In vivo Assessment of Bone Quality without X-rays

by

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Abstract

Purpose of Review: This review summarizes recent advances in the assessment of bone quality using non-X-ray techniques.

Recent Findings: Quantitative ultrasound (QUS) provides multiple measurements of bone characteristics based on the propagation of sound through bone, the attenuation of that sound, and different processing techniques. QUS parameters and model predictions based on backscattered signals can discriminate non-fracture from fracture cases with accuracy comparable to standard bone mineral density (BMD). With advances in magnetic resonance imaging (MRI), bound water and pore water, or a porosity index, can be quantified in several long bones in vivo. Since such imaging-derived measurements correlate with the fracture resistance of bone, they potentially provide new BMD-independent predictors of fracture risk. While numerous measurements of mineral, organic matrix, and bound water by Raman spectroscopy correlate with the strength and toughness of cortical bone, the clinical assessment of person's bone quality using spatially offset Raman spectroscopy (SORS) requires advanced spectral processing techniques that minimize contaminating signals from fat, skin, and blood.

Summary: Limiting exposure of patients to ionizing radiation, QUS, MRI, and SORS have the potential to improve the assessment of fracture risk and track changes of new therapies that target bone matrix and micro-structure.

Introduction

There is a long-standing recognition that the clinical assessment of a person's risk of an osteoporotic fracture would benefit from measurements of bone quality [1]. This is because areal bone mineral density (aBMD in g/cm^2) from dual-energy X-ray absorptiometry (DXA) scans of the hip and spine misses many individuals at risk of a fragility fracture when based on T-score thresholds [2]. To date, the most common clinical measurement of bone quality is the trabecular bone score (TBS) [3]. Interpreted as an indicator of trabecular microarchitecture, TBS comes from a texture analysis of the L1-L4 images in a DXA scan of the spine [4]. It is the slope of the variogram or gray-level variations vs. distance between pixels in the DXA image. As such in its current use, clinical TBS requires images generated by ionizing radiation, albeit at a minimal dose (~9 μSv). It also does not assess the contribution of the organic matrix of bone to age- and post-menopausal increase in fracture risk.

For direct measurements of trabecular microarchitecture and cortical microstructure, high resolution, peripheral quantitative computed tomography (HR-pQCT) scanners can generate three-dimensional (3D) images of the distal radius and tibia diaphysis with an isotropic voxel size of ~60 μm and a low radiation dose (~3 μSv) [5]. The resolution is sufficient for image processing algorithms to determine multiple quantitative parameters such as trabecular bone volume fraction (%), cortical thickness (mm), and volumetric bone mineral density (vBMD in mgHA/cm^3). More importantly, these parameters are relatively accurate in discriminating patients with an incident fracture and those without a fragility fracture [6,7]. Nonetheless, like DXA, it is only sensitive to the mineral phase of bone as attenuation of X-rays by the organic matrix is negligible.

To advance the assessment of fracture risk and the prevention of fragility fractures, new *clinically* viable techniques are needed that do not require ionizing radiation and are sensitive to the contribution of the bone matrix, not just aBMD or vBMD, to the fracture resistance of bone. From numerous studies investigating the mechanical behavior of bone, the organic matrix of bone confers plasticity to bone or the ability to inelastically deform and to resist crack growth without the bone breaking (for reviews, see [8,9]). Despite the long-standing observation that bone toughness (or lack of brittleness) decreases to a greater extent than bone strength with aging [10–12], the clinical determination of whether a patient has osteoporosis does not include any direct indicator of the inherent integrity of the organic matrix. Without being able to track changes in the fibrillar, interconnected network of collagen I, water, and various non-collagenous proteins, the effectiveness of osteoporosis medications that target the extracellular matrix of bone cannot be assessed without costly clinical trials with fracture as the endpoint.

Herein, we summarize the latest findings from studies that applied 3 different non-X-ray techniques to bone: quantitative ultrasound (QUS), magnetic resonance imaging (MRI), and spatially offset Raman spectroscopy (SORS). While there are other laboratory techniques that assess characteristics of bone and its hierarchical organization (Fig. 1A), these three can assess bone quality in vivo and are clinically viable.

The Use of Ultrasound to Measure Bone Quality

QUS is a cost-effective and portable modality that can measure bone properties at peripheral skeletal sites using sub-audible, high-frequency sound waves. IT analyzes bone by calculating ultrasound velocity (speed of sound, SOS, in m/s) and attenuation (broadband ultrasound attenuation, BUA, in dB/MHz) parameters. Specifically, SOS is the sound wave transmission time divided by the length (i.e., the distance between transducer and receiver) of the tissue being studied (e.g., bone), and BUA is the slope of the frequency-dependent attenuation vs. frequency as the signal travels through bone. SOS is indicative of mechanical and structural characteristics of bone, including elasticity, strength, and trabecular microarchitecture [13], while BUA is influenced by structural properties (bone size, volume, and trabecular orientation; Fig. 1A) because of sound wave absorption and scattering [14]. SOS and BUA can be combined to provide additional information, such as the stiffness index (SI) or quantitative ultrasound index (QUI) using GE Healthcare or Hologic QUS devices, respectively. Thus, changes in bone composition and mechanical properties are reflected in alterations of the speed, shape, and intensity of the propagating ultrasound waves, offering valuable insights into microarchitecture, cortical thickness, elasticity, and strength (Fig. 1B) [15,16].

While DXA is the gold standard for bone density measurements, QUS offers a non-ionizing radiation-free alternative for screening and monitoring bone health. Correlations between QUS-derived SOS and BUA and DXA-derived BMD have been extensively explored since the technology's application in bone was documented in 1984 [17,18]. Subsequently, numerous prospective and/or cross-sectional clinical studies have focused on the potential of QUS to predict fracture risk or discriminate individuals who had previously fractured across age and disease, with perhaps the best performance in predicting fracture risk in Caucasian and Asian women over the age of 55 [19,20]. Trabecular sound transmission QUS at the calcaneus, known as heel-QUS, is the most utilized method to determine bone density and is supported by empirical evidence that it is sensitive to age-related bone loss [21]. In a recent heel-QUS study, a 1 standard deviation (SD) in SOS, BUA, or SI was significantly associated with a higher risk of a major osteoporotic fracture (MOF) over subsequent 6.7 years after adjusting for FRAX MOF score, treatment, femoral neck BMD, and/or TBS [22]••. However, in the same study, the percent change in these QUS parameters over 2.5 years was not associated with the odds of experiencing a MOF in the next 5 years. Nonetheless, QUS has the potential to detect changes in bone density over short periods of physical activity [23]. While heel-QUS can monitor treatment-related changes in bone density, it may require longer timeframes (3-4 years) compared to DXA (6-12 months) [24,25]. Moreover, stand-alone QUS is not recommended for treatment decisions in osteoporosis, and establishing specific thresholds for QUS devices and parameters through cross-validation is crucial to ensure a well-defined and reliable clinical use of QUS [26].

Research finds that SOS and BUA are negatively correlated with one another as osteoporosis worsens [22] and age increases [27] and can differentially respond to disease and bone-targeting treatments [28]. These observations suggest that QUS may be sensitive to intrinsic bone properties beyond traditional BMD measurements. Moreover, unlike other imaging modalities, ultrasound waves are naturally suitable for assessing bone mechanical properties [29] providing a unique opportunity to derive mechanical properties non-invasively.

Relating Mechanical and Material Properties to QUS parameters

Hans et al. described the relationship between the velocity of ultrasound waves propagating through bone (i.e., SOS) and the mechanical properties of bone as follows:

$SOS = (E/\rho)^{0.5}$

where E is the elastic modulus of bone and ρ is bone density [30]. Several early ex vivo studies described relationships between SOS parameters and mechanical properties of bone [15,31,32]. In vitro, heel-QUS BUA demonstrated a moderate correlation with elastic modulus ($r^2=0.64$, $p<0.0001$) as measured through compression tests on calcaneal trabecular bone [33]. Additionally, both heel-QUS BUA and SOS were correlated with proximal femoral strength ($r^2=0.70$ and 0.67, respectively, both $p<0.0001$) in femoral specimens subjected to failure simulations of a fall [34]. Using femoral donor specimens from elderly individuals, Peralta et al. demonstrated that ultrasound velocities propagating along or perpendicular to osteons were correlated with compressive strength [35]. Linear regression modeling showed that a decrease in ultrasound velocity of 100 m/s corresponded to an approximate loss of 10% in the maximum observed strength, with waves propagating along the osteons (shear) having a slightly higher sensitivity.

Fracture toughness, the material characteristic that represents the bone's ability to resist crack propagation or fracture, is an essential component of understanding fracture risk and is distinct from bone strength [36]. Cook et al. evaluated the relationship between QUS parameters at four sites (calcaneus, phalanx, radius, mid-shaft tibia) and fracture toughness at the femoral neck in 20 patients undergoing emergency surgery for a fractured femoral neck [37]. The study found significant relationships between in vivo QUS and fracture toughness values in femoral head specimens, particularly when notched across the main trabecular direction. Moving in vivo, impact micro-indentation (IMI) measures of the bone material strength index (BMSi) using the OsteoProbe in 377 men (33-96 years) were taken and compared to QUS measures of SOS, BUA, and SI acquired at the calcaneus [38]. The authors observed a positive association between QUS parameters and BMSi, which were not independent of age. Although the positive correlation coefficients were small, the observation is promising since IMI determines the ability of cortical bone to resist micro-indentation at high loading rate in vivo and is thought to be related to bone quality aspects such as microporosity, collagen, degree of mineralization, bone water, and tissue homogeneity [39,40]. Studies are needed to establish the sensitivity of QUS measurements to specific aspects of bone across its length scales.

Cortical Microstructural Assessment by QUS

Cortical QUS can be obtained using various modes, including transverse transmission and its variations, as well as axial transmission and its variants, such as bi-directional axial transmission, with several groups working towards methodologies to calculate cortical porosity, cortical pore density, and cortical pore size with QUS. The QUS ultrasound wave propagation at cortical sites is influenced by bone micro-structure, primarily due to the acoustic impedance contrast between the pores and the solid cortical matrix. The acoustic impedance difference causes the ultrasound wave to take random paths as scattering occurs within the heterogeneous bone. When ultrasound waves scatter, energy is lost; and in cases of multiple scattering, a diffusive regime, or diffusion constant, is observed. Numerical simulation studies indicate that an increase in pore density and pore size each cause a decrease in the ultrasound diffusion constant [41]. The statistical parameter Shannon Entropy quantifies uncertainty in a system, and when applied to measure the randomness of ultrasound backscatter in a numerical simulation study of cortical bone, the authors report that a positive correlation between cortical porosity and entropy was observed for bone having a porosity less than 15% [42]. When higher porosity was present in cortical bone, entropy plateaued. In a phantom study with porosity ranging from 0-25%, Grasel et al. utilized axial transmission and separately measured velocities in three different directions (axial=0° and

±37.5°) to calculate an anisotropy index which could estimate porosity with an accuracy error of 1.5% [43].

Raum and colleagues have introduced the cortical bone backscatter model, which utilizes the frequency-dependent and backscatter coefficients at the tibial site to estimate the distribution of cortical porosity and pore sizes [44]. In an ex vivo study on human bones, the combination of backscatter parameters could predict all cortical pore properties including pore density and diameter with an adjusted R^2 of ≤ 0.59 [44]. In a cross-sectional pilot study conducted in vivo at the tibial midshaft, the cortical pore size distribution parameters derived from the model exhibited good discriminatory ability for fragility fractures and showed moderate agreement with site-matched HR-pQCT values [45]. In another in vivo study, bi-directional axial transmission QUS acquired at the radius to measure cortical porosity in vivo showed a strong positive association between cortical porosity and all non-traumatic fractures in the cohort of postmenopausal women [46]. The association was independent of femoral neck DXA-derived aBMD. The bi-directional axial transmission technique unfortunately fails when there is too much soft tissue (particularly in cases where BMI > 28 kg.m-2) [46]. Although promising, current research on assessing cortical porosity has predominantly involved numerical modeling, ex vivo phantom and cadaveric studies, and smaller in vivo proof-of-concept work. Further investigations are needed to evaluate the effectiveness of quantifying cortical porosity in vivo using QUS and determine if this measure can serve as an additional promising application of QUS.

QUS and Bone Matrix Collagen

There is little information regarding how alterations in the organic matrix (collagen I) of bone impact QUS parameters, but several early studies have documented associations between collagen abnormalities and mutations and QUS. A 1999 study by Cheng et al. evaluated females with two diseases related to collagen mutations, Ehlers-Danlos syndrome type III and systemic sclerosis, using QUS [47]. The authors reported that Ehlers-Danlos syndrome patients had lower BUA than controls but BMD values within the normal range. The difference in BUA remained even after the authors adjusted for height, weight, and BMD. Systemic sclerosis patients also had lower BUA compared to controls, normal BMD of the spine, and lower aBMD of the hip. The authors discussed the interesting disparity between BUA via ultrasound and aBMD and questioned whether quantitative ultrasound measurement is 'a function of more than just the mineral component (perhaps collagen matrix and bone fluid are involved)?'. A 2002 study systematically evaluated the impact of collagen and mineral on QUS parameters of SOS and BUA using chemically treated specimens to deproteinate or demineralize bone [48]. Deproteinated samples had a 10-12% decrease in SOS and a 35-75% increase in BUA. The demineralized samples had a 19-39% decrease in SOS and a 44-58% decrease in BUA. They conclude that QUS may yield valuable information about the content, and perhaps organization, of both collagen and mineral in bone. Kann et al. examined the ability of SOS measurements to detect changes in collagen and, consequently, fracture risk in individuals with G to T polymorphism in the Sp1 binding site of collagen I alpha 1 (COLIA1) [49]. The study found a connection between SOS and the COLIA1 Sp1 polymorphic genotype, indicating that the mutation primarily affects the elastic properties of bone rather than its structural aspects, as measured by BMD. More studies are needed to systematically determine the impact of collagen and bone hydration on ultrasound velocity and attenuation in bone.

Fracture Risk Assessment by Radiofrequency Echographic Multi-Spectrometry (REMS)

REMS utilizes the raw, unfiltered backscattered ultrasound pulses (e.g., the radiofrequency signals, RF) to obtain spectral measurements, which retain all feature information from the target tissue. REMS is employed along with conventional echography, which produces images where the region of interest (e.g., the bone) can be automatically segmented for analysis (Fig. 1A). REMS has permitted the direct investigation of commonly fractured axial skeletal sites, such as the lumbar spine and the femoral neck, with excellent repeatability [50,51], although, in theory, any bone site could be measured. For clinical interpretation, the acquired multi-spectra are compared to anthropometrically matched databases (>15,000 people) of the corresponding model for the disease being evaluated, namely osteoporosis, or against otherwise healthy bones [52,53].

Processing the raw unfiltered REMS spectra yields the Osteoporosis Score, which is used to derive the REMS-BMD value through a linear equation [52,53]. Early prospective studies demonstrate good agreement between REMS-BMD and DXA-derived BMD, with low inter-operator variability (RMS-CV) reported at the femoral neck and lumbar vertebra sites (0.32% and 0.38%, respectively) [54]. REMS demonstrated strong discriminative ability for fragility fractures in patients aged between 30 and 90 years, achieving high sensitivity and specificity with areas under the curve (AUC) of the receiver operating characteristic (ROC) of 0.63 and 0.68 ($p<0.0001$) for femoral neck (young and old, respectively), and 0.60 and 0.64 ($p=0.0002$) for lumbar (young and old, respectively) spine [55]. Quarta et al. demonstrated the potential of REMS-BMD in monitoring short-term skeletal changes induced by denosumab treatment, as REMS, along with DXA-BMD, successfully detected changes at the lumbar spine after one year, with REMS showing continued skeletal improvements at 18 months, offering a promising alternative to DXA for shorter follow-up intervals (abstract [56]). In addition to REMS-BMD, REMS calculates the Fragility Score (FS). The REMS FS, a BMD-independent fracture risk estimator, is proposed as a valuable tool for assessing bone strength and quality [57]. The FS ranges from 1 to 100 and utilizes reference models for patient comparisons between those that have fractured exclusively on the hip or had a major osteoporotic fracture and subjects without a fragility fracture [58]. It has demonstrated a significant correlation with FRAX scores [57] and higher sensitivity and specificity than DXA in distinguishing fracture and non-fracture cases [59,60]. In a cross-sectional study by Pisani et al., the Fragility Score could discriminate between post-menopausal Caucasian women with osteoporotic fractures at the lumbar spine and age- and BMI-matched women without a fracture (AUCs): 0.76 (L1-L4 aBMD) and 0.76 (FS) [59]. In a follow up, prospective study of incident fragility fractures at any site over 5 years, the AUC of the FS, as determined from REMS of the lumbar spine and femoral neck, was 0.811 ($p<0.0001$) and 0.780 ($p<0.001$), respectively, for females and 0.780 ($p<0.0001$) and 0.809 ($p<0.0001$), respectively, for males [61]••.

While the current body of research on REMS has predominantly focused on its application in osteoporosis, REMS has the potential to be effectively utilized in discerning fracture risk in other diseases, particularly those where DXA has clear limitations. In patients with type 2 diabetes mellitus (T2DM), DXA is known to have limited predictive value [62]•. To address this, a recent study aimed to assess the utility of REMS compared to DXA in evaluating bone status in postmenopausal women with T2DM [63]. While DXA-derived BMD values were unsurprisingly higher in T2DM participants compared to controls, REMS-estimated BMD values were lower in T2DM participants, resulting in a higher classification of osteoporotic T2DM women compared to DXA. In young women with anorexia nervosa, despite the increased risk of fragility fracture, studies have revealed a lack of correlation between DXA-derived BMD and fracture risk [64,65]. Caffarelli et al. showed that REMS BMD at the hip was significantly lower in anorexic women with a previous vertebral fracture but not DXA-derived BMD [66], suggesting REMS could provide information about bone quality changes in anorexic women, but further studies are needed. For patients with chronic kidney disease (CKD) who were on dialysis, REMS agreed with DXA measures at both the lumbar spine and femoral neck sites [67]. Sensitivity of REMS to the early skeletal changes in CKD, characterized by collagen modifications, bone hydration, and significant cortical microarchitecture changes, is unknown. Further research is required to elucidate the specific aspects of bone quality and microstructural characteristics that significantly influence REMS values and determine the extent of change necessary to be detected by REMS.

The Use of Magnetic Resonance Imaging to Improve the Assessment of Fracture Risk

MRI is a versatile imaging modality which can be used to visualize and quantitatively characterize tissue structure, but it can also be used to probe molecular and microstructural composition of tissue. There is a long history of investigating MRI as a tool for evaluating bone fracture risk, with much of which is captured in previous reviews [68–70]. Here, we describe three categories of clinically viable MRI measurements of bone and discuss recently reported experimental and clinical findings associated with these categories.

Standard clinical MRI provides essentially no signal from cortical bone or the trabeculae within cancellous bone, but these structures can be identified and quantitatively evaluated as the void or hypointense regions within surrounding soft-tissue signals. A challenge for such measurements is that typical MRI image resolution provides voxel volumes > 1 mm^3, which is insufficient to characterize some cortical bone and all trabecular micro-architecture/structure. The ability to achieve higher resolution, particularly in trabecular regions, is helped by the relatively high fat content of bone marrow: the relatively fast longitudinal relaxation of fat proton magnetization allows for rapid acquisition repetition and, in-turn, relatively high resolution. Nonetheless, reaching sufficient resolution and signal-to-noise ratio in a reasonable scan time has been a challenge, which initially limited scans to peripheral sites, such as the distal radius [71,72], calcaneus [73], or distal tibia (Fig. 1B) [74]. Through methodological and technical developments, studies have extended to the proximal femur [75–78], and one recent study made use of parallel imaging and compressed sensing to reach 400 μm isotropic resolution in 6 min [79]•.

Similar to analyses of HR-pQCT scans of trabecular bone, various quantitative metrics can be derived from high resolution MRI scans of trabecular bone (e.g., bone volume fraction, trabecular thickness, erosion index, etc.), and finite element analysis (FEA) of the bone volume fraction maps can be used to estimate elastic modulus. For example, some trabecular architecture metrics from MRI of the proximal femur were different between a small cohort of HIV infected men and controls, consistent with similar measures in the distal tibia by HR-pQCT but in contrast to aBMD measures by DXA [78]. Another study used MRI of the proximal femur and subsequent FEA and found a lower elastic modulus in post-menopausal women with fractures compared to those without, while no differences in DXA T-scores was found between the two groups [80]. A similar story was found between long-term glucocorticoid users and controls, with differences in both metrics of trabecular architecture (trabecular number and plate-to-rod ratio) and modulus from FEA; and again, there was no difference in DXA T-scores between the groups [77].

In addition to the direct evaluation of bone structure, MRI has been used to investigate the composition of bone, particularly cortical bone, for the purpose of characterizing its material properties. This approach builds off non-localized NMR studies of the proton relaxation characteristics of cortical bone specimens [81–85]. Amongst other findings, these early studies established that the proton NMR signals from cortical bone can be largely attributed to one of three sources: i) tightly bound collagen protons, ii) water bound within the collagen matrix (bound water), and iii) free water within the bone pore network (pore water). These three signals can be distinguished by their different transverse relaxation characteristics: the non-aqueous protons decay extremely rapidly, with signals that largely disappear within 100 μs; bound water magnetization has a rapid and highly reproducible transverse relaxation time constant, $T_2 \approx 400$ μs; and pore water magnetization exhibits T_2s that vary widely, between ≈ 1 ms and ≈ 1 s, presumably due to the widely varied size of pore spaces within cortical bone (Fig. 1A). Importantly, both bound and pore water concentrations have been found to reflect bone mechanical properties of bone specimens [86–88], making them potential biomarkers for bone fracture risk. As bone ages and deteriorates, the mineral and matrix are lost and pore space increases, resulting in lower concentrations of bound water and higher concentrations of pore water. Further, mechanical properties of bone may be reflected in bound water concentration through dehydration [89] or alterations of collagen integrity that accompany the age-related increase in bone brittleness [90,91]. However, measuring bound and pore water concentrations in a clinical setting brings its own challenges.

As noted above, conventional MRI provides little or no signal from cortical bone, partly due to the relatively low proton density of bone, but more importantly, due to the rapid decay of most of the transverse magnetization. However, the advancement of ultra-short echo time (UTE)

MRI methods has enabled quantitative MRI studies of cortical bone [92–94]. With these acquisition methods and leveraging the understanding of bound and pore water relaxation characteristics resulting from the aforementioned non-localized studies, an assortment of UTE-MRI methods have been developed to measure bound and/or pore water contents in cortical bone.

One approach for bound and pore water imaging involves the use of T_2-selective adiabatic RF pulses to suppress signal from either bound or pore water in order to directly image the other signal [95–97]. For the case of imaging bound water, the adiabatic inversion recovery (AIR) method uses a T_2-selective adiabatic inversion pulse to invert the relatively long-T_2 pore water magnetization while saturating the short-T_2 magnetization of bound water. Following a suitable inversion-recovery delay, the pore water magnetization recovers to near zero, while the bound water magnetization substantially recovers towards its equilibrium state. A subsequent UTE-MRI acquisition thus produces an image that is largely derived from bound water. Similarly, to image pore water, the double adiabatic full passage (DAFP) method makes use of two consecutive T_2-selective adiabatic inversion pulses to saturate the bound water magnetization while rotating pore water magnetization though 360°, finishing near its equilibrium state. A subsequent UTE-MRI acquisition thus produces an image that is largely derived from pore water.

Both AIR and DAFP rely on signal from a reference marker and known or assumed relaxation characteristics of bound and pore water magnetization to convert the AIR or DAFP image intensity into a measure of bound or pore water concentration, respectively. The use of the reference marker is manageable and has been used in earlier quantitative MRI measurements of total bone water concentration [93,94], but it does introduce a cost in precision and potentially bias. The relaxation characteristics of bound water are reasonably well known and highly consistent across individuals, but pore water relaxation characteristics vary widely within bones and between individuals [98]. The variations in pore water T_1 primarily impact the accuracy of bound water measurements, but the errors tend to result in overestimating bound water concentration in bones with more bound water and underestimating in bones with less bound water, thereby presumably retaining the monotonic relationships between measured bound water concentration and various material properties of bone [98].

Several alternative approaches have been developed for investigating the bound and pore water contents of cortical bone with MRI. One alternative to DAFP is to compute pore water concentration from the difference between bound water concentration (from AIR) and total water concentration from proton density weighted UTE acquisition [99,100]. A similar approach could involve using only DAFP and proton density UTE, avoiding the AIR acquisition. Either approach would potentially minimize the effect of inaccurate relaxation assumptions because those would have less effect on the proton density weighted image, but to our knowledge, this has not been rigorously investigated. Another approach altogether involves the use of bi- or tri-component signal model to fit bound and pore water signal amplitudes from multiple images with varied T_2* weighting [101–104]. This approach does not require a reference marker signal because the relative amplitudes of the bound and pore water concentrations are typically estimated as percentage; however, bound and pore water signals may not be well separated by T_2* at magnetic field strengths of 3 T and above [95,105]. Lastly, simpler metrics based on the ratio of two images have also been used to track porosity: the ratio of unsuppressed and long-T_2 suppressed (e.g., AIR) images provides a metric known as the "saturation ratio" [106], and the ratio of long echo time and UTE images provides the "porosity index" [107,108], and the ratio of two UTE images acquired with different repetition times has been used to estimate T_1 [109], which will tend to increase with increasing porosity. These image ratio measures, like the bi-/tri-component fitting, avoid the need for a reference signal, but they lack the ability to provide independent measures of bound and pore water concentrations.

While these UTE-MRI methods of interrogating bound and pore water concentrations have been under development for more than a decade, only recently have they been investigated in vivo in patient populations. A 2021 publication reported porosity index as correlating with categorical definitions of the stage of chronic kidney disease [110]●, and four 2023 publications report studies of patients with osteoporosis. Jerban et al. found both porosity index and saturation ratio measured from the tibia mid-shaft to distinguish individuals with osteoporosis (DXA T-score < -2.5) from those with osteopenia (DXA T-score between -2.5 and -1), as well as from normal healthy controls [111]. Similarly, Jones et al. used a version of AIR and proton density weighted UTE in the tibia of patients with osteoporosis and healthy controls and found differences in pore water concentration and total water concentration, but not in bound water concentration [100]. Finally, Nyman et al. reported results from AIR and DAFP imaging in the tibia and the radius of patients with osteoporosis and healthy controls [112]●●. In the studies of the tibia, bound water but not pore water concentration was found to distinguish patients (DXA T-score < -2.5) from controls (DXA T-scores > -1). In the studies of the radius, both bound and pore water concentrations distinguished patients with recent fragility fracture from controls, and the *combination* of bound and pore water concentrations was found to be the best overall classifier of fracture vs controls, including in comparison to models using DXA measures as predictors.

The Translation of Raman Spectroscopy toward the Assessment of Bone Matrix Quality

As described in recent reviews of spectroscopy techniques applied to bone [113,114], Raman spectroscopy (RS) captures the intensities of photons after they interact with molecular bonds of a material like bone and does so as a function of the shift in wavelength of the incident light (e.g., 785 nm laser). The shift is the result of the energy loss that occurs during *inelastic* scatter of incident photons as they encounter different molecular vibrations in the material. Thus, one advantage of RS is the molecular specificity that it provides. On the other hand, one disadvantage of RS is the weak signal of the *inelastic* scatter that necessitates well aligned optics and an efficient detector. Most of the incident light undergoes *elastic* scatter in which photons do not undergo a change in energy. Due to numerous advances in RS over the past several decades, multiple Raman spectra of bone with well characterized peaks can be acquired within 2-to-15 minutes. The quality of the spectra generally improves with acquisition time.

As applied to bone, other advantages of RS include: i) non-destructive with minimal sample preparation [114], sensitivity to mineral [115], collagen [116], and water [117] such that it quantifies various compositional characteristics (e.g., mineral-to-matrix ratio, type B carbonate substitution, crystallinity, collagen-bound water) that correlate with the fracture resistance of cortical bone [114,118], ii) ability to quantify glycosaminoglycans [119], advanced glycation end-products [120], and lipid content [121], and iii) amendable to in vivo assessment of bone [122]. Other disadvantages of RS include: i) relative, not absolute, quantification of bone composition, ii) expert knowledge in the acquisition and processing of the inherently noisy Raman spectra in which background fluorescence must be removed, and iii) numerous overlapping bands (i.e., different compounds with similar vibrational characteristics) that can obscure important peaks of bone [114].

Spatially offset Raman spectroscopy (SORS) is the primary technique by which RS is being translated into a clinical tool for diagnostic purposes [123]. In this technique, fiber optics transmit the laser to the surface of skin, and the acquisition of the Raman signals from bone involves fiber optics that are offset a set distance from the excitation source. The depth of light interactions with molecular bonds increases as the offset distance increases because Raman-scattered photons from deep layers migrate further in the lateral direction (i.e., away from incident light) than Raman-scattered photons from superficial layers [124]. There are still signals from skin, fat, blood, and possibly muscle that interfere with key Raman peaks of bone. Moreover, affecting the quality of Raman spectra, the laser power must not exceed the maximum permissible exposure limit for skin.

In the first in vivo transcutaneous acquisition of Raman signals from human bone, Matousek et al. developed a SORS probe in which a ring of 26 fibers (diameter of silica = 200 μm) had a 3 mm offset from the fiber optic transmitting the input laser (827 nm), and they collected Raman signals from the distal thumb of one volunteer using an acquisition time of 200 s and a laser power of 2 mW [125]. Although the acquired Raman spectrum had noise, the probe detected mineral peaks ($v_1PO_4^{3-}$ and CO_3^{2-}) and several collagen-related peaks (Amide III, CH_2-wag, and Amide I). Since their SORS probe also collected Raman signals with a ring of 6 fibers with no offset (i.e., distributed adjacent to excitation fiber), the difference spectra – 3 mm offset spectrum minus 0 mm offset spectrum – minimized the interference from the skin. In a follow up study by the same group, transcutaneous spectra were acquired from the phalangeal bones (finger) and the anteromedial surface of the proximal tibiae (shin) using a SORS probe with 33 fibers (200 μm in diameter) at the center, while the laser was delivered through a ring of fibers (1 mm in diameter) with an offset distance from the center equal to either 5 mm (finger) or 8 mm (lower limb) [126]. In this inverse SORS approach, the laser power was higher (180 mW) than the previous study because of the higher illumination area. Using a 60 s acquisition time, SORS spectra were acquired from 10 post-menopausal women being treated for osteoporosis (hip or spine T-scores < -2.5) with a bisphosphonate and 6 adults without osteoporosis (4 men and 2 women). Upon spectral decomposition by band target entropy minimization (BTEM) to minimize contamination from skin, fat, and blood, prominent peaks of bone were detected ($v_1PO_4^{3-}$ CO_3^{2-}, Amide III, CH_2-wag) with noticeable variance among the 16 subjects. However, in this small cohort study, no significant differences in Raman properties were detected [126].

The development of SORS for the in vivo assessment of bone quality has primarily been done using pre-clinical models or cadaveric bone (see previous reviews [114,124]). One promising approach to come out of these studies is the simultaneous, over-constrained, library-based decomposition (SOLD) of SORS-derived spectra involving transcutaneous acquisition of Raman spectra. In the approach, the decomposition or unmixing of the contaminated signal (overlying soft tissue) from the target signal (bone) involves fitting the SORS spectra to spectra acquired directly from bone tissue and overlying soft tissues (dermal, adipose, and muscle) that contaminates the bone signal [127]. Applying “top layer subtracted or tls” technique to SOLD based on spectral libraries generated from ex vivo Raman spectra of 108 mouse femurs and tibiae as well as 165 soft tissue samples (leg muscle, cartilage, skin, and fat), Chen et al. acquired SORS spectra (3 rings of fibers surrounding excitation fiber at 0.2 mm to 0.5 mm) through skin from the tibia of live mice between 4 weeks and 23 week of age [128]••. Using partial least squares regression models, the "tls" spectra, which is derived from SOLD fitting, could explain 95% of the variance in vBMD (ex vivo micro-computed tomography) and 68% of the variance in bone strength (ex vivo torsional testing) [128]. By subtracting off the top layer estimate, as given by the SOLD algorithm, from the SORS signal of the outer ring (primarily the bone tissue), "tls" essentially retains both Raman characteristics of bone, as given by the SOLD algorithm, and residual information that is specific to the limb being analyzed. To date, SOLD and "tls" have not be developed for human bone and human soft tissue, but work by the same group involving SORS-derived spectra from 2 cadaveric hands and murine-derived SOLD spectral libraries indicates that an offset of 4 mm to 6 mm from the incident light maximizes the ratio of phosphate Raman signal-to-noise [129]•.

Lastly, two recent SORS studies investigated whether a probe with 3 rings of fiber optics at offsets of 5 mm, 6 mm, and 7 mm from a central excitation source could detected ex vivo treatment effects on traditional Raman properties [130] and sub-peak ratios within the Amide I band [131]. The selected treatment was autoclaving 10 cadaveric femur mid-shafts in water because the increase in pressure and temperature reduced the ultimate stress of cortical beams from the 10 donors [130]••. Acquiring SORS signals through tissue phantoms as function of increasing layers between the bone surface and SORS probe and doings before and after autoclaving, a cut-off in the thickness equal to 4 mm was found for $v_1PO_4^{3-}$/Amide III and $v_1PO_4^{3-}$/(proline+OH-proline), irrespective of offset. The relative change in these band area ratios before treatment and after treatment matched direct measurements when SORS probe contact the bone surface [130]. The cut-off for the Amide I sub-peak ratios was 2 mm [131]. This highlights the challenges of acquiring Raman signals through overlying tissues when those tissues have overlapping bands with bone.

The development of SORS toward the clinic likely requires advanced spectral processing techniques like SOLD.

Conclusions

As originally developed, QUS provides measurements of bone mass or density at peripheral sites, namely the heel (calcaneus), but decades of research have related the propagation of sound waves through the tissue to the mechanical properties of bone. The ability of QUS to assess bone quality lies in the complex attenuation patterns of ultrasound waves as each phase of the bone matrix (mineral, collagen, and water) and the various pore sizes in bone affect the transmission of signals from transducer to receiver. With additional clinical studies comparing fracture to no fracture or changes before and after treatment, the value of processing complex echo signals can be ascertained.

MRI was initially developed to assess bone quality as parameters of trabecular architecture that can be derived from the absence of signal (i.e., high resolution imaging of bone that is surrounded by marrow with signal). More recently, MRI techniques on clinical scanners have been developed to quantify the amount of free water occupying pores within cortical bone (i.e., micro-structural quality) and the amount of water bound to the bone matrix (i.e., tissue quality). At present, these techniques can be applied to peripheral sites like the tibia or radius. Additional research is required to ascertain which techniques – those needing a phantom and those not needing a phantom – best predict fracture risk.

Spatially offset Raman spectroscopy holds promise in providing the clinical assessment of different attributes of the matrix (mineral, collagen, and water separately), but additional research is needed to figure out how to disentangle overlapping Raman signals from bone and soft tissue (fat and skin).

Figure 1 caption: Clinical tools for the assessment of bone mass (mineral density) and bone quality (micro-structure and various tissue characteristics) with and without ionizing

radiation. The hierarchical arrangement of bone, ranging from the macro-structure (left) to the ultra-structure (right), confers multiple 'toughening' mechanisms to keep bone from breaking upon a fall to the ground or due to daily cyclic loading that accumulates damage over time (A). As such, multiple techniques are being developed to quantify different attributes of bone at key length scales of the bone hierarchy (B). The μMRI image was reproduced from Rajapakse, C.S., Leonard, M.B., Bhagat, Y.A., Sun, W., Magland, J.F., and F.W. Wehrli. Micro–MR Imaging–based Computational Biomechanics Demonstrates Reduction in Cortical and Trabecular Bone Strength after Renal Transplantation. Radiology. 262(3):912-20, 2012 (published by the Radiological Society of North America). The QUS image was reproduced from Raum, K., Laugier, P. (2022). Clinical Devices for Bone Assessment. In: Laugier, P., Grimal, Q. (eds) Bone Quantitative Ultrasound. Advances in Experimental Medicine and Biology, vol 1364. (published by Springer)

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Competing Interests

Two authors M.D.D and J.S.N. have patent, entitled "System and method for determining mechanical properties of bone structures", that describes the measuring bound water and pore water concentration using T2 signals. To date, they have not received any money from this patent.

References

1. Lester G. Bone quality: summary of NIH/ASBMR meeting. J Musculoskel Neuronal Interact. 2005;5:309.

2. Siris ES, Chen Y-T, Abbott TA, Barrett-Connor E, Miller PD, Wehren LE, et al. Bone Mineral Density Thresholds for Pharmacological Intervention to Prevent Fractures. Arch Intern Med. 2004;164:1108–12.

3. Shevroja E, Cafarelli FP, Guglielmi G, Hans D. DXA parameters, Trabecular Bone Score (TBS) and Bone Mineral Density (BMD), in fracture risk prediction in endocrine-mediated secondary osteoporosis. Endocrine. 2021;74:20–8.

4. Silva BC, Leslie WD, Resch H, Lamy O, Lesnyak O, Binkley N, et al. Trabecular Bone Score: A Noninvasive Analytical Method Based Upon the DXA Image. J Bone Miner Res. 2014;29:518–30.

5. Whittier DE, Boyd SK, Burghardt AJ, Paccou J, Ghasem-Zadeh A, Chapurlat R, et al. Guidelines for the assessment of bone density and microarchitecture in vivo using high-resolution peripheral quantitative computed tomography. Osteoporosis Int. 2020;31:1607–27.

6. Mikolajewicz N, Bishop N, Burghardt AJ, Folkestad L, Hall A, Kozloff KM, et al. HR-pQCT Measures of Bone Microarchitecture Predict Fracture: Systematic Review and Meta-Analysis. J Bone Miner Res. 2020;35:446–59.

7. Cappelle SI, Moreau M, Karmali R, Iconaru L, Baleanu F, Kinnard V, et al. Discriminating value of HR-pQCT for fractures in women with similar FRAX scores: A substudy of the FRISBEE cohort. Bone. 2021;143:115613.

8. Unal M, Creecy A, Nyman JS. The Role of Matrix Composition in the Mechanical Behavior of Bone. Curr Osteoporos Rep. 2018;16:205–15.

9. Burr DB. Changes in bone matrix properties with aging. Bone. 2019;120:85–93.

10. Burstein AH, Reilly DT, Martens M. Aging of bone tissue: mechanical properties. J Bone Joint Surg Am. 1976;58:82–6.

11. Zioupos P, Currey JD. Changes in the Stiffness, Strength, and Toughness of Human Cortical Bone With Age. Bone. 1998;22:57–66.

12. Nyman JS, Roy A, Tyler JH, Acuna RL, Gayle HJ, Wang X. Age-related factors affecting the postyield energy dissipation of human cortical bone. J Orthopaed Res. 2007;25:646–55.

13. Guglielmi G, Adams J, Link TM. Quantitative ultrasound in the assessment of skeletal status. Eur Radiol. 2009;19:1837–48.

14. Nicholson PHF, Strelitzki R, Cleveland RO, Bouxsein ML. Scattering of ultrasound in cancellous bone: predictions from a theoretical model. J Biomech. 2000;33:503–6.

15. Hans D, Wu C, Njeh CF, Zhao S, Augat P, Newitt D, et al. Ultrasound Velocity of Trabecular Cubes Reflects Mainly Bone Density and Elasticity. Calcif Tissue Int. 1999;64:18–23.

16. Minh HN, Du J, Raum K. Estimation of Thickness and Speed of Sound in Cortical Bone Using Multifocus Pulse-Echo Ultrasound. IEEE Trans Ultrason, Ferroelectr, Freq Control. 2020;67:568–79.

17. Langton CM, Palmer SB, Porter RW. The Measurement of Broadband Ultrasonic Attenuation in Cancellous Bone. Eng Med. 1984;13:89–91.

18. Swinton PA, Elliott-Sale KJ, Sale C. Comparative analysis of bone outcomes between quantitative ultrasound and dual-energy x-ray absorptiometry from the UK Biobank cohort. Arch Osteoporos. 2023;18:77.

19. McCloskey EV, Kanis JA, Odén A, Harvey NC, Bauer D, González-Macias J, et al. Predictive ability of heel quantitative ultrasound for incident fractures: an individual-level meta-analysis. Osteoporos Int. 2015;26:1979–87.

20. Fu Y, Li C, Luo W, Chen Z, Liu Z, Ding Y. Fragility fracture discriminative ability of radius quantitative ultrasound: a systematic review and meta-analysis. Osteoporos Int. 2021;32:23–38.

21. Imashuku Y, Takada M, Murata K. Comparisons of bone mass measurements on various skeletal sites including quantitative ultrasonography of the calcaneus for assessing age-related losses, their correlations, and diagnostic agreement using the Japanese and WHO criteria for osteoporosis. Radiat Med. 2007;25:148–54.

22. Métrailler A, Hans D, Lamy O, Rodriguez EG, Shevroja E. Heel quantitative ultrasound (QUS) predicts incident fractures independently of trabecular bone score (TBS), bone mineral density (BMD), and FRAX: the OsteoLaus Study. Osteoporos Int. 2023;34:1401–9. ●●The study found that Heel-QUS could predict major osteoporotic fractures independently of FRAX, BMD, and the trabecular bone score. This underscores its potential as a pre-screening tool for osteoporosis management.

23. Strässle M, Grossmann J, Eppenberger P, Faas A, Jerkovic I, Floris J, et al. Short-termed changes in quantitative ultrasound estimated bone density among young men in an 18-weeks follow-up during their basic training for the Swiss Armed Forces. PeerJ. 2023;11:e15205.

24. Sahota O, San P, Cawte SA, Pearson D, Hosking DJ. A Comparison of the Longitudinal Changes in Quantitative Ultrasound with Dual-Energy X-ray Absorptiometry: The Four-Year Effects of Hormone Replacement Therapy. Osteoporos Int. 2000;11:52–8.

25. Gonnelli S, Cepollaro C, Montagnani A, Martini S, Gennari. L, Mangeri M, et al. Heel Ultrasonography in Monitoring Alendronate Therapy: A Four-Year Longitudinal Study. Osteoporos Int. 2002;13:415–21.

26. Hans D, Métrailler A, Rodriguez EG, Lamy O, Shevroja E. Bone Quantitative Ultrasound, New Horizons. Adv Exp Med Biol. 2022;1364:7–34.

27. Moris M, Peretz A, Tjeka R, Negaban N, Wouters M, Bergmann P. Quantitative ultrasound bone measurements: Normal values and comparison with bone mineral density by dual X-ray absorptiometry. Calcif Tissue Int. 1995;57:6–10.

28. Rosenthall L, Caminis J, Tenehouse A. Calcaneal Ultrasonometry: Response to Treatment in Comparison with Dual X-ray Absorptiometry Measurements of the Lumbar Spine and Femur. Calcif Tissue Int. 1999;64:200–4.

29. Töyräs J, Nieminen MT, Kröger H, Jurvelin JS. Bone mineral density, ultrasound velocity, and broadband attenuation predict mechanical properties of trabecular bone differently. Bone. 2002;31:503–7.

30. Hans D, Fuerst T, Uffmann M. Bone density and quality measurement using ultrasound. Curr Opin Rheumatol. 1996;8:370–5.

31. ABENDSCHEIN W, HYATT GW. 33 Ultrasonics and Selected Physical Properties of Bone. Clin Orthop Relat Res. 1970;69:294–301.

32. Rho JY, Ashman RB, Turner CH. Young's modulus of trabecular and cortical bone material: Ultrasonic and microtensile measurements. J Biomech. 1993;26:111–9.

33. Bouxsein ML, Radloff SE. Quantitative Ultrasound of the Calcaneus Reflects the Mechanical Properties of Calcaneal Trabecular Bone. J Bone Miner Res. 1997;12:839–46.

34. Bouxsein ML, Coan BS, Lee SC. Prediction of the strength of the elderly proximal femur by bone mineral density and quantitative ultrasound measurements of the heel and tibia. Bone. 1999;25:49–54.

35. Peralta L, Redin JDM, Fan F, Cai X, Laugier P, Schneider J, et al. Bulk Wave Velocities in Cortical Bone Reflect Porosity and Compression Strength. Ultrasound Med Biol. 2021;47:799–808.

36. Hernandez CJ, Meulen MC van der. Understanding Bone Strength Is Not Enough. J Bone Miner Res. 2017;32:1157–62.

37. Cook RB, Curwen C, Tasker T, Zioupos P. Fracture toughness and compressive properties of cancellous bone at the head of the femur and relationships to non-invasive skeletal assessment measurements. Méd Eng Phys. 2010;32:991–7.

38. Rufus-Membere P, Holloway-Kew KL, Diez-Perez A, Kotowicz MA, Pasco JA. Associations between Bone Material Strength Index, Calcaneal Quantitative Ultrasound and Bone Mineral Density in Men. J Endocr Soc. 2020;5:bvaa179-.

39. Bridges D, Randall C, Hansma PK. A new device for performing reference point indentation without a reference probe. The Review of scientific instruments. 2012;83:044301.

40. Abraham AC, Agarwalla A, Yadavalli A, Liu JY, Tang SY. Microstructural and compositional contributions towards the mechanical behavior of aging human bone measured by cyclic and impact reference point indentation. Bone. 2016;87:37–43.

41. Karbalaeisadegh Y, Yousefian O, Iori G, Raum K, Muller M. Acoustic diffusion constant of cortical bone: Numerical simulation study of the effect of pore size and pore density on multiple scattering. J Acoust Soc Am. 2019;146:1015–23.

42. Karbalaeisadegh Y, Yao S, Zhu Y, Grimal Q, Muller M. Ultrasound Characterization of Cortical Bone Using Shannon Entropy. Ultrasound Med Biol. 2023;49:1824–9.

43. Gräsel M, Glüer C-C, Barkmann R. Characterization of a new ultrasound device designed for measuring cortical porosity at the human tibia: A phantom study. Ultrasonics. 2017;76:183–91.

44. Iori G, Du J, Hackenbeck J, Kilappa V, Raum K. Estimation of Cortical Bone Microstructure From Ultrasound Backscatter. IEEE Trans Ultrason, Ferroelectr, Freq Control. 2021;68:1081–95.

45. Armbrecht G, Minh HN, Massmann J, Raum K. Pore-Size Distribution and Frequency-Dependent Attenuation in Human Cortical Tibia Bone Discriminate Fragility Fractures in Postmenopausal Women With Low Bone Mineral Density. JBMR Plus. 2021;5:e10536.

46. Minonzio J, Bochud N, Vallet Q, Ramiandrisoa D, Etcheto A, Briot K, et al. Ultrasound-Based Estimates of Cortical Bone Thickness and Porosity Are Associated With Nontraumatic Fractures in Postmenopausal Women: A Pilot Study. J Bone Miner Res. 2019;34:1585–96.

47. Cheng S, Tylavsky FA, Orwoll ES, Rho J-Y, Carbone LD. The Role of Collagen Abnormalities in Ultrasound and Densitometry Assessment: In VivoEvidence. Calcif Tissue Int. 1999;64:470–6.

48. Hoffmeister BK, Whitten SA, Kaste SC, Rho JY. Effect of Collagen and Mineral Content on the High-Frequency Ultrasonic Properties of Human Cancellous Bone. Osteoporos Int. 2002;13:26–32.

49. Kann P, Bergink AP, Fang Y, Daele PLA van, Hofman A, Leeuwen JPTM van, et al. The Collagen Ia1 SP1 Polymorphism is Associated With Differences in Ultrasound Transmission Velocity in the Calcaneus in Postmenopausal Women. Calcif Tissue Int. 2002;70:450–6.

50. Lalli P, Mautino C, Busso C, Bardesono F, Monaco MD, Lippi L, et al. Reproducibility and Accuracy of the Radiofrequency Echographic Multi-Spectrometry for Femoral Mineral Density Estimation and Discriminative Power of the Femoral Fragility Score in Patients with Primary and Disuse-Related Osteoporosis. J Clin Med. 2022;11:3761.

51. Messina C, Gitto S, Colombo R, Fusco S, Guagliardo G, Piazza M, et al. Short-Term Precision and Repeatability of Radiofrequency Echographic Multi Spectrometry (REMS) on Lumbar Spine and Proximal Femur: An In Vivo Study. J imaging. 2023;9.

52. Conversano F, Franchini R, Greco A, Soloperto G, Chiriacò F, Casciaro E, et al. A Novel Ultrasound Methodology for Estimating Spine Mineral Density. Ultrasound Med Biol. 2015;41:281–300.

53. Casciaro S, Peccarisi M, Pisani P, Franchini R, Greco A, Marco TD, et al. An Advanced Quantitative Echosound Methodology for Femoral Neck Densitometry. Ultrasound Med Biol. 2016;42:1337–56.

54. Sergio R-O, Nayelli RGE. Evaluation of the bone mineral density in the Mexican female population using the Radiofrequency Echographic Multi Spectrometry (REMS) technology. Arch Osteoporos. 2022;17:43.

55. Cortet B, Dennison E, Diez-Perez A, Locquet M, Muratore M, Nogués X, et al. Radiofrequency Echographic Multi Spectrometry (REMS) for the diagnosis of osteoporosis in a European multicenter clinical context. Bone. 2021;143:115786.

56. Quarta E, Ciardo D, Ciccarese M, Conversano F, Paola MD, Forcignanò R, et al. SAT0461 SHORT-TERM MONITORING OF DENOSUMAB EFFECT IN BREAST CANCER PATIENTS RECEIVING AROMATASE INHIBITORS USING REMS TECHNOLOGY ON LUMBAR SPINE. Ann Rheum Dis. 2020;79:1187.2-1188.

57. Greco A, Pisani P, Conversano F, Soloperto G, Renna MD, Muratore M, et al. Ultrasound Fragility Score: An innovative approach for the assessment of bone fragility. Measurement. 2017;101:236–42.

58. Diez-Perez A, Brandi ML, Al-Daghri N, Branco JC, Bruyère O, Cavalli L, et al. Radiofrequency echographic multi-spectrometry for the in-vivo assessment of bone strength: state of the art—outcomes of an expert consensus meeting organized by the European Society for Clinical and Economic Aspects of Osteoporosis, Osteoarthritis and Musculoskeletal Diseases (ESCEO). Aging Clin Exp Res. 2019;31:1375–89.

59. Pisani P, Greco A, Conversano F, Renna MD, Casciaro E, Quarta L, et al. A quantitative ultrasound approach to estimate bone fragility: A first comparison with dual X-ray absorptiometry. Measurement. 2017;101:243–9.

60. Caffarelli C, Pitinca MDT, Refaie AA, Vita MD, Catapano S, Gonnelli S. Could radiofrequency echographic multispectrometry (REMS) overcome the overestimation in BMD by dual-energy X-ray absorptiometry (DXA) at the lumbar spine? BMC Musculoskelet Disord. 2022;23:469.

61. Pisani P, Conversano F, Muratore M, Adami G, Brandi ML, Caffarelli C, et al. Fragility Score: a REMS-based indicator for the prediction of incident fragility fractures at 5 years. Aging Clin Exp Res. 2023;35:763–73. ●●The Fragility Score from REMS outperformed DXA BMD in identifying individuals at risk for fragility fracture for both males and females at both sites. This indicates that backscattered echo signals in ultrasound likely asses bone quality.

62. Schacter GI, Leslie WD. DXA-Based Measurements in Diabetes: Can They Predict Fracture Risk? Calcif Tissue Int. 2017;100:150–64. ●While women with type 2 diabetes exhibited an expected higher DXA-derived BMD value compared to control women, the REMS-estimated BMD was lower in the women with diabets. This allowed for more women with T2DM to be classified as 'osteoporotic' when using REMS, highlighting its potential value as a diagnostic tool in diseases where DXA-derived BMD falls short.

63. Caffarelli C, Pitinca MDT, Refaie AA, Ceccarelli E, Gonnelli S. Ability of radiofrequency echographic multispectrometry to identify osteoporosis status in elderly women with type 2 diabetes. Aging Clin Exp Res. 2022;34:121–7.

64. Faje AT, Fazeli PK, Miller KK, Katzman DK, Ebrahimi S, Lee H, et al. Fracture risk and areal bone mineral density in adolescent females with anorexia nervosa. Int J Eat Disord. 2014;47:458–66.

65. Workman C, Blalock DV, Mehler PS. Bone density status in a large population of patients with anorexia nervosa. Bone. 2020;131:115161.

66. Caffarelli C, Refaie AA, Vita MD, Pitinca MDT, Goracci A, Fagiolini A, et al. Radiofrequency echographic multispectrometry (REMS): an innovative technique for the assessment of bone status in young women with anorexia nervosa. Eat Weight Disord - Stud Anorex, Bulim Obes. 2022;27:3207–13.

67. Fassio A, Andreola S, Gatti D, Bianco B, Gatti M, Gambaro G, et al. Radiofrequency echographic multi-spectrometry and DXA for the evaluation of bone mineral density in a peritoneal dialysis setting. Aging Clin Exp Res. 2023;35:185–92.

68. Sollmann N, Löffler MT, Kronthaler S, Böhm C, Dieckmeyer M, Ruschke S, et al. MRI-Based Quantitative Osteoporosis Imaging at the Spine and Femur. J Magn Reson Imaging. 2021;54:12–35.

69. Jerban S, Alenezi S, Afsahi AM, Ma Y, Du J, Chung CB, et al. MRI-based mechanical competence assessment of bone using micro finite element analysis (micro-FEA): Review. Magn Reson Imaging. 2022;88:9–19.

70. Nyman JS, Does MD. Bound Water and Pore Water in Osteoporosis. In: Du J, Bydder GM, editors. MRI of Short and Ultrashort-T2 Tissues: Making the Invisible Visible. Spinger; 2023.

71. Majumdar S, Genant HK, Grampp S, Newitt DC, Truong V -H., Lin JC, et al. Correlation of Trabecular Bone Structure with Age, Bone Mineral Density, and Osteoporotic Status: In Vivo Studies in the Distal Radius Using High Resolution Magnetic Resonance Imaging. J Bone Miner Res. 1997;12:111–8.

72. Wehrli FW, Hwang SN, Ma J, Song HK, Ford JC, Haddad JG. Cancellous bone volume and structure in the forearm: noninvasive assessment with MR microimaging and image processing. Radiology. 1998;206:347–57

73. Link TM, Majumdar S, Augat P, Lin JC, Newitt D, Lu Y, et al. In Vivo High Resolution MRI of the Calcaneus: Differences in Trabecular Structure in Osteoporosis Patients. J Bone Miner Res. 1998;13:1175–82.

74. Benito M, Gomberg B, Wehrli FW, Weening RH, Zemel B, Wright AC, et al. Deterioration of Trabecular Architecture in Hypogonadal Men. J Clin Endocrinol Metab. 2003;88:1497–502.

75. Krug R, Banerjee S, Han ET, Newitt DC, Link TM, Majumdar S. Feasibility of in vivo structural analysis of high-resolution magnetic resonance images of the proximal femur. Osteoporos Int. 2005;16:1307–14.

76. Chang G, Deniz CM, Honig S, Rajapakse CS, Egol K, Regatte RR, et al. Feasibility of three-dimensional MRI of proximal femur microarchitecture at 3 tesla using 26 receive elements without and with parallel imaging. J Magn Reson Imaging. 2014;40:229–38.

77. Chang G, Rajapakse CS, Regatte RR, Babb J, Saxena A, Belmont HM, et al. 3 Tesla MRI detects deterioration in proximal femur microarchitecture and strength in long-term glucocorticoid users compared with controls. J Magn Reson Imaging. 2015;42:1489–96.

78. Kazakia GJ, Carballido-Gamio J, Lai A, Nardo L, Facchetti L, Pasco C, et al. Trabecular bone microstructure is impaired in the proximal femur of human immunodeficiency virus-infected men with normal bone mineral density. Quant Imaging Med Surg. 2017;8:5–13.

79. Vu B-TD, Jones BC, Lee H, Kamona N, Deshpande RS, Wehrli FW, et al. Six-minute, in vivo MRI quantification of proximal femur trabecular bone 3D microstructure. Bone.

2023;177:116900. ●This study provides the current state-of-the art for clinical evaluation of trabecular microarchitecture in the proximal femur.

80. Chang G, Honig S, Brown R, Deniz CM, Egol KA, Babb JS, et al. Finite element analysis applied to 3-T MR imaging of proximal femur microarchitecture: lower bone strength in patients with fragility fractures compared with control subjects. Radiology. 2014;272:464 474.

81. Fernández-Seara MA, Wehrli SL, Takahashi M, Wehrli FW. Water Content Measured by Proton-Deuteron Exchange NMR Predicts Bone Mineral Density and Mechanical Properties. J Bone Miner Res [Internet]. 2004;19:289–96.

82. Fantazzini P, Brown RJS, Borgia GC. Bone tissue and porous media: common features and differences studied by NMR relaxation. Magn Reson Imaging. 2003;21:227–34.

83. Wang X, Ni Q. Determination of cortical bone porosity and pore size distribution using a low field pulsed NMR approach. J Orthopaed Res. 2003;21:312–9.

84. Ni Q, King JD, Wang X. The characterization of human compact bone structure changes by low-field nuclear magnetic resonance. Meas Sci Technol. 2004;15:58.

85. Horch RA, Nyman JS, Gochberg DF, Dortch RD, Does MD. Characterization of 1H NMR signal in human cortical bone for magnetic resonance imaging. Magnet Reson Med. 2010;64:680–7.

86. Granke M, Makowski AJ, Uppuganti S, Does MD, Nyman JS. Identifying Novel Clinical Surrogates to Assess Human Bone Fracture Toughness. J Bone Miner Res. 2015;30:1290–300.

87. Manhard MK, Uppuganti S, Granke M, Gochberg DF, Nyman JS, Does MD. MRI-derived bound and pore water concentrations as predictors of fracture resistance. Bone. 2016;87:1–10.

88. Jerban S, Lu X, Dorthe EW, Alenezi S, Ma Y, Kakos L, et al. Correlations of cortical bone microstructural and mechanical properties with water proton fractions obtained from ultrashort echo time (UTE) MRI tricomponent T2* model. Nmr Biomed. 2020;33:e4233.

89. Nyman JS, Roy A, Shen X, Acuna RL, Tyler JH, Wang X. The influence of water removal on the strength and toughness of cortical bone. J Biomech. 2006;39:931–8.

90. Zioupos P, Currey JD, Hamer AJ. The role of collagen in the declining mechanical properties of aging human cortical bone. J Biomed Mater Res. 1999;45:108–16.

91. Wang X, Shen X, Li X, Agrawal CM. Age-related changes in the collagen network and toughness of bone. Bone. 2002;31:1 7.

92. Robson MD, Bydder GM. Clinical ultrashort echo time imaging of bone and other connective tissues. Nmr Biomed. 2006;19:765 780.

93. Techawiboonwong A, Song HK, Leonard MB, Wehrli FW. Cortical Bone Water: In Vivo Quantification with Ultrashort Echo-Time MR Imaging1. Radiology. 2008;248:824–33.

94. Du J, Carl M, Bydder M, Takahashi A, Chung CB, Bydder GM. Qualitative and quantitative ultrashort echo time (UTE) imaging of cortical bone. J Magn Reson [Internet]. 2010;207:304–11.

95. Horch RA, Gochberg DF, Nyman JS, Does MD. Clinically compatible MRI strategies for discriminating bound and pore water in cortical bone. Magnet Reson Med. 2012;68:1774–84.

96. Manhard MK, Horch RA, Harkins KD, Gochberg DF, Nyman JS, Does MD. Validation of quantitative bound- and pore-water imaging in cortical bone. Magnet Reson Med. 2014;71:2166–71.

97. Manhard MK, Horch RA, Gochberg DF, Nyman JS, Does MD. In Vivo Quantitative MR Imaging of Bound and Pore Water in Cortical Bone. Radiology. 2015;277:221–9.

98. Ketsiri T, Uppuganti S, Harkins KD, Gochberg DF, Nyman JS, Does MD. T1 relaxation of bound and pore water in cortical bone. Nmr Biomed. 2023;36:e4878.

99. Zhao X, Song HK, Seifert AC, Li C, Wehrli FW. Feasibility of assessing bone matrix and mineral properties in vivo by combined solid-state 1H and 31P MRI. Vashishth D, editor. Plos One. 2017;12:e0173995.

100. Jones BC, Lee H, Cheng C-C, Mukaddam M al, Song HK, Snyder PJ, et al. MRI Quantification of Cortical Bone Porosity, Mineralization, and Morphologic Structure in Postmenopausal Osteoporosis. Radiology. 2023;307:e221810.

101. Bae WC, Chen PC, Chung CB, Masuda K, D'Lima D, Du J. Quantitative ultrashort echo time (UTE) MRI of human cortical bone: Correlation with porosity and biomechanical properties. J Bone Miner Res. 2012;27:848–57.

102. Biswas R, Bae W, Diaz E, Masuda K, Chung CB, Bydder GM, et al. Ultrashort echo time (UTE) imaging with bi-component analysis: Bound and free water evaluation of bovine cortical bone subject to sequential drying. Bone. 2012;50:749–55.

103. Chen J, Carl M, Ma Y, Shao H, Lu X, Chen B, et al. Fast volumetric imaging of bound and pore water in cortical bone using three-dimensional ultrashort-TE (UTE) and inversion recovery UTE sequences. Nmr Biomed. 2016;29:1373–80.

104. Lu X, Jerban S, Wan L, Ma Y, Jang H, Le N, et al. Three-dimensional ultrashort echo time imaging with tricomponent analysis for human cortical bone. Magnet Reson Med. 2019;82:348–55.

105. Seifert AC, Wehrli SL, Wehrli FW. Bi-component T2* analysis of bound and pore bone water fractions fails at high field strengths. Nmr Biomed. 2015;28:861–72.

106. Li C, Seifert AC, Rad HS, Bhagat YA, Rajapakse CS, Sun W, et al. Cortical Bone Water Concentration: Dependence of MR Imaging Measures on Age and Pore Volume Fraction. Radiology. 2014;272:796–806.

107. Rajapakse CS, Bashoor-Zadeh M, Li C, Sun W, Wright AC, Wehrli FW. Volumetric Cortical Bone Porosity Assessment with MR Imaging: Validation and Clinical Feasibility. Radiology. 2015;276:526–35.

108. Jones BC, Jia S, Lee H, Feng A, Shetye SS, Batzdorf A, et al. MRI-derived porosity index is associated with whole-bone stiffness and mineral density in human cadaveric femora. Bone. 2021;143:115774.

109. Abbasi-Rad S, Rad HS. Quantification of Human Cortical Bone Bound and Free Water in Vivo with Ultrashort Echo Time MR Imaging: A Model-based Approach. Radiology. 2017;283:160780.

110. Xiong Y, He T, Wang Y, Liu WV, Hu S, Zhang Y, et al. CKD Stages, Bone Metabolism Markers, and Cortical Porosity Index: Associations and Mediation Effects Analysis. Front Endocrinol. 2021;12:775066. ●The is the first in vivo patient study involving MRI measures of cortical bone material properties.

111. Jerban S, Ma Y, Moazamian D, Athertya J, Dwek S, Jang H, et al. MRI-based porosity index (PI) and suppression ratio (SR) in the tibial cortex show significant differences between normal, osteopenic, and osteoporotic female subjects. Front Endocrinol. 2023;14:1148345.

112. Nyman JS, Ketsiri T, Louie EA, Harkins KD, Manhard MK, Gochberg DF, et al. Toward the use of MRI measurements of bound and pore water in fracture risk assessment. Bone. 2023;176:116863. ●●In this case-control study, the combination of bound and pore water concentrations in the radius significantly predicted patients with a fragility fracture and subjects without a history of osteoporosis.

113. Taylor EA, Donnelly E. Raman and Fourier transform infrared imaging for characterization of bone material properties. Bone. 2020;139:115490.

114. Unal M, Ahmed R, Mahadevan-Jansen A, Nyman JS. Compositional assessment of bone by Raman spectroscopy. Analyst. 2021;146:7464–90.

115. Roschger A, Gamsjaeger S, Hofstetter B, Masic A, Blouin S, Messmer P, et al. Relationship between the v2PO4/amide III ratio assessed by Raman spectroscopy and the calcium content measured by quantitative backscattered electron microscopy in healthy human osteonal bone. J Biomed Opt. 2014;19:065002–065002.

116. Mandair GS, Akhter MP, Esmonde-White FWL, Lappe JM, Bare SP, Lloyd WR, et al. Altered collagen chemical compositional structure in osteopenic women with past fractures: A case-control Raman spectroscopic study. Bone. 2021;148:115962.

117. Unal M, Yang S, Akkus O. Molecular spectroscopic identification of the water compartments in bone. Bone. 2014;67:228–36.

118. Unal M, Akkus O. Raman spectral classification of mineral- and collagen-bound water's associations to elastic and post-yield mechanical properties of cortical bone. Bone. 2015;81:315–26.

119. Heath S, Han Y, Hua R, Roy A, Jiang J, Nyman JS, et al. Assessment of glycosaminoglycan content in bone using Raman spectroscopy. Bone. 2023;171:116751.

120. Shitole P, Choubey A, Mondal P, Ghosh R. Influence of Low Dose Naltrexone on Raman Assisted Bone Quality, Skeletal Advanced Glycation End-products and Nano-mechanical Properties in Type 2 Diabetic Mice Bone. Mater Sci Eng C. 2021;123:112011.

121. Gamsjaeger S, Brozek W, Recker R, Klaushofer K, Paschalis EP. Transmenopausal Changes in Trabecular Bone Quality. J Bone Miner Res. 2014;29:608–17.

122. Matousek P, Clark IP, Draper ERC, Morris MD, Goodship AE, Everall N, et al. Subsurface Probing in Diffusely Scattering Media Using Spatially Offset Raman Spectroscopy. Appl Spectrosc. 2005;59:393–400.

123. Mosca S, Conti C, Stone N, Matousek P. Spatially offset Raman spectroscopy. Nat Rev Methods Primers. 2021;1:21.

124. Nicolson F, Kircher MF, Stone N, Matousek P. Spatially offset Raman spectroscopy for biomedical applications. Chem Soc Rev. 2021;50:556–68.

125. Matousek P, Draper ERC, Goodship AE, Clark IP, Ronayne KL, Parker AW. Noninvasive Raman spectroscopy of human tissue in vivo. Applied spectroscopy. 2006;60:758–63.

126. Buckley K, Kerns JG, Vinton J, Gikas PD, Smith C, Parker AW, et al. Towards the in vivo prediction of fragility fractures with Raman spectroscopy. J Raman Spectrosc. 2015;46:610–8.

127. Maher JR, Inzana JA, Awad HA, Berger AJ. Overconstrained library-based fitting method reveals age- and disease-related differences in transcutaneous Raman spectra of murine bones. J Biomed Opt. 2013;18:077001–077001.

128. Chen K, Massie C, Berger AJ. Soft-tissue spectral subtraction improves transcutaneous Raman estimates of murine bone strength in vivo. J Biophotonics. 2020;13:e202000256. ●●This pre-clinical study shows how a spectral library of Raman spectra from different tissues (bone, skin, fat) can be used to derive meaningful spectra from transcutaneous in vivo acquisition of Raman signals from the hindlimb (tibia) of mice between 4 to 23 weeks of age using SORS.

129. Chen K, Massie C, Awad HA, Berger AJ. Determination of best Raman spectroscopy spatial offsets for transcutaneous bone quality assessments in human hands. Biomed Opt Express. 2021;12:7517. ●●In a translational study involving SORS acquisition of Raman signals from a cadaveric hand (phalanx and metacarpal), increasing the offset distance increased Raman peaks specific to bone at the cost of lower signal-to-noise.

130. Gautam R, Ahmed R, Haugen E, Unal M, Fitzgerald S, Uppuganti S, et al. Assessment of spatially offset Raman spectroscopy to detect differences in bone matrix quality. Spectrochim Acta Part A: Mol Biomol Spectrosc. 2023;303:123240. ●●In this cadaver study, SORS detected changes in bone composition that were related to a loss in bone strength, but only few Raman bands could be detected when tissue phantom layers with a thickness of 4 mm or higher were placed between the bone and the probe.

131. Ahmed R, Unal M, Gautam R, Uppuganti S, Derasari S, Mahadevan-Jansen A, et al. Sensitivity of the amide I band to matrix manipulation in bone: a Raman micro-spectroscopy and spatially offset Raman spectroscopy study. Analyst. 2023;148:4799–809.