent a structure that will be more difficult (if not impossible) to restore to its original health architecture for any given therapy. For a more detailed description of methods used in three-dimensional micro–CT scanning, please see the studies by Muller [13••], Hildebrand *et al.* [18], and Odgaard [19].

Sensitivity of micro-CT to quantify changes in trabecular morphology

High-resolution micro-CT has been instrumental in providing quantitative, three-dimensional data on baseline bone morphology, as well as the structural changes, that result from anabolic or catabolic stimuli. Recently, we used this methodology as a critical assay to test the hypothesis that short durations (20 minutes per day) of a low-magnitude (< 10 microstrain), high-frequency (30 Hz) mechanical stimulus can influence the quality of the trabecular bone in weight-bearing regions of the skeleton [15]. This can be viewed as an indication of the range of data that can be derived from micro-CT scanning. High-resolution three-dimensional models were made of the medial condyle of the distal femur, using a microtomographic fan-beam imaging system (micro-CT 20, Scanco, Switzerland). For each 8.3 mm cube of bone, a total of 300 microtomographic slices were acquired. These three-dimensional reconstructions were used to model the bone structure, with the data demonstrating a 10.6% greater trabecular bone volume fraction in the experimental animals subject to 1 year of the stimulus ($P < 0.05$) (Table 1). This increase in the density of the condyle was achieved by an 8.3% increase in trabecular number ($P < 0.01$) and an 11.8% decrease in trabecular spacing ($P < 0.01$). Not only did this stimulus increase bone quantity, but also the decrease in the trabecular bone pattern factor by 24.2% in the experimental animals ($P < 0.03$) reflected an increase in bone quality.

As another index of bone quality, the micro-CT data were used to establish the structural anisotropy (directionality) of the adaptive response by calculating the mean intercept length (MIL). MIL decreased significantly in the longitudinal direction (-8%) when compared with control trabecular samples ($P < 0.01$), while changes in the two transverse directions were less pronounced (< 4%). The quantity and quality changes measured in sheep bone is one of many examples that demonstrates the great morphologic and structural detail that data derived from micro-CT imaging can provide. In this specific case, these changes evident in trabecular bone were essentially missed when DXA scanning was used, perhaps because the relative amount of trabecular to cortical bone makes the DXA approach insensitive to more subtle changes in bone quantity realized by adaptation specific to cancellous regions.

The sensitivity of micro-CT has also been shown in disease pathogeneses where catabolic stimuli are present. For example, in an experimental model of osteoarthritis, clinical CT was able to detect a large decrease in bone fractional volume (40%) following a knee joint injury [20]; however, the exact morphologic changes that occurred could not be determined from those low-resolution data. Subsequently, in the same bones, micro-CT was able to quantify the precise morphologic changes underlying those data [21], and through the combined use of specifically designed mechanical testing methods with finite element modeling, it was possible to provide insight into the pathogenesis of this experimental model. As an example, it was established that despite the large morphologic changes, there were no associated detectable changes in bone tissue properties [14], thus indicating that architectural changes played a primary role in the early pathogenesis of osteoarthritis.

In vivo micro-CT scanning

A limitation of most recent micro-CT scanners has been their inability to scan tissues in vivo, resulting from relatively slow scanning speeds and high exposure of the sample to ionizing radiation. Currently, a number of in vivo scanners are becoming commercially available and are able to scan small animals at high resolutions and scanning speeds [22]. With this technology, scanning resolutions of up to 10 microns and scanning speeds of up to 20 times greater can be achieved, compared with models from only 3 years ago. For instance, the in vivo scan time

Table 1. Bone morphometric indices medial condyle of the femur following 1 year of 30 Hz mechanical stimulation at 0.3 g for 20 minutes per day*

	Difference, %	*P* value
BV/TV (%)	10.60	< 0.05
Trabecular number (1/mm)	8.30	< 0.01
Spacing (mm)	- 11.80	< 0.01
TBPf (1/mm)	- 24.20	< 0.03
MIL, long (mm)	- 7.80	< 0.01
MIL, A-P (mm)	- 3.50	< 0.1
MIL, M-L (mm)	- 3.90	< 0.01

*The increase in bone fractional bone volume was achieved by decreased trabecular spacing and increased number of trabeculae leading to an improved trabecular bone pattern factor (TBPf). The adaptive response, however, was directional, as the mean intercept length (MIL) showed significant difference in the longitudinal direction (long) and medial-lateral (M-L) direction, with no significant changes in the anterior-posterior (A-P) directions.
BV/TV—bone fractional volume.

for 100 sections at a 20 micron resolution, a resolution high enough for imaging trabecular bone in a mouse, can be as little as 4 minutes.

The in vivo capabilities of scanners offer many exciting opportunities previously not available, such as the use of fewer animals and the assessment of longitudinal changes within a bone for the testing of mechanistic relations between a specific stimulus and altered bone quantity and quality. The size of current scanning chambers restricts high-resolution in vivo scanning to small animals such as mice or rats. The effect of the radiation dose of scanners with in vivo capabilities has to be explored in greater detail, however, by traditional standards (based on survival), it may be considered low. The average dose per scan of 200 slices is approximately 25 mGy, compared with the lethal dose at which 50% of mice die (LD50), which is several orders of magnitude higher (2 Gy). While it is unclear at what threshold x-ray radiation affects organ and tissue metabolism, the effects of x-ray exposure on the animal can be minimized with scanners that only expose the very small volume of interest to radiation (rather than the entire animal).

For the clinician, the prediction of fracture risk has so far been restricted to the evaluation of BMD because radiation doses from CT scanning at high resolutions in humans have been prohibitive. Recently, resolutions of up to 80 microns have been achieved at a tolerable radiation limit, at least for peripheral sites. Such a resolution is high enough for a full characterization of trabecular bone morphology, and the future integration of high-resolution bone morphologic indices with estimates of its mechanical properties may ultimately facilitate the prediction of fracture risk both in real time and in the clinic [23].

Effect of altered bone architecture on mechanical properties

Assessing bone's structural and mechanical properties will identify skeletal weaknesses and give critical information on the efficacy of osteoporosis treatments. In the absence of imaging modalities that directly assess the strength of a bone, investigators have relied on determining mechanical properties such as stiffness and strength via direct mechanical testing [24]. Not only is this type of destructive testing confined to cadaveric or animal models, it can also suffer from a large degree of variability (30%–60%) associated with strain inhomogeneity, frictional end effects, as well as storage conditions and damage during machining [24–27].

Alternatively, micro-CT imaging allows the generation of micromechanical models of the scanned tissue by directly converting bone voxels to mechanical elements that can be analyzed by the finite element (FE) method. With the voxel-conversion technique, bone voxels are converted to equally shaped brick elements such that the resulting FE model has exactly the same geometry as the reconstruction it is derived from (Fig. 1). FE modeling has been successfully applied to mechanically model bone tissue [14,23,28,29], enabling the simulation of the mechanical behavior of the scanned voxel structures in silico. Similar to a true mechanical test, it can calculate the overall (apparent) mechanical properties of the structure. For instance, the FE software can simulate a mechanical compression test on a specimen, calculating the apparent stiffness as well as apparent strains and stresses for a given force. Unlike mechanical tests, however, FE modeling also has the capability to calculate tissue-level and mechanical loading conditions (*eg*, stresses, strains) for each element within the bone. High accuracy of FE models can be achieved at even relatively low scanning resolutions [30]. However, the mechanical reconstruction of large volumes can make the computational process overwhelming if not impossible. For example, generating an FE mesh of a small trabecular bone cube (8.3 x 8.3 x 8.3 mm^3, 68 µm nominal isotropic resolution) with a bone fractional volume of 50% requires approximately one million elements and significant computational power. Extrapolating the vital central processing unit power from such a small bone sample to the simulation of the mechanical behavior of a large bone (*eg*, human femur), it becomes clear that compromises with respect to scanning resolution have to be made until more advanced computer solutions are available.

As an example of the data that can be obtained from FE modeling, we tested the mechanical implications of the anabolic low-magnitude mechanical intervention described above. Specifically, we asked whether structural bone adaptations would directionally increase mechanical stiffness and affect strain and stress distribution within individual trabeculae [31]. FE models were directly generated from the previously generated micro-CT data (Fig. 1) and FE software tested each trabecular bone cube in axial compression in the three orthogonal directions. The

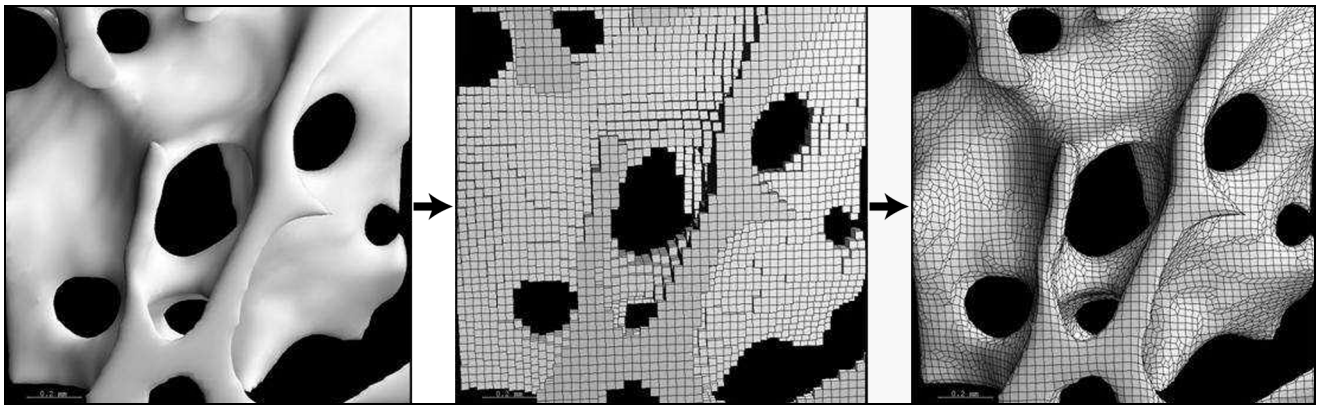

Figure 1. Transformation of the three-dimensional visualization of micro–computed tomography data from sheep trabecular bone at 34 μm resolution (*left panel*) to a voxel-based hexahedron finite element mesh (*middle panel*) to a hexahedron finite element mesh employing a surface smoothing algorithm (*right panel*).

analyses revealed that low-level mechanical vibration for 20 minutes per day significantly increased the apparent trabecular elastic modulus of the femoral condyle in all three loading directions: 17% larger in the longitudinal direction ($P < 0.01$), 29% larger in the anterior-posterior direction ($P < 0.02$), and 37% larger in the medial-lateral direction ($P < 0.01$). For a given apparent input stress in the off-axis loading directions, the resultant stresses and strains within trabeculae were more uniformly distributed in cubes of mechanically loaded sheep. There were fewer elements that were subject to extremely low strain magnitude and more elements were subject to intermediate strains. These data suggested that altered trabecular bone quantity and microarchitecture induced by low-level mechanical vibration produced large nonuniform increases in mechanical stiffness in the three orthogonal directions, and decreased strain and stress levels throughout individual trabeculae for a given applied load. The morphologic trabecular changes reduced skewness in the distributions of stress and strain, perhaps a goal of the adaptive process of bone to increased mechanical loading [8]. Thus, combining micro-CT data with FE modeling allowed the conclusion that adaptations seen in this study may effectively protect the skeleton from large off-axis loading events and lead to reduced fracture rates, the ultimate measure of any anabolic treatment with clinical potential.

Physical Properties and Bone Quality

It is apparent that the incorporation of micro-CT data into (micro)-mechanical models can be a powerful tool for assessing mechanical strength and quantifying tissue stress and strain distributions under physiologic and applied loading conditions. However, it is important to realize that only the spatial integration of physical material properties into these models will give a true estimate of bone quality and bone strength. Otherwise, changes in bone strength would be purely based on altered geometric properties and an assumed isotropic homogenous material property, which of course is not the case. Thus, bone quality is not solely dependent on bone architecture, but is also influenced by the physical properties of the material that are manifested in bone's organic and mineral components.

Chemical properties and bone mineral density

The organic matrix of bone consists primarily of collagen type I, which provides a great degree of the tensile strength of bone. Similar to reinforcing bar embedded in concrete, the collagen also provides bone matrix toughness, resilience to fracture, and ductility [32]. Changes in the content, organization, and quality of the collagen will have marked effects on the material properties of the bone, not only in terms of metabolic bone diseases such as osteogenesis imperfecta or osteomalacia [33], but on functional parameters such as a high collagen content providing resilience to impact (as needed in the horns of rams), or low collagen content providing high acoustic impedance (as is needed in the tympanic bulla) [34]. While the quantity and quality of the collagen directly influences the quality and quantity of bone, neither DXA nor CT scanning is sensitive to such changes.

Compressive strength of the bone comes primarily from the inorganic, or mineral phase of the bone, composed primarily of poorly crystalline hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. Mechanically, the ratio of matrix to mineral concentration is very important. As bone crystals increase in size, the stiffness of bone increases [35]. In addition to the mineral protein ratio, substitutions into the mineral lattice can affect bone strength as well. For example, the calcium site can be substituted by cations such as strontium (Sr^{2+}) and lead (Pb^{2+}) and the anionic sites can be substituted with carbonate (CO_3^{2-}), acid phosphate (HPO_4^{2-}), and fluoride (F^-) [36]. Fluoride increases mineral density by stabilizing the apatite lattice. Strontium also increases bone mass; however, it decreases BMD by loosening the apatite lattice and increasing bone mineral solubility [37]. These data strongly suggest that at

the molecular level, bone chemistry has powerful effects on bone quality.

Infrared spectroscopy is a valuable technique for studying the chemical composition of bone because the protein and mineral components of bone absorb infrared light at different frequencies [38]. The intensities and frequencies of all of these bands are sensitive to mineral content, crystallinity, and the content/nature of the organic matrix. Previous methods for preparing bone samples for infrared analysis included homogenization of the samples, thus precluding in situ measurements. Synchrotron infrared microspectroscopy (SIRMS) was recently developed for the quantification of both collagen and mineral content and composition, collagen cross-linking, and mineral crystallinity in situ [38,39]. Using the high brightness of a synchrotron infrared light source, this technique provides a spatial resolution of 5 to 10 µm, small enough to characterize individual trabeculae and osteons.

Because bone chemistry is related to bone's mechanical properties, it is not surprising that chemical analyses have found a lack of correlation of BMD with bone quality at the molecular material level. For instance, we have used SIRMS in situ to test whether levels of carbonate are related to changes in BMD induced by pharmaceutical treatment of monkeys for osteoporosis [40••]. Bone from untreated animals indicated a positive correlation between carbonate content and BMD (r = 0.68). However, in animals that were treated with nandrolone decanoate, an anabolic steroid known to increase bone density, the carbonate content was negatively correlated with BMD (r = -0.57) (Fig. 2). The stark differences between the treated and untreated groups dramatically demonstrate that BMD does not provide any information on changes in bone composition, and thus is not a good measure of bone quality, as represented by bone strength. These data emphasize that chemical properties are a potent determinant of bone quality, yet specific relations between chemical bone compositional parameters and bone's mechanical properties are elusive.

Characterization of mechanical material properties

It is apparent that changes in chemical properties will be reflected in changes in mechanical properties at the level of the material. Elucidation of this link in situ will provide important data on how the chemical composition of bone affects bone quality and, ultimately, fracture rates. Tackling this research question, however, will require an accurate assessment of mechanical properties at the microscale. Such an assay is also essential for determining changes in micromechanical properties of the bone matrix, which will improve our understanding of the etiology of osteoporosis as well as provide a more complete assessment of the efficacy of anabolic and antiresorptive interventions. The importance of quantifying the material mechanical properties of bone is accentuated by correlations between micro-CT/FE modeling that demonstrate that a portion of the variability in mechanical properties remains unexplained by these relations.

Over the past decade, there has been significant advances in accurately assessing the nano/micro mechanical properties of biologic tissues, such as cortical and trabecular bone, using nanoindentation. Nanoindentation evolved from techniques testing the hardness of a material by calculating its resistance to penetration of a very fine indentor using small forces (0.2–2 mN). The ability of nanoindentation to measure mechanical properties at a very small scale have led to precise measurements of mechanical properties at the material level enabling the quantification of tiny ultrastructural features in bone [41••,42].

The necessity of mechanical measurements at the level of the material becomes further apparent when considering that measurements of the bulk elastic modulus of trabecular bone not only vary by orders of magnitude (0.02–5 GPa), but trabecular moduli are much smaller than that of cortical bone (approximately 15 GPa) [24]. Clearly, bulk mechanical properties are dependent on the amount and architecture of the bone present as well as on the material properties of the trabeculae themselves. It has been presumed previously that the material properties of trabecular bone are less than those of cortical bone because of differences in structure and mineralization. However, recent data using nanaoindentation suggest that the elastic moduli from trabecular and cortical bone are, indeed, similar [43].

Nanoindentation also permits the comparison of the stiffness of adjacent lamellae, or the properties of newly added bone perhaps stimulated by a pharmaceutical agent, relative to pre-existing bone. Recent studies using nanoindentation have successfully determined the variation in material modulus across individuals and anatomic sites [44,45], providing insight into the regulation of the spatial distribution of ultrastructural mechanical properties by genetic and environmental factors. Similar to the substantial influence of variations in trabecular thickness on bone strength [46], it is obvious that variations in ultrastructural tissue moduli will influence the overall mechanical properties of bone. Thus, nanoindentation will provide insight into the spatial distributions of material properties including ultrastructural porosity or permeability of bone, and thus will help to better define how mechanical loads are tolerated by the skeleton. The generation of rigorous spatial material maps that can be incorporated into micro-CT–generated high-resolution FE models to provide estimates of bone strength, however, remains a challenge.

The complexities inherent in developing an integrative assessment of bone quality

The previous sections have described the influence of architectural, chemical, and physical factors that contribute to bone quality. However, a complete assessment of bone

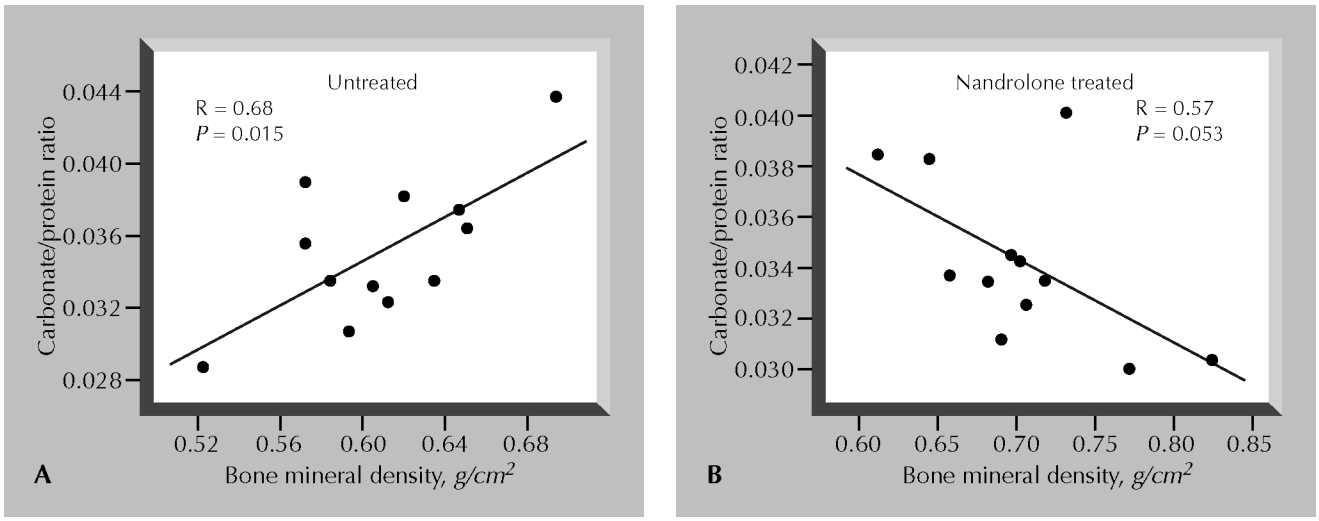

Figure 2. Infrared microspectroscopy data demonstrating the relationship between carbonate content and bone mineral density in animals that were untreated (**A**) or treated with nandrolone (**B**) for osteoporosis.

quality must consider other factors, equally important, but less readily quantified. For example, microdamage in bone, accumulated by repetitive loading (and perhaps perturbed by antiresorptive intervention), and its effect on skeletal integrity have received considerable attention in recent years [47]. While the mechanical and biologic consequences of microdamage on bone can be grave, little is known about the in vivo functional environments that induce microdamage and the mechanisms that prevent its repair. Certainly, the development of techniques, noninvasive or invasive, that will allow a more direct assessment of microdamage will enhance our ability to determine the degree to which this parameter is, or is not, relevant to bone quality.

In addition to microdamage, there are a great number of other parameters that may directly or indirectly affect bone quality including, but certainly not limited to, the spatial distribution and density of secondary haversian remodeling [48], collagen orientation and morphology [49], or proteoglycan orientation [50].

On top of physical and morphologic factors, biologic factors such as cell viability or the integrity of gap-junctional proteins for cell communication are typically neglected in the discussion on bone quality but, at least for a wider definition of bone quality, should be included. For example, bisphosphonates exert their bone sparing effects essentially by poisoning osteoclasts [51], stabilizing bone quantity but potentially jeopardizing specific aspects of bone quality by disrupting biologic processes critical to long-term bone health such as osteoclast-osteoblast coupling and remodeling activity. At the level of the DNA, we have recently demonstrated that variations in genetic make-up influence the responsivity of the skeleton to either anabolic or catabolic stimuli [52]. Because such relationships will help us to understand why some people ultimately suffer from osteoporosis while others do not, genotypic differences also reflect an aspect of bone quality.

It may be argued that disturbances in biologic factors of bone quality will ultimately affect physical and morphologic parameters of bone quality. However, a better understanding of the contributions of these biologic factors to bone quality may present an opportunity for a very early assessment of deteriorating bone quality or an inability to improve by interventions of low bone mass.

Conclusions

Bone strength is a central predictor of fracture risk, and a better understanding of the factors influencing strength will lead to an improved diagnosis of osteoporosis and the ability to assess the efficacy of treatments of the disease. Current clinical skeletal assessments by DXA focus on bone quantity, yet it is becoming more and more apparent that assessing bone quality is as critical for accurately estimating fracture risk. Bone architectural indices, combined with the chemical and mechanical features of the bone matrix, have a significant influence on bone quality, but the precise effects of both morphologic (*eg*, connectivity density or trabecular thickness) and material (*eg*, crystallinity, phosphate content, or material modulus) effects remain incompletely understood. Even more subtle parameters such as collagen orientation, microdamage, and cell responsivity may contribute significantly to the quality of bone, yet it is not yet clear how to assess this in a coherent, integrative fashion. Future research elucidating the link between these variables together with technologic advances in the assessment of bone's morphologic and physical material properties, performed noninvasively in humans, will ultimately facilitate the evaluation of bone strength both in the clinic and perhaps in real time.

Scanning and ultrasound technology known as acoustic confocal ultrasound scanning has the potential to make significant progress toward this goal in the near future [7]. In contrast to radiologic techniques, ultrasound is capable of characterizing bone's physical properties, and recent advances in these technologies now permit confocal scanning at resolutions of 500 microns. However, even with advances in technology and in our understanding of bone quality, it must be appreciated that factors well beyond bone quantity and quality contribute to fracture risk. Thus, systems as diverse as vision, vestibular, muscular, or neural will also have to be considered to obtain an accurate estimate of fracture risk.

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