COMPARISON OF hr-pQCT & MRTA TO DXA & QUS
FOR THE EX-VIVO ASSESSMENT OF BONE STRENGTH

by

Idrees Ally

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ABSTRACT

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There is a pressing need for better assessment of bone strength as current clinical tools do not directly measure bone mechanical properties, but offer only surrogate measures of bone strength. We conducted an ex-vivo study of emu bones to examine how two investigative devices, hr-pQCT and MRTA, compare to current clinical tools (DXA and QUS) in predicting true bone mechanical properties. We found that hr-pQCT parameters were able to assess bone strength as well as DXA and better than QUS, while MRTA was able to predict bone strength well in low-density but not high-density bones. Our results suggest that both hr-pQCT, which has the unique ability to specifically assess the various determinants of bone strength, and MRTA, which measures a bone mechanical property (stiffness), have great potential for use as clinical tools that can assess various components of bone strength not measured by current devices.
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<td>Areal bone mineral density</td>
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<td>BMC</td>
<td>Bone mineral content</td>
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<td>DXA</td>
<td>Dual-energy X-ray absorptiometry</td>
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<td>EI</td>
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<td>hr-pQCT</td>
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<td>MRTA</td>
<td>Mechanical response tissue analyzer</td>
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<tr>
<td>NHP</td>
<td>Nonhuman primate</td>
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<td>OVX</td>
<td>Ovariectomized</td>
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INTRODUCTORY REMARKS & RATIONALE

The results of a large cohort study by a group at McMaster University in Hamilton, – Ontario were recently published, providing new insight into the relationship between osteoporotic fractures and mortality (Ioannidis et al. 2009). The results are alarming: a patient over the age of fifty who suffers a hip fracture has almost a twenty-five percent chance of dying within one year of the fracture (Ioannidis et al. 2009, Ashe & Khan 2009). This amounts to approximately three times the risk of death compared to persons who have not fractured (Ashe & Khan 2009). When we consider that more than forty percent of women over fifty will suffer from a fracture at some point in their lives, we realize that the human cost of osteoporotic fractures is extremely high.

The authors suggest what should be self-evident to all: in order to lessen the probability of death by fracture, interventions must be implemented to ward off fractures in the first place (Ioannidis et al. 2009). Some of the interventions they suggest, such as the use of pharmaceuticals, require the specific ability to identify patients at high risk of fracture.

How to do so is the question at hand. Past definitions of osteoporosis, characterizing the disorder as one of low bone mass, have lent support to a density-based approach to assessing fracture risk. More recently, however, it has become well recognized that the overall state of bone is dependent on much more than its density alone. Newer definitions of osteoporosis, making reference to terms such as “bone strength,” reflect an awareness that the quality of bone is dependent on a complex interplay of its many material and structural characteristics (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001). The
goal, then, in assessing fracture risk, would be to look beyond density alone, and consider the various properties of bone that together characterize the overall strength of the bone.

Current devices such as dual-energy x-ray absorptiometry (DXA) and quantitative ultrasound (QUS) favour a density-based approach to assess fracture risk and are widely used in the clinical setting. Thus, to properly assess bone strength, there is a need for the development of new diagnostic devices for clinical use that can accurately assess bone strength or its determinants.

Two devices that have shown some potential for assessing bone strength are the high-resolution peripheral quantitative computed tomography (hr-pQCT) scanner and the mechanical response tissue analyzer (MRTA). Any interest in developing these tools for osteoporosis diagnosis must be preceded by a thorough understanding of how measures obtained from these tools reflect true bone strength. Thus, we ask here: how well do hr-pQCT and MRTA measures reflect true mechanical properties of bone, compared to DXA and QUS? We hypothesize that both hr-pQCT, measuring both material and structural properties of bone (the determinants of bone strength), and MRTA, measuring bone stiffness (a mechanical property of bone), can reflect true mechanical properties of bone better than either DXA or QUS.

In order to test this, it is first necessary to consider a new animal model that can overcome some of the serious limitations of current models when it comes to assessing bone strength, especially on human-sized devices. After a review of background topics in Chapter 1, presented in Chapter 2 is a review of current models used in osteoporosis, as well as the proposal of the emu as a suitable ex-vivo animal model for the assessment bone strength.

Chapter 3 takes a close look at the hr-pQCT and its ability to assess the determinants of bone strength. We present results of an ex-vivo study of emu bones examining how hr-pQCT compares to DXA and QUS in predicting true bone mechanical properties.
In Chapter 4, we build on the previous chapter by examining the ability of both hr-pQCT and MRTA to assess bone strength, this time in highly demineralized bones. Findings from this study are key to understanding the utility of hr-pQCT and MRTA in assessing bone strength across bones that vary in their densities and structures.

Chapters 2-4 have been written up as manuscripts for publication. We follow those three chapters with a general discussion and summary of our findings, as we consider the work as a whole in light of the original hypothesis.
CHAPTER 1: Background

Osteoporosis

Osteoporosis, literally meaning “porous bone,” is a systemic metabolic bone disorder characterized by a decrease in both the density and quality of bone (Burnell et al. 1982, Kleerekoper et al. 1985, Parfitt 1992). Normally, bone tissue is dynamically involved in a process of turnover, as bone tissue is resorbed and reformed. The pathology of osteoporosis arises when there is a greater rate of bone resorption than there is bone formation. The result is a progressive deterioration of bone tissue until its mechanical integrity is severely compromised. This loss in bone strength invariably leads to an increase in the risk of fractures, especially atraumatic ones at the wrist, spine, and hip.

Defining osteoporosis can often be tricky, especially when the definition forms the basis for diagnosis. The 1994 World Health Organization (WHO) definition describes osteoporosis as “a systemic skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue, with a consequent increase in both bone fragility and susceptibility to fractures,” and the WHO uses bone density as a basic criterion for diagnosing osteoporosis (Leslie et al. 2006). More recently, however, greater attention has been given to the argument that overall bone strength is dependent on more than bone mass and density alone (Hernandez & Keaveny 2006, Friedman 2006), and newer definitions reflect this. The 2000 National Institutes of Health (NIH) definition describes osteoporosis as “a skeletal disorder characterized by compromised bone strength predisposing a person to an increased risk of fracture. Bone strength reflects the integration of two main features: bone density and bone quality (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001).”
Regardless of how we define osteoporosis, however, it is no secret that the disorder and the fractures it causes are a significant health burden. Osteoporosis affects approximately one in two women and one in five men over the age of 50 (Keen 2007), and osteoporotic fractures have devastating outcomes. At best, they can lead to pain and disability that results in disfigurement, decreased mobility, loss of independence, and fear of falling (Burge et al. 2007, Robbins et al. 2006, Adachi et al. 2001, Ensrud et al. 2000, Greendale et al. 1995). At worst, they can lead to death via a number of routes; a recent study showed that a patient over the age of 50 who suffers a hip fracture has almost a twenty-five percent chance of dying within one year – this represents almost a three-fold risk of death over non-fractured individuals (Ioannidis et al. 2009, Ashe & Khan 2009). These high human costs result in major economic ones: fractures cost the United States approximately 17 billion dollars per year, with the costs projected to rise by almost 50% by 2025 (Burge et al. 2007).

Fracture risk

The human and economic costs of osteoporosis and related fractures are very high. Given this, there is a great need to be able to prevent osteoporotic fractures from occurring. Whereas good bone health can be maintained by non-specific means such as through a healthful diet or regular exercise, there is also a need for specific interventions (such as the use of pharmaceuticals) to ward off fractures in high-risk individuals (Ioannidis et al. 2009). In order to implement these therapeutic or preventative strategies, physicians are first interested in assessing the fracture risk of individuals and populations.

Fracture risk is, essentially, the probability of the occurrence of osteoporotic fractures in a specific population, and can be expressed either as a percentage or in terms of relative risk. To
determine fracture risk, physicians rely on a variety of bone assessment techniques which attempt to make determinations about the health of the bone based on measurements of some of the parameters that affect bone strength.

Because fractures are mechanical events, resulting from situations where the applied load exceeds the strength of the bone, the interest should be in assessing the mechanical competence of bone, and not other parameters of bone per se (Cheung & Detsky 2008). However, direct biomechanical tests involve measuring the response of materials under stress due to an applied force. Clearly, these are not feasible methods of assessment in a clinical setting.

It is understandable then that the main methods of fracture risk assessment available clinically involve the measurement of parameters (such as bone mass and density) that affect overall bone mechanical competence, or bone strength. The two tools for assessing fracture risk that are widely used clinically, DXA and QUS, shall be described in greater detail pending a brief discussion of the concept of bone strength.

**Bone strength**

**Definition**

As a technical term, bone strength is rather hard to define, as it refers neither to a specific nor measurable property of bone. Rather, it refers conceptually to the overall mechanical competence of bone, or its ability to withstand failure, such that bones fracture when applied loads exceed their strength (Cheung & Detsky 2008). The NIH, in defining osteoporosis, describes bone strength as being “the integration of two main features: bone density and bone quality.” The term thus reflects current understandings of the fact that bone is much too complex to be characterized only by the amount of mineral that it contains and instead needs to be
considered in terms of its overall ability to function in a healthy manner (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001).

**Bone quality**

A related term is that of bone quality. Like bone strength, bone quality is not precisely defined and remains a vague notion rather than a measurable property of bone (Cheung & Detsky 2008, Licata 2009). The term is sometimes used in contradistinction to bone density in order to reflect more recent understandings of whole bone strength that are not solely density-based (as in the NIH definition). However, the term bone quality is an encompassing term that can refer to all of the multiple factors that help bone resist fracture, including density (Licata 2009).

**Factors that affect bone strength**

These many factors that collectively determine bone strength can be summarized as being either material properties or structural properties (Cheung & Detsky 2008, Seeman & Delmas 2006 Seeman 2008, Chavassieux et al. 2007). Material properties are those having to do with the nature and composition of the bone material itself. The material properties of bone are affected by the amount of the bone mineral hydroxyapatite (the degree of mineralization of the bone tissue), the size of the mineral crystals, the amount of other minerals (such as fluoride), the amount, type, and quality of collagen, as well as a host of other factors (Cheung & Detsky 2008, Licata 2009, Seeman & Delmas 2006. The structural properties, on the other hand, reflect the arrangement of the material in space, and therefore depend on both the size and the shape of the bone. The amount of trabecular struts, their thickness, and the amount of separation between
them collectively help determine how well the trabecular network as a whole is able to support against crushing forces. The thickness of cortical bone, its porosity, and how it is distributed about a central axis in long bones (cross-sectional moment of inertia) also collectively help determine the overall strength of bone. The presence and amount of accumulated microscopic cracks in bone are also structural properties that affect the quality of bone and its ability to resist failure (Cheung & Detsky 2008, Licata 2009, Augat & Schorlemmer 2006). All in all, we continue to learn that bone is a complex structure whose ability to function in a structurally supportive role depends on a wide variety of factors, of which material density is only one.

**Mechanical properties of bone**

While the clinical world uses the word bone strength to describe the overall mechanical competence of bone, the world of biomechanics has more specific terms to describe the behaviour of materials and structures.

The ultimate load is the maximum load or force the bone can sustain before breaking. At times the terms ultimate strength and bone breaking strength are also used, though these terms are not necessarily interchangeable for materials other than bone (Turner & Burr 1993. The load at which bone breaks (and the other mechanical properties of bone, for that matter) depends on how the bone was loaded: a vertebral body loaded in compression would not have the same ultimate load as a tibia loaded in bending, nor would the same bone have the same ultimate load if loaded in different directions. The static strength of bones, describing the load at which a bone would fail if loaded slowly, would also be very different from the fatigue strength of bone, describing the load at which the bone would fail if continuously repeatedly loaded with a light load (Turner & Burr 1993 Currey 2001).
What is referred to by the term stiffness is measured in engineering tests as E, known as Young’s modulus or the elastic modulus (Currey 2001). This is the rate at which a material deforms (or experiences displacement) in response to an applied load, and refers to the intrinsic stiffness of the bone material (Turner & Burr 1993). In the context of whole bones, stiffness can refer also to the overall stiffness of the whole structure (Currey 2001). For trabecular bone, each of the trabeculae would have an intrinsic stiffness, while the network of trabeculae as a whole would have a stiffness relating to the entire structure (Turner & Burr 1993).

The toughness of bone is the amount of energy a bone can absorb before failing. If someone falls on their wrist, for example, their forearm needs to be able to absorb the energy of the fall; the greater the ability to absorb the impact, the tougher the bone (Currey 2001).

Typically bone scientists use the term bone strength to refer to ultimate load, though in actuality different mechanical properties of bone describe a bone’s mechanical competence in different scenarios.

**Dual-energy x-ray absorptiometry (DXA)**

Dual-energy x-ray absorptiometry is currently the technology of choice when it comes to bone assessment. As with older absorptiometry techniques, DXA uses the attenuation (i.e. reduction in intensity) profiles of an incident beam passing through the site of interest to quantify the degree of tissue mineralization. The greater the amount and density of tissue present in the path between the incident beam source and the detector, greater is the attenuation of the beam. Although the amounts of ionizing radiation are low (approximately 1/10 of a chest x-ray), the mere presence of radiation is one disadvantage of this device, especially with regards to pregnant women.
Technical principles of DXA

DXA employs an x-ray tube as its energy source, which does away with concerns regarding source decay and resultant drifts in patient values which would have been present in older methods employing radioactive sources. DXA applies two x-ray beams of different energies (140 kVp and 70 kVp), either by using alternating pulses or by filtering a single beam. Whichever the method, the result is that two beams of differing energies are passed through the region of interest, such that two attenuation profiles are generated. Both x-ray beams are equally attenuated by soft tissue (musculature, fat, marrow, etc) present in the region of interest.

However, one beam, being of a lower energy, is more preferentially attenuated by bone than the high energy beam. Thus, by subtracting the attenuation profile of the low energy beam from the attenuation profile of the high energy beam, it is possible to remove the effects of soft tissue. The remaining attenuation profile, being that of bone mineral, can then be quantified to produce measures of bone density. The degree of attenuation can be used to calculate the bone mineral content (BMC; g), and by dividing the BMC by the projected area of bone, a measure of bone mineral density (BMD; g/cm²) is obtained. Because DXA uses a two-dimensional projected area to determine density (which should really be a volumetric measure), BMD reported by DXA is often referred to as areal BMD, or aBMD. It is this notation that we use here to differentiate DXA-based density measures from other volumetric measures of bone density.

Clinical use of DXA

Dual-energy x-ray absorptiometry was first introduced in the late eighties, and is now the gold-standard method for the non-invasive assessment of skeletal integrity (Blake & Fogelman 2007). It has both excellent accuracy (3 - 5%) and precision (0.5 - 2%) (Mirsky & Einhorn
11998), and can be used to measure any skeletal site, though mainly central sites such as the lumbar spine or proximal femur are measured. DXA measures of aBMD, used in the diagnosis of osteoporosis according to WHO criteria (2.5 standard deviations below the mean for a healthy 30 year old woman), have gained widespread acceptance clinically.

Ex vivo studies have shown that there is a high correlation ($R^2 = 0.4 - 0.9$) between aBMD and the force needed to break a bone (Cummings et al. 2002). In addition to this, aBMD has been shown to be able to predict the risk of low-trauma (osteoporotic) fractures. Cummings et al. found that for one standard deviation decrease in BMD of the femoral neck, the age-adjusted hip fracture risk increased by 2.6 times (Cummings et al. 1993). A meta-analysis of eleven large, prospective cohort studies, confirmed that a decrease in aBMD is associated with an increased risk of fracture (Khan et al. 2002, Marshall et al. 1996). For a one standard deviation decrease in aBMD at the femoral neck, there was an age-adjusted relative risk of a hip fracture of 2.6, and a one standard deviation decrease in aBMD at the spine corresponding to an age-related risk of a spine fracture of 2.3. A one standard deviation decrease in aBMD at either site corresponded to a relative risk of 1.6 at the other site, showing that while measurements at each site best predicts fracture at that same site, they could still be effective in predicting fracture occurrence at the other site as well (Marshall et al. 1996). The large body of research on the relationship between aBMD and fracture risk suggest that aBMD is a “continuous risk factor: the lower the aBMD, the higher the risk of fracture” (Cummings et al. 2002).
**DXA and bone strength**

Despite the reported benefits of DXA and the usefulness of aBMD in predicting fracture risk (Marshall et al. 1996, Stone et al. 2003), there are many difficulties with using DXA-based aBMD to assess whole bone strength.

For one, bone density is only one aspect of bone strength (Cheung & Detsky 2008). Studies on the use of sodium fluoride to treat osteoporosis showed that sodium fluoride caused large increases in bone mass and density, but the strength of the bone did not properly reflect these increases. Bones became more brittle as a result of the fluoride treatments, and thus fractured more despite having greater densities (Riggs et al. 1990). DXA, which obtains a density measurement based on a user-defined area, cannot tell us much about the brittleness of bone, or any material or structural properties of bone per se. Because DXA cannot directly assess bone material or structural properties, aBMD is used as a surrogate measure of bone strength. However, aBMD cannot discriminate between cortical and trabecular bone, which have very different rates of remodelling/metabolism and maintain very distinct structural functions. Instead, both types of bone are assessed together to produce a single density measurement (Liu et al. 2007). Furthermore, DXA-based aBMD cannot encompass the anisotropy of bone microarchitecture (Cheung & Detsky 2008), nor can it assess changes in microarchitecture (Nazarian et al. 2009).

The fact that DXA produces an area-based density measure (i.e. a density value based on the two-dimensional projected area of the bone rather than its volume) complicates the interpretation and the accuracy of the results. In growing bones, such as those of children and young adults who have not yet reached peak bone mass, this is a concern, especially when trying to interpret changes in aBMD. Longitudinal studies involving growing mice have shown that the growth of long bones could artificially increase the aBMD even if the actual density of the bone
remains the same (Grynpas et al. 2000). Simply put, aBMD would be “higher if the bone is
bigger, even if the degree of mineralization of the bone is the same” (Cheung & Detsky 2008).
Even in an elderly population where there is greater susceptibility to osteoporotic fractures,
density measures could be affected. For example, an elderly osteoporotic patient may, over time,
show an increase in aBMD even though he is experiencing atraumatic fractures in his lumbar
spine due to deteriorating bone health. While this seems strange at the outset, a person whose
vertebrae have fractured and collapsed under their own body weight would have a smaller
projected area than non-fractured vertebrae. In some cases, vertebral collapse could occur not
due to a significant amount of bone loss, but rather to a small amount of bone loss in strategic
areas, such as tiny trabecular cross-bridges that work to support the overall weight-bearing
capacity of the vertebra. Areal BMD, being a function of area, would be affected greatly by the
change in size of the vertebrae, and very minimally by the negligent decrease in bone mass, and
aBMD would be artificially overestimated; for example, aBMD could increase over time despite
the presence of low-trauma fractures (Blake & Fogelman 2007).

Although DXA-based aBMD can be used to predict fractures, aBMD cannot be used to
identify patients who will fracture. The authors of the 1996 meta-analysis which showed rather
conclusively that aBMD decreases are associated with increases in fracture risk note that they
“cannot recommend a screening programme for osteoporosis by measuring bone density”
(Marshall et al. 1996). This is because there is significant overlap between the aBMD
distribution curves of persons who fracture and those who don’t (Marshall et al. 1996, Jergas &
Gluer 1997). This is due to the fact, as mentioned previously, that aBMD is but one determinant
of future fracture. Other aspects of bone quality that contribute to its overall strength as well as
external factors (such as propensity to fall) would affect whether or not a person develops
fractures. A young (35 year old), healthy female individual might have a low aBMD as
measured by DXA (more than 2.5 standard deviations below the mean), yet her bone strength could be higher than that of an elderly (85 year old) who has the exact same aBMD but poor bone quality. The two individuals, having two very distinct conditions, would require very different plans for maintaining healthy bones, and use of aBMD alone would not help in differentiating them. Areal BMD, then, can be used as a predictor of fracture risk, but cannot be used to definitively identify patients who will later fracture (Marshall et al. 1996).

The link between areal BMD and bone strength is further challenged by the fact that post-therapy changes in fracture risk cannot be properly accounted for by the corresponding changes in aBMD. In several studies examining the role of antiresorptive agents in decreasing fracturing risk, it was found that the drugs prevented fractures more effectively than would be expected from their minimal effects on bone density (Licata 2009). Salmon calcitonin nasal spray (200IU for 5 years), for example, decreased the occurrence of new fractures by 33% even though bone density increased by only 0.6% (Chesnut et al. 2000). Raloxifene (60 mg for 3 years) decreased fracture risk by 35% with only a 2.6% increase in density (Ettinger et al. 1999), while Risedronate (5 mg for 3 years) decreased fracture risk by 41% while only increasing aBMD by 4.3% (Harris et al. 1999). Cummings et al. (2002) performed a meta-analysis of these three and nine other studies to describe the relationship between improvements in spine bone density after therapy and the reduction in spine fracture risk (Cummings et al. 2002). A regression model from this meta-analysis was applied to data from the Fracture Intervention Trial where 1 year of alendronate treatment caused a 3.9% increase in aBMD corresponded to a decrease in fracture risk of 47% (Black et al. 1996, Cummings et al. 1998). The authors conclude from their analysis that the change in aBMD with alendronate treatment accounts for only 16% of the change in fracture risk, and note that while an increase in spine density helps reduce spine fracture risk, aBMD measured by DXA “substantially underestimates the degree” to which the drugs reduce
fracture risk (Cummings et al. 2002). Studies of the bone formation agent Teriparatide (PTH 1-34) had similar findings to those of antiresorptives, with a 9.7% increase in spine aBMD corresponding to 65% reduction in fracture risk. These findings collectively indicate that post-therapy changes in fracture risk occur not solely nor primarily due to the addition of bone mass or increases in density, but rather to the overall improvement of bone quality due to characteristics independent of bone density. It is likely, especially in the case of antiresorptives, that bones become stronger before they become denser due to a reduction of osteoclast activity and a subsequent shift in the bone remodelling balance away from resorption. This leads to the preservation of architecture and other aspects of bone quality even before bone mass increases (Licata 2009).

**Quantitative Ultrasound (QUS)**

While DXA is the gold standard in the clinical assessment of bone, QUS can also be used as an inexpensive, highly accessible device to assess the quality of bone (Djokoto et al. 2004 Lewiecki et al. 2006, Hans & Krieg 2009). The device transmits sound waves through bone and measures both the speed of the sound through bone, as well as the attenuation of sound through bone.

**Technical principles of QUS**

Ultrasonic waves are sound waves that fall outside the limit of human hearing (Hans & Krieg 2009). First used during the Second World War to detect submarines under water, most people know of the clinical use of ultrasound technology in the context of obstetrics, when assessing fetal development. However, in contrast to this use of qualitative ultrasound (which
Ultrasonic waves pass freely through fluid and other soft tissues. Upon hitting bone, however, the waves are altered in terms of their shape, intensity, and speed (Hans & Krieg 2009). QUS works by passing high-frequency sound waves, between 0.1 and 1.0 MHz, through bone and quantifying the changes in the characteristics of the signal upon contact with bone (Lewiecki et al. 2006). The device produces and detects the sound signals using highly efficient piezoelectric transducers, which are coupled to the skin with ultrasound coupling gel, silicone pads, or even a water bath, depending on the model used (Lewiecki et al. 2006). Different models of QUS devices exist; however they can be classified into three main types. Most devices use trabecular sound transmission to pass sound through the calcaneus (heel). Cortical transverse transmission is used in some devices for assessment of phalanges, while cortical axial transmission is also used in devices to assess the phalanges and long bones such as the radius, ulna, or tibia (Hans & Krieg 2009). The difference between the cortical transverse devices and the cortical axial devices is that the sound waves pass entirely through the bone with transverse transmission, whereas the waves run longitudinally across the bone with axial transmission.

QUS devices produce two main measures: speed of sound through bone (SOS) and broadband ultrasound attenuations (BUA). SOS is, simply, the speed of the sound wave that passes through the bone, and is measured in metres per second (m/s). Typical values for cortical bone are 3000 to 3600 m/s and are 1650 to 2300 m/s for trabecular bone (Lewiecki et al. 2006). BUA is a measure of the loss of energy that the sound wave experiences while travelling through the bone, and is measured in decibels per megahertz (dB/MHz) (Lewiecki et al. 2006). The higher these numbers are the higher the density of the bone, and for this reason QUS devices are generally capable of producing an estimate of bone density. Many devices combine the
measurements of SOS and BUA to produce a composite measure, referred to as the “stiffness index” (SI) or the “quantitative ultrasound index” (QUI) (Lewiecki et al. 2006, Hans & Krieg 2009).

Clinical use of QUS

QUS was first reported as being useful for bone assessment in 1984, when it was shown that it could differentiate between population of elderly women with a history of hip fractures and a population of elderly women with no history of hip fractures (Lewiecki et al. 2006, Knapp 2009). Because QUS is much cheaper to obtain, operate, and maintain than DXA, it enjoys widespread usage in places especially where there is limited access to DXA (Knapp 2009).

Numerous studies have shown that QUS, especially when done at the calcaneus, can be used to predict fracture risk in both elderly men and women (Hans & Krieg 2008, Bauer et al. 1997, Gluer et al. 1996, Bauer et al. 1995, Njeh et al. 1997, Siris et al. 2001). The EPIDOS study of 5662 elderly women found that one standard deviation below the mean for SOS corresponded to a relative risk of fracture of 1.7, while a one standard deviation below the mean for BUA corresponded to a relative risk of fracture of 2.0 (Hans et al. 1996). By comparison, a one standard deviation below the mean for DXA-based aBMD corresponded to a relative risk of 1.9 (Hans et al. 1996). The Study of Osteoporotic Fractures (SOF) found that a one standard deviation reduction in BUA corresponded to a relative risk of fracture of 2.0, while the relative risk of fracture was 2.6 for a one-SD decrease in bone density (Bauer et al. 1997). In the National Osteoporosis Risk Assessment (NORA), hip fracture relative risk was 1.28 for a one-SD decrease in BMD estimated from QUS. Although it is difficult to compare the results from various studies of the relationship between QUS and fractures due to methodological issues,
Hans and Krieg (2009), after reviewing thirteen such studies, state that the relative risk of fracture for each one-SD decrease in the stiffness index (SI) is approximately 2.0 for the hip and spine specifically, and approximately 1.5 for all fractures. They conclude that QUS of the calcaneus is similar to aBMD measured by DXA in its ability to predict hip and spine fracture risk.

**QUS and bone strength**

Like DXA, QUS does not directly measure bone mechanical properties but instead provides only an indirect assessment of bone strength (Djokoto et al. 2004). However, because the sound waves of QUS behave in an essentially distinct way from the x-rays of DXA upon contact with bone, QUS has the potential to inform on more aspects of the quality and mechanical integrity of bone.

The speed of sound through bone in particular is affected by properties of the bone material that affect its mechanical competence (Hodgkinson et al. 1997, Greenfield et al. 1981). A sound wave travelling through a solid medium is described by the formula $v = \sqrt{E/\rho}$, where $E$ is the modulus of elasticity and $\rho$ is the material density. This is also true for bone (Hodgkinson et al. 1997, Greenfield et al. 1981). Thus, SOS is related to both the density and material stiffness of bone, both of which affect the overall strength of bone.

Numerous authors have used QUS to investigate a wide range of bone quality parameters, such as density, cortical thickness, and microstructure (Gluer et al. 1993, Njeh et al. 2001a, Sievanen et al. 2001, Funck et al. 1996, Knapp et al. 2001, Gluer et al. 1994). In one ex vivo study with bovine bone, ultrasound velocity was found to correlate strongly ($R = 0.753$) with values of cancellous bone yield strength (Turner & Eich 1991). Another study found that
ultrasound velocity explained 95% of the variance in the elastic modulus of cancellous bone (Hodgkinson et al. 1997). Several studies have reported that QUS parameters are significantly related to bone structural parameters independent of density (Gluer et al. 1994, Cortet et al. 2004, Wuster et al. 2005, Portero et al. 2005). Ultrasound attenuation has also been reported to be affected by bone structural parameters in addition to being dependent on bone density (Njeh et al. 2001a).

Unfortunately, although QUS measures are affected by bone quality parameters more than DXA-derived measures, it is not entirely clear how different material or structural properties of bone end up contributing to the SOS or BUA values (Njeh et al. 2001b). Because of this, interpretation is difficult, as differences between QUS measures between individuals or populations, or even in a single individual over time, can be due to a wide variety of reasons. In cortical bone, for example, many properties would affect QUS measures such as cortical thickness, degree and quality of mineralization, and porosity. At present there is much ambiguity surrounding the degree that these variables would affect both QUS measures and bone strength (Muller et al. 2003, Lee et al. 1997, Barkmann et al. 2000, Sakata et al. 2004). For this reason, unlike DXA, QUS cannot be used to initiate treatment for osteoporosis (Hans & Krieg 2009). Furthermore, although QUS measures have strong correlations to DXA measures at the same sites, there are huge variations in QUS values between skeletal sites (Hans & Krieg 2009). As such, there is not enough evidence to allow QUS measures, which are typically taken at peripheral sites, to be used as estimates of central (hip or spine) DXA measures in the diagnosis of osteoporosis (Lewiecki et al. 2006, Hans & Krieg 2009). Thus, while QUS measures reflect bone quality to some degree, it cannot be used effectively to assess individual properties or changes in bone, nor can it be used to diagnose individuals with osteoporosis. Both of these abilities are required for the purposes of initiating treatment and preventing fractures before they
occur. In North America, DXA measures at central skeletal sites remain as the standard in the
diagnosis of osteoporosis.

**High-resolution peripheral quantitative computed tomography (hr-pQCT)**

High-resolution peripheral quantitative computed tomography is a relatively new device
that can make assessments of bone density and geometry in three dimensions. Currently used as
an investigative device, it has shown potential for better assessment of bone strength than either
DXA or QUS. As with DXA, ionizing radiation (approximately 1/5 of amounts present in a
chest x-ray) is present.

**Technical principles of hr-pQCT**

High-resolution pQCT is based on the same technical principles as quantitative computed
tomography (QCT). Similar to DXA, hr-pQCT utilizes an x-ray tube to transmit x-rays through
the region of interest, which are then received by a detector on the opposite side of the sample
(Kazakia & Majumdar 2006). Unlike DXA, however, both the source and detector rotate around
the sample so as to obtain a series of attenuation profiles in 360 degrees, thereby creating one
“slice.” Algorithms are used to reconstruct data from multiple slices into a three-dimensional
data matrix representing all of the variation in x-ray attenuation within the sample (Kazakia &
Majumdar 2006). Because the device is precalibrated with a phantom of known density, the
attenuation data can be converted to measures of mineral density (Kazakia & Majumdar 2006).
In addition to data acquisition, hr-pQCT software can also perform 3D image reconstruction, and
provide compartmentalized volumetric density measurements (i.e. separate measures for cortical
and trabecular bone), and measures of bone geometry at a high resolution (approximately 82
microns). Typically, hr-pQCT scans are performed at the distal forearm or the tibia (Kazakia & Majumdar 2006).

High-resolution pQCT and bone strength

High-resolution pQCT shows great potential for overcoming many of the limitations presented by both DXA and QUS in assessing bone strength. Because it has a relatively high resolution of 82 microns, it is able to resolve individual trabeculae (Kazakia & Majumdar 2006, Boutroy et al. 2008, Varga & Zysset 2009), which typically range in size from 60 to 150 microns (Schnitzler et al. 1996). Because it assesses bone in three-dimensions, it can provide true volumetric density measures, as opposed to areal measures obtained with DXA. High-resolution pQCT can separate out the effects of trabecular and cortical bone. This is of great importance in assessing bone quality and in understanding bone physiology, as trabecular bone is metabolically more active than compact bone. By failing to assess each type of bone separately, the sheer volume of cortical bone can sometimes mask clinically important changes in trabecular mass or quality (Kazakia & Majumdar 2006). Furthermore, being able to assess each type of bone separately can lead to new insights into the cause of fracture – was it due to trabecular deterioration or to cortical thinning? – which can help guide decisions on therapeutic interventions. High-resolution pQCT can provide measures of both of the determinants of bone strength: bone material properties (such as density) and bone structural properties (such as cortical thickness, trabecular thickness, and trabecular separation) (Muller et al. 1996a, Muller et al. 1996b).

Currently, hr-pQCT is being evaluated in long-term clinical studies to assess its utility in assessing bone strength (Kazakia & Majumdar 2006). One recent study using hr-pQCT found
that at the radius and tibia, postmenopausal women had lower density, trabecular number, and
cortical thickness than younger women (Boutroy et al. 2005). Furthermore, lower density,
cortical thickness, and higher trabecular separation was found for osteoporotic women compared
to osteopenic women. Lastly, a significantly lower trabecular density was found at the distal
radius for fractured osteopenic women compared to unfractured osteopenic women, even though
aBMD was not able to differentiate between the two groups (Boutroy et al. 2005). More recently
the same group found that atraumatic fractures were associated with low volumetric BMD and
alterations of cortical and trabecular bone measured by hr-pQCT, partially independent of any
decrease in aBMD measured by DXA (Sornay-Rendu et al. 2007).

A recent study of human cadaver radii with low bone mass found that volumetric BMD
measured by hr-pQCT correlated strongly to DXA-based aBMD (R² = 0.69) (MacNeil & Boyd
2007). In addition, the structural parameters measured by hr-pQCT were validated against
measurements obtained by microCT, showing that hr-pQCT provides accurate assessments of
bone quality that can be of great clinical utility (MacNeil & Boyd 2007). Many other ex-vivo
studies have used pQCT (having lower resolutions than hr-pQCT) to assess the quality of small
animal or human bone (Gasser et al. 2008, Silva & Touhey 2007, McCann et al. 2008,
Lochmuller et al. 2002). While these studies can provide some insight into the relationship
between pQCT measures and bone strength, results must be accepted with caution, as the lower
resolution of older pQCT devices precludes proper analysis of trabecular architecture, especially
in smaller animals where the cortical shells might be thinner than the voxel size of the scanner.
**Mechanical response tissue analyzer (MRTA)**

MRTA, unlike DXA and QUS, does provide a mechanical measurement of bone (Djokoto et al. 2004. Developed by NASA to assess the effect of space travel on astronauts’ bones, MRTA is a vibration-based device that can provide a measure of the cross-sectional bending stiffness (EI) of long bones.

**Technical principles of MRTA**

MRTA uses a random signal generator to cause an electromagnetic shaker to deliver a low-frequency vibration stimulus (<1600 Hz) to bone via a small impedance head. A transducer, which is also connected to the impedance head, measures the dynamic response of the bone (force and acceleration) and uses a software algorithm developed by Gaitscan and NASA to calculate the lateral stiffness of the bone (Djokoto et al. 2004. The vibration stimulus is applied at the midpoint of the long bone, such that the bone is “loaded” in a three-point bending setup. Based on the formula $EI = kL^3/48$, where $k$ is the lateral stiffness and $L$ is the length of the bone, EI, or the cross-sectional bending stiffness of the bone can be computed (Djokoto et al. 2004.

The MRTA was developed for in vivo use, where skin and musculature can affect the computation of EI by dampening the vibration stimulus and the dynamic response of the bone. To account for this stiffness of soft tissue between the impedance head and the bone, the electromagnetic shaker and probe are weighted so as to press down on the bone at the point of contact, compressing the skin, thereby increasing its stiffness, which helps to minimize its dampening effects by increasing the transmission of the vibration stimulus to the bone (Young et al. 1976, Saha & Lakes 1977). In addition, MRTA uses a seven-parameter mathematical model
to determine bone stiffness that models skin and bone as two springs in series, each with their own stiffness (Djokoto et al. 2004. The model contains terms to account for the stiffness and damping effects of both skin and bone (Steele et al. 1988).

### MRTA and bone strength

The cross-sectional bending stiffness of the bone (EI) that is measured by MRTA is a product of E, the intrinsic stiffness or modulus of the material, and I, the cross-sectional moment of inertia. As such, MRTA produces a measure of a measure of a mechanical property of bone. Cross-sectional bending stiffness by its very definition reflects the mechanical competence of the bone, taking into account both the intrinsic properties of the bone material, as well as the geometry of the bone (Djokoto et al. 2004. EI tells us about the bone’s ability to withstand the bending forces that can ultimately lead to fracture. By taking into account both bone material properties as well as bone structural properties, we can expect that MRTA would tell us more about bone strength than do DXA or QUS.

The MRTA has previously been shown to be a good indicator of true bone mechanical properties as measured in three-point bending tests (Djokoto et al. 2004. Roberts et al (1996) showed that for padded aluminum rods, there was an extremely strong relationship ($R^2 = 0.999$) between EI measured by MRTA and the theoretical EI values for the rods (Roberts et al. 1996). In animal studies, EI as measured by MRTA has been found to have a strong correlation to the three-point bending stiffness of the same excised bones ($R = 0.753$ to $0.975$) (Roberts et al. 1996, Norrdin et al. 1995, Hutchinson et al. 2001). In addition, EI as measured by MRTA has been found to correlate strongly ($R^2 = 0.92$) to the ultimate load (the bone’s breaking strength) for the same excised bones tested in a three-point bending setup (Roberts et al. 1996). Collectively,
these animal studies indicate that EI measured by MRTA does reflect bone strength to a great degree.

In clinical populations, MRTA measurements have shown significant amounts of variation between healthy and osteoporotic women (Kiebzak et al. 1999). In addition, the MRTA has shown some ability to distinguish between some subject groups based on age or gender (Kiebzak et al. 1999).
CHAPTER 2:

A new ex-vivo animal model for the evaluation of bone strength

Idrees Ally, Luisa Moreno, PhD, Jeremy Lau, Christina Djokoto, MSc,

and Angela M. Cheung, MD, PhD

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Several animal models are currently used in the preclinical study of osteoporosis, but none are specifically used to test new clinical diagnostic tools. With the increased focus on trying to measure bone quality and bone strength, there is a need to develop new models to assess new diagnostic tools that would examine these factors and not just bone density in the clinical setting. This paper presents the emu as a suitable ex-vivo animal model for the assessment of bone strength. The emu is a large, bipedal, flightless bird whose tibiae are similar in size to human long bones. They have large medullary cavities which make them amenable to ex vivo modeling of endocortical bone mineral loss and breakdown of collagen. Lastly, unlike many other smaller models in use, their size makes them well suited for testing on clinical diagnostic devices, thus allowing for further development of these tools for clinical use.
Introduction

Osteoporosis is a metabolic bone disorder characterized by a systemic decrease in the density and quality of bone (Burnell et al. 1982, Kleerekoper et al. 1985, Parfitt 1992). The disease, which affects approximately one in two women and one in five men over the age of 50 (Keen 2007), causes progressive deterioration of bone tissue until its mechanical integrity is compromised. This results in decreased bone strength, the consequence of which is an increased risk of fracture, especially at the wrist, spine, and hip. Osteoporotic fractures are a major health burden, with significant pain, disability and increased mortality, especially after a hip fracture (Keen 2007).

The decreases in bone strength seen in osteoporosis are associated with compromised material and structural properties (Seeman & Delmas 2006 Chavassieux et al. 2007). Material properties are affected by the amount of the bone mineral hydroxyapatite, the collagen content, and the amount of other crystals. Structural properties reflect the arrangement of the material in space – structural parameters such as bone size, cortical thickness, and cross-sectional moment of inertia have all been used as predictors of bone strength (Augat & Schorlemmer 2006). Increasingly, more attention is being given to the argument that bone strength depends on more than just bone mass and density (Hernandez & Keaveny 2006, Friedman 2006). While osteoporosis was previously defined as a “skeletal disorder characterized by low bone mass”, recent definitions describe it as being “characterized by compromised bone strength predisposing to an increased risk of fracture” (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001).

In the clinical setting, there is interest in improving the prediction of fracture risk in individuals so as to take the necessary preventative steps in those at high-risk. As such, it would
be useful to be able to measure one’s bone strength. However, no accurate measures of bone strength currently exist (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001, Grynpas et al. 2000). Clinical tools employed in diagnosing osteoporosis are still largely based on areal bone mineral density (aBMD) measurements, which account for some (30%) but not all of bone strength. In order to better predict fracture risk, it is highly desirable that new clinical diagnostic tools are developed to measure bone strength in humans. To do so, a new animal model is needed, one that can overcome the limitations of current models. We propose here the emu as an ex vivo model that can be used to assess bone strength.

**Animal Models in the Study of Osteoporosis**

Davidson et al. (Davidson et al. 1987) have outlined nine criteria for the selection of an animal model for research: “1) appropriateness as an analog, 2) transferability of information, 3) genetic uniformity of organisms, where applicable, 4) background knowledge of biological properties, 5) cost and availability, 6) generalizability of the results, 7) ease of and adaptability to experimental manipulation, 8) ecological consequences, and 9) ethical implications.” However, an important consideration is the “realization that different questions may require different models,” especially when considering a complex, multifactorial disease such as osteoporosis, where a single model may not be sufficient to address all of the characteristics of the disease. A reductionist approach would thus divide a complex disease into several components, and analogous animals would be chosen to model each component (Davidson et al. 1987).

For osteoporosis, these include smaller animals such as mice and rats, and larger models such as dogs, sheep, swine, and non-human primates (Kimmel et al. 1999). Rodent models have
practical advantages such as being readily available, inexpensive, and easy to care for (Turner 2001, Rodgers et al. 1993, Mosekilde 1995). The mouse is used mostly in experiments in which manipulation of the genome is required (Kimmel et al. 1999, Turner 2001), and transgenic mice have been used to identify and characterize genes involved in regulating osteoclast activity (Kimmel et al. 1999). The rat is the most commonly used animal model in the preclinical study of osteoporosis (Grynpas et al. 2000, Turner 2001). Having been used to model a wide variety of human conditions, including skeletal disorders, there exists extensive information regarding their skeletal physiology and mineral metabolism (Rodgers et al. 1993). In addition, standardized strains and feeds are available, lending additional credibility to results (Rodgers et al. 1993, Mosekilde 1995). Ovariectomized (OVX) rats are most commonly used, as they experience increased turnover and site-specific trabecular bone loss that closely mimics that of human postmenopausal osteoporosis (Kimmel et al. 1999, Turner 2001). In addition, they have been found to respond positively to treatment with bisphosphonates, calcitonin, and selective estrogen receptor modulators, just as humans do. However, because they lack Haversian systems, they have little intracortical remodeling, and their cortices are therefore less responsive to ovariectomy than human cortices (Grynpas et al. 2000, Turner 2001, Rodgers et al. 1993, Mosekilde 1995). Two OVX rat models are used: mature rats and aged rats, where the aged rats are those less responsive to ovariectomy as their skeletal growth has plateaued (Grynpas et al. 2000, Rodgers et al. 1993). As with other animal models used in the preclinical study of osteoporosis, rats do not develop fragility fractures spontaneously (Grynpas et al. 2000, Kimmel et al. 1999), and ex vivo mechanical testing is thus required to determine the amount of load required to cause a rat bone to fracture – more load translates to stronger bones (Grynpas et al. 2000, Kimmel et al. 1999). Notwithstanding, the rat is an excellent model for postmenopausal
bone loss, and can be used to evaluate new therapies and their effects on bone quality (Grynpas et al. 2000, Mosekilde 1995).

The dog is another commonly used model, having a skeleton that is similar to that of humans, with Haversian systems and similar patterns of remodeling. Although some studies have indicated that OVX dogs lose around 8-10% of their bone mass per annum, these results have not been reproduced consistently, and as such the OVX dog has not been used much as a model (Kimmel et al. 1999, Turner 2001). Adult dogs can, however, be used to test the effects of anabolic agents on Haversian remodeling (Kimmel et al. 1999, Turner 2001).

Sheep and pigs have been used as larger animal models of osteoporosis, and have the advantage of not being subject to the same level of emotional attachment as dogs are (Turner 2001). Sheep have been found to experience selective bone loss following ovariectomy (Kimmel et al. 1999, Turner 2001, Rodgers et al. 1993) that can be corrected by estrogen replacement therapy and selective estrogen receptor modulators (Turner 2001). Biochemical markers of bone turnover have also been found to increase in OVX ewes, indicating accelerated remodeling (Kimmel et al. 1999, Turner 2001). However, because of inconsistencies in data, further studies are required to validate the sheep as a model (Kimmel et al. 1999). The osteology of the pig is similar to that of the human in several aspects. The pig displays extensive Haversian remodeling, and bone remodeling in both cortical and cancellous bone occurs at a rate similar to that of humans (Turner 2001, Rodgers et al. 1993). Like humans, pigs have lamellar bone and can also develop spontaneous vertebral fractures on rare occasions (Turner 2001). Studies have shown that ovariectomy combined with calcium restriction causes bone loss (Turner 2001, Rodgers et al. 1993). However, the effects are minor, and like the sheep, more studies are needed for further acceptance of the pig as a model for postmenopausal bone loss (Kimmel et al.
These two models, being larger animal models, are also more expensive to obtain and maintain, which is an important consideration in larger studies.

The nonhuman primate (NHP), such as the macaque monkey, is the most analogous of all models to humans due to morphological and physiological similarities, and is thus the large animal model of choice when intracortical remodeling is a necessity (Grynpas et al. 2000, Kimmel et al. 1999). Female NHPs exhibit monthly estrous cycles and experience menopause, displaying similar endocrine profiles to human females (Grynpas et al. 2000, Kimmel et al. 1999, Turner 2001, Rodgers et al. 1993). Furthermore, their bone biomechanics may be similar to human biomechanics, as anatomic similarities at the hip suggest similar patterns of stress distribution (Grynpas et al. 2000, Rodgers et al. 1993). NHPs have been found to experience decreased bone mass and bone strength after ovariectomy (Kimmel et al. 1999, Rodgers et al. 1993), as well as increased amounts of biochemical markers of turnover (Turner 2001). Bisphosphonates have also been shown to prevent bone loss in OVX NHPs (Kimmel et al. 1999). The aged, non-OVX NHP also experiences age-related osteopenia, with progressive bone loss after reaching skeletal maturity (Grynpas et al. 2000, Kimmel et al. 1999). Limitations of the NHP as a model include the practical difficulties and expenses incurred in maintaining them for the lengths of time required to obtain results. Public reluctance to housing NHPs in captivity is also of concern (Grynpas et al. 2000, Turner 2001). Also, these animals rarely sustain spontaneous fractures, so biomechanical tests of excised bone are still necessary to determine bone strength.
The Emu: Basic Biology

The emu (*Dromaius novaehollandiae*) is a large, bipedal, ostrich-like bird, one of the few remaining species of flightless birds. It is a member of the ratite family, one that includes ostriches, kiwis, cassowaries, and rheas (Reed & Brown 2001). Emus are indigenous to mainland Australia, although they are also found in North America where they are farmed commercially (Reed & Brown 2001). In their native habitat, emus feed on a diet consisting mainly of plants, including flowers, seeds, fruits, and growing shoots, though they do at times consume insects and small vertebrates (Davies & Bamford 2002). Emus are nomadic, congregating in areas of food abundance, and dispersing after consuming the available supply (Davies & Bamford 2002). They are seasonal breeders, laying their eggs each autumn (Davies & Bamford 2002), with adults eventually reaching skeletal maturity at around one year of age (Conzemius et al. 2002). They can grow up to 2.5 meters, though they are generally approximately 1.5 meters tall (Reed & Brown 2001, Davies & Bamford 2002).

As birds, their bones should follow the general structural patterns of avian bones, which are pneumatic, being filled with hollow air spaces that are connected to the respiratory system (Proctor & Lynch 1993). Although avian bones are strong relative to their masses (Proctor & Lynch 1993), they tend to be lighter than mammalian bones, and are more weak and compliant (Reed & Brown 2001). Reed and Brown, though, suggest the possibility that emus may have evolved bones that are mechanically more similar to mammalian bone than avian bone as a result of their continued “terrestrial bipedal ambulation” (Reed & Brown 2001). In their study of emu cortical bone, they found the elastic modulus of emu cortical bone to be $13.05 \pm 3.94$ GPa, which is similar to that of ostriches, geese, and other domestic fowl, and is about 25% less than the elastic modulus of human bone. They also found in bending tests that emu cortical bone
typically had similar mechanical properties to those produced during bending tests of human
cortical bone. However, there are morphological differences between human and emu bone. For
example, the emu femoral cortex is relatively thinner than in humans, and the orientation of
trabeculae is likely different as well (Reed & Brown 2001).

The Emu as a Model

The tibia of the emu (Figure 1) presents itself as a potential new animal model for the assessment
of bone strength. The excised tibiae can be used to evaluate the mechanical properties of bone
under various experimental conditions. At the same time, the excised tibia can be modified in
order to elicit various characteristics of osteoporosis such as decreased mineral content, and the
mechanical properties can be assessed by mechanical loading tests. While, as mentioned
previously, other animal models currently exist, the ex-vivo emu model overcomes some of the
serious limitations that affect application of conclusions from these models to the human
condition. According to Davidson, this is the reductionist approach to research: different models
are utilized in assessing different symptoms or component aspects of a disease state. The
overriding consideration in choosing a model then, should be “the realization that different
questions may require different models” (Davidson et al. 1987).
Figure 1. Emu Tibia
The benefit of the emu as a model for assessing bone strength stems mainly from its size (Reed & Brown 2001, Conzemius et al. 2002), which makes for better assessment of its mechanical properties, better induction of pathophysiologic conditions, and increased suitability for use on human devices. Because of its length, the emu tibia allows for more accurate assessment of mechanical properties. In bending tests, as described by Turner and Burr (Turner & Burr 1993), the length of the segment of the bone being loaded must be sufficiently long in order for results to be as accurate representation of the true modulus of the bone. In cases where the bone is not of a sufficient length, the calculated modulus will be affected by the fact that the displacement of the bone would be highly influenced by shear stresses. Turner and Burr recommend a length-to-width ratio of at least 16:1, which they also note is difficult to attain with most whole-bone specimens (Turner & Burr 1993). Some researchers resort to using machined samples in order to achieve the desired ratio, but the behaviour of these samples under loading cannot be taken as being representative of the behaviour of whole bones, nor can there be any guarantee that the machining process does not result in unwanted or unquantifiable amounts of mechanical damage to the specimen.

The size of the emu tibia also makes it easier to induce experimental conditions that are more analogous to certain characteristics of pathological bone loss in humans. Our group has utilized emu tibiae to model human bone loss. The epiphyses of the tibiae were removed, as well as the trabeculae and marrow, leaving a cylindrical shell of cortical bone. By preparing the samples such that the ends were completely sealed, except for tubes entering and exiting the bone to supply a demineralizing agent, a novel system was set up to model endocortical bone loss. This is analogous to the human condition, where remodeling takes place endocortically and from within Haversian systems. In previous experiments (Shah et al. 1995, Broz et al. 1995, Bowman et al. 1996), bone loss was induced by bathing the entire whole-bone or machined
specimen in the demineralizing agent, resulting in bone that while demineralized, differs from its original state in ways far greater than does human osteoporotic bone. The change in the mechanical integrity of a specimen demineralized as in previous experiments cannot, therefore, completely reflect the changes that would be expected due to endocortical bone loss.

Though it is possible to some extent with other models to assess mechanical properties and to induce experimental conditions that are analogous to the human condition, the ex vivo emu model is unique in its suitability for testing on human-scale clinical devices. It is of clinical relevance to be able to assess not only changes in mechanical properties of the bone, but also the effect of these changes on clinical measures of bone quality. The ex-vivo emu model confers an advantage here, as its size allows for it to be tested on clinical devices, including dual-energy x-ray absorptiometry (DXA), high resolution peripheral quantitative computed tomography (HR-pQCT), quantitative ultrasound (QUS), and the mechanical response tissue analyzer (MRTA) – a direct mechanical measure of bone strength. While some of these devices are also manufactured at smaller sizes for the purposes of testing small animals, there are many issues that complicate the transferability of information from small animal models to the human condition. For example, with DXA, a small animal scan mode has previously been validated for measurement of bone density at a higher resolution in smaller specimens (Koo et al. 2004, Casez et al. 1994, Norris et al. 2000, Nagy et al. 2001). However, because DXA calculates bone mineral density as a function of a two-dimensional region of interest, rather than the true volume of the bone, it is possible that artifacts could be produced that complicate interpretation of results. In longitudinal studies involving growing mice, for example, the growth of long bones could artificially increase the aBMD even if the actual density of the bone remains the same (Grynpas et al. 2000). This could also prove to be a concern with machined or whole-bone specimens that are bathed in a demineralizing solution, as the external dimensions of the sample could change, creating
unwanted artifacts. With the ex-vivo emu model, however, the sample is of a fixed size, and by having treatments applied to the medullary cavity, the outer dimensions remain the same after treatment, reducing the possibility of having such artifacts.

QUS has also been used on small animals by using a probe of an appropriate size (Giavresi et al. 2004). The benefit of the emu model with regards to QUS lies in the fact that it is an ex-vivo model, making it easier to isolate and identify factors that cause changes in bone quality. QUS, while being a useful tool in predicting fracture risk, provides a measure of the speed of sound (SOS) passing through the bone, which reflects a host of factors including bone density, cortical thickness, elasticity of the bone, and microarchitecture (Djokoto et al. 2004). The downside is that interpretation of QUS results are difficult in humans or in vivo animal models, as on its own, one cannot tell how the different factors interact to affect SOS. With ex-vivo testing, as in the emu model, it is possible to manipulate these factors independently, to see how they affect QUS results in isolation of each other.

With regards to its ability to be tested on human devices, the emu model confers the greatest benefit with MRTA. The MRTA is a non-invasive device that is currently being developed for the direct assessment of bone strength in long bones such as the ulna or tibia. It applies a low-frequency vibration stimulus to a long bone modeled in a three-point bending setup. Based on the dynamic response of the bone to the stimulus, it provides a calculated measure of the bone’s cross-sectional bending stiffness (EI). This measure reflects both the intrinsic stiffness of the bone, while at the same time taking the geometry of the bone into account (Djokoto et al. 2004 Roberts et al. 1996, Myburgh et al. 1992). Results from our preliminary studies suggest that this device will have a greater predictive ability for fractures than other current devices used in the diagnosis of osteoporosis and fracture risk assessment.
Because this device was built for use on humans, testing and optimization for clinical use requires bones of an animal model that are of a similar size. The emu tibia is best suited as a model, as most other animal models used in the study of osteoporosis are of a smaller size.

In addition to the main advantages of the ex-vivo emu model that result from its size, there are also several other important reasons for the emu’s suitability as a model for human orthopaedic diseases. Because the birds are bipedal, they are similar to humans in that their high levels of activity place more of a biomechanical burden on weight-bearing bones such as the femur than in quadrupeds, where several limbs are available to bear body weight, even in cases where function of one limb is limited due to disease or injury (Conzemius et al. 2002). Conzemius et al. evaluated the emu’s potential as a suitable model for femoral head osteonecrosis, and found that emus in which femoral head osteonecrosis was induced progressed to end-stage structural collapse of the femoral head at a higher rate than what is seen with quadruped models of the same disease. Because they reproduce this feature of human osteonecrosis, emus show potential as an in vivo model for testing and developing methods for preventing mechanical failure of the femoral head (Conzemius et al. 2002). Finally, although not as a primary consideration, it is important to note that emu tibiae are available at low costs, though not in large numbers. Because they are already farmed commercially for their meat, feathers, and oil, there is little ethical concern or social resistance to their use.

Our proposed emu model does have its limitations. Avian osteology is significantly different from human osteology. Mammalian bone is more analogous to human bone in terms of composition and structure, although emus have bones that are closer in size to human bones. Studies of pharmacological treatments for human osteoporosis would still be best performed in mammalian models in which there is known intracortical remodeling. The US Food and Drug
Administration requires that agents intended to treat osteoporosis be tested first in a small animal model as well as a large animal model with known Haversian remodeling before they can be approved for clinical trials (Kimmel et al. 1999, Turner 2001, Mosekilde 1995). Most commonly used in these studies of potential therapies are the ovariectomized rat and non-human primate models. Lastly, the emu is a large animal, making it difficult to house under controlled conditions if used as an in vivo model.

**Conclusion**

The benefits outlined above support the emu as a potential ex vivo model for assessing bone strength. Further studies examining the composition and the structural and biomechanical properties of emu bones (especially weight-bearing bones) would be helpful for bone strength research.
CHAPTER 3:

Comparison of hr-pQCT to DXA and QUS

for the ex-vivo assessment of bone strength

Idrees Ally, Jeremy Lau, George Tomlinson, Claudia Chan, Jean Zu, Angela Cheung
Current clinical tools used to assess fracture risk do not directly assess bone mechanical properties but instead offer surrogate measures of bone strength. We conducted an ex-vivo study of emu bones to examine how a new tool, hr-pQCT, compares to current clinical tools (DXA and QUS) in predicting true bone mechanical properties.

We used DXA, QUS, and hr-pQCT to obtain measures of bone quality from emu tibiae (n=37), and performed four-point bending tests to obtain bone mechanical properties. We examined relationships between bone mechanical properties and individual measures obtained from DXA, QUS, and hr-pQCT using Pearson correlations. Linear regression modeling was used to predict mechanical properties from hr-pQCT parameters in order to compare the devices’ predictive abilities. We also built regression models to determine whether hr-pQCT measures improve on prediction of mechanical properties beyond DXA-derived measures alone.

We found that the bone breaking strength (ultimate load) correlates most strongly with areal bone mineral density (aBMD) and bone mineral content (BMC) measured by DXA, as well as cortical thickness and cortical cross-sectional area measured by hr-pQCT. Bone cross-sectional bending stiffness (EI-4pt) correlates most strongly with BMC by DXA, and polar moment of inertia, cross-sectional moment of inertia, and cortical cross-sectional area by hr-pQCT. Bone toughness correlates most strongly with aBMD and cortical thickness.

We also found that a model containing the hr-pQCT parameters cortical thickness and polar moment of inertia correlated to ultimate load as well as aBMD ($R^2 = 0.588$; $p < 0.001$ for hr-pQCT parameters vs. $R^2 = 0.605$; $p < 0.001$ for aBMD). Furthermore, a model containing the hr-pQCT parameters volumetric cortical BMD, polar moment of inertia, cross-sectional moment of inertia, and total cross-sectional area had a better correlation to EI-4pt than did DXA-derived
BMC ($R^2 = 0.893; p < 0.001$ for hr-pQCT parameters vs. $R^2 = 0.689; p < 0.001$ for BMC).

While adding hr-pQCT measures to aBMD did not increase prediction of ultimate load, adding hr-pQCT measures to aBMD increased prediction of EI-4pt.

Our results suggest that hr-pQCT parameters can predict bone strength as well as parameters obtained from DXA, using mechanical properties as the gold standard. As such, hr-pQCT shows great potential as a clinical device that can inform clinicians regarding determinants of bone strength and fracture risk not obtained by DXA and QUS.
INTRODUCTION

Osteoporosis and osteoporotic fractures are a major health burden (Burge et al. 2007) and a significant cause of pain, disability, and death (Robbins et al. 2006, Adachi et al. 2001, Ensrud et al. 2000, Greendale et al. 1995). Untreated, osteoporosis will lead to a reduction in the mechanical integrity of bone, resulting in fractures. Currently, there is much interest in improving the identification of individuals at risk of fractures so that preventative or therapeutic strategies can be implemented.

Fractures are mechanical events (Cheung & Detsky 2008, Silva 2007), and therefore proper assessment of fracture risk depends on the ability to properly assess bone mechanical competence, or bone strength. However, no accurate measures of bone strength currently exist in the clinical setting (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001). Although the mechanical competence of a material can be measured in an ex-vivo setup using mechanical testing, these tests are often destructive and are not appropriate in the clinical setting. Thus, in the clinical setting, accurately assessing bone strength entails being able to assess bone material and structural properties non-invasively.

Current tools used to diagnose osteoporosis and assess fracture risk, such as dual energy x-ray absorptiometry (DXA) and quantitative ultrasound (QUS), attempt to do so by assessing some of the determinants of bone strength (Djokoto et al. 2004). In doing so, they do not directly measure bone mechanical properties but instead offer surrogate measures that account for only some of the properties of overall bone strength. DXA, for example, measures the density of bone in a specified region of interest based on x-ray attenuation profiles. However, while the density of bone material is a useful predictor of fracture risk (Marshall et al. 1996, Stone et al. 2003), bone density alone does not take into account all of the factors that affect bone strength.
(Cheung & Detsky 2008), nor is it sufficient to completely explain changes in fracture risk with treatment (Harris et al. 1999, Chesnut et al. 2000, Ettinger et al. 1999, Benhamou 2007). QUS, on the other hand, measures the speed of sound (SOS) that passes through bone tissue. While affected by the quality of the bone, this measure is of limited value clinically in terms of diagnosing osteoporosis or monitoring therapy. Current tools, then, are based primarily on bone material properties (and to some extent bone size for DXA) and do not measure bone biomechanical properties directly (Djokoto et al. 2004). In order to better predict fracture risk, it is highly desirable that new clinical diagnostic tools are developed to measure bone strength in humans.

The high-resolution peripheral quantitative computed tomography (hr-pQCT) scanner is one tool that shows potential for better assessment of bone strength than currently used devices. hr-pQCT is a high-resolution (100 μm) 3D imaging method that can quantitatively provide detailed information about bone material and structural properties. Unlike the DXA scanner, which provides areal density measures, the hr-pQCT scanner can provide measures of true volumetric density, and can do so for each of the trabecular and cortical components of bone. In addition, it can quantify various parameters of bone structural integrity – cortical thickness, average thickness of trabeculae, average separation between trabeculae, and bone cross-sectional area are but a few such parameters. Since hr-pQCT can provide information about both the material and structural properties of bone, it is reasonable to hypothesize that information gathered from hr-pQCT scans can provide us with more information about bone strength. Thus, in this study, we aimed to investigate the utility of hr-pQCT in predicting true bone mechanical properties, as compared to DXA and QUS. We employed an ex-vivo emu model that we previously developed for assessing changes in bone strength due to endocortical bone loss.
METHODS

We obtained fresh-frozen emu legs (male and female, aged 3-5 years) from local farms with most of the musculature already removed. We dissected out the tibia and removed any remaining skin, musculature, and connective tissue, such that we were left with the bare bone. Care was taken to remove the periosteum without damaging the bone surface.

The ends of the bone were then removed. Using a high-speed circular saw, fifteen percent of the length of the tibia was removed from the proximal end, and ten percent removed from the distal end. Any remaining epiphyseal trabeculae were removed using a drill. Care was taken to ensure that the integrity of the cortical shell was not compromised by use of the drill. Marrow and marrow fat were flushed out with water, leaving intact a cylindrical shell of cortical bone. A total of thirty-seven bones were prepared, then soaked in saline-soaked gauze and stored at -20°C.

We then obtained measures of bone quality using DXA and QUS, the two commonly used technologies for assessing fracture risk. In addition, we obtained bone quality measures using hr-pQCT, and performed mechanical tests on the bones to obtain their true mechanical properties.

Dual-energy X-ray Absorptiometry (DXA) Scans

We used the Hologic DiscoveryA scanner to obtain measures of bone mineral content (BMC) and areal bone mineral density (aBMD) for a twenty-four centimeter region of interest centered about the midpoint of the bone. We scanned bones once in lumbar spine mode, taking care to ensure identical positioning along the centre of the fan beam to avoid potential artefacts. Bones were scanned while submerged in water that was used to simulate soft tissue.
Quantitative Ultrasound (QUS)

A Sunlight Omnisense sonometer was used to obtain measures of the speed of sound (SOS) through the bone. We positioned the ultrasound probe at the midpoint of the bone; wave transmission was axial. The probe was coupled to the bone with ultrasound coupling gel and thin slices (0.5 cm) of extra-firm tofu. Previous work by J. Wu (2001) has shown that tofu can be used to mimic soft tissue for the purposes of ex-vivo QUS testing (Wu 2001).

High-resolution peripheral Quantitative Computed Tomography (hr-pQCT)

We used the Scanco XtremeCT hr-pQCT scanner. We placed bones in a Plexiglas box filled with water. We positioned the box inside a carbon fiber tube designed to ensure proper placement in the scanner. Bones were scanned once in a scout view to identify the region of interest to be scanned in detail. A small piece of wire was fixed to the bone and used to identify the region of interest on the scout view. Because of size limitations of the hr-pQCT scanner, we could not scan the midpoint of the bone. Instead, we scanned two regions, approximately six centimeters of either side of the midpoint and averaged the values. Images were segmented using the device’s semi-automatic contouring procedure. Measures of volumetric density (cortical and total density) and geometric parameters (cortical thickness, cortical and total cross-sectional area) were obtained from these scans. Cross-sectional moment of inertia measures, which reflect bending, and polar moment of inertia measures, which reflect twisting, were also obtained.

Mechanical Tests

To obtain information about the bones’ true mechanical properties, we conducted four-point bending tests. On an Instron material testing system, bones were positioned on a four point
bending setup, with two lower points at thirteen centimeters on either side of center, and two upper points at either side of six centimeters from center. Bones were loaded at 0.005 m/s until failure occurred. To stabilize the bone and to prevent rotation or other movement during loading, we preloaded the bones to 100 N before beginning the tests. We collected force versus displacement data from the tests, from which we obtained the bones’ ultimate load, stiffness, and toughness. Using the equation \( EI = \frac{1}{12d} (F a^2)(3L-4a) \), where ‘\( L \)’ is the distance between the two lower points, ‘\( a \)’ is the distance between the lower and upper point on each side of centre, and ‘\( F/d \)’ is the stiffness measure obtained from the test, we computed the bones’ cross-sectional bending stiffness (Turner & Burr 1993), a measure of a bone’s ability to withstand bending forces.

**Statistical Analyses**

We used Pearson correlations to examine the relationships between measures for mechanical bending tests and measures of bone quality obtained from DXA, QUS, and hr-pQCT. In addition, we used linear regression modeling to predict the mechanical properties of bone from surrogate measures of bone strength, in order to determine the relative utility of DXA, QUS, and hr-pQCT in assessing bone strength. We combined hr-pQCT parameters that were significantly related to the mechanical properties measured in the four-point tests. Then, we successively removed the least significant terms until reaching our final model: the largest model with all predictors significant. In consideration of the limitations of stepwise model selection in the presence of collinearity among predictors, we performed additional analyses to assess the robustness of our model selection (Appendix A).

Additionally, we created predictive models including aBMD in addition to hr-pQCT parameters, in order to determine whether hr-pQCT measures of bone quality provide added
information of significance beyond aBMD. We chose $\alpha = 0.157$ as a measure of significance. Statistical analyses were performed using R for Windows version 2.6.2.

RESULTS

Correlation Analysis

Table 1 highlights some of the characteristics of the emu bones. Results of correlation analyses are displayed in Figure 2. We found that ultimate load, a common measure of bone strength, was best related to the hr-pQCT parameters cortical thickness ($R = 0.70$, $p < 0.001$) and bone cross-sectional area ($R = 0.74$, $p < 0.001$), and to the DXA parameters aBMD ($R = 0.78$, $p < 0.001$) and BMC ($R = 0.73$, $p < 0.001$). It is interesting to note that aBMD was equal to if not better than the individual hr-pQCT parameters in prediction of ultimate load. We found also that total volumetric density, as opposed to cortical volumetric density, was related to ultimate load ($R = 0.61$, $p < 0.001$ and $R = 0.11$, $p = 0.533$ for total and cortical volumetric density, respectively).

Cross-sectional bending stiffness was strongly related to moment of inertia measures obtained by hr-pQCT ($R = 0.82$, $p < 0.001$ for polar moment of inertia and $R = 0.75$, $p < 0.001$ for cross-sectional moment of inertia), as well as measures of cross-sectional area ($R = 0.74$, $p < 0.001$ for bone area and $R = 0.72$, $p < 0.001$ for total area). Bending stiffness was found to be more strongly related to volumetric cortical density ($R = 0.64$, $p < 0.001$) than to volumetric total density ($R = 0.23$, $p = 0.179$). Measures of bone quality obtained by DXA, such as BMC and aBMD, also had strong relationships with bending stiffness ($R = 0.83$, $p < 0.001$ and $R = 0.72$, $p < 0.001$ for BMC and aBMD, respectively).
Bone toughness was most strongly related to the DXA measures of aBMD (\( R = 0.63, p < 0.001 \)) and BMC (\( R = 0.56, p < 0.001 \)), as well as to the hr-pQCT measures of cortical thickness (\( R = 0.60, p < 0.001 \)) and bone cross-sectional area (\( R = 0.58, p < 0.001 \)). Toughness was moderately correlated to total volumetric density (\( R = 0.51, p = 0.001 \)).

We found that SOS measured by QUS had no significant relationships with any of the measures of bone mechanical properties.

**Multiple Regression Analysis**

Several parameters of bone material and geometric properties, assessed by hr-pQCT, were used to create a single model predicting bone strength. For ultimate load, these were cortical thickness, total volumetric density, polar and cross-sectional moment of inertia, and bone cross-sectional area; for cross-sectional bending stiffness, these were cortical thickness, cortical volumetric density, polar and cross-sectional moment of inertia, bone cross-sectional area, and total cross-sectional area. We found that a multivariable model containing both cortical thickness and polar moment of inertia correlated strongly to ultimate load (\( R^2 = 0.588, p < 0.001 \)). Bootstrap sampling showed that this is comparable to the correlation of aBMD to ultimate load (Figure 3); approximately 50% of the time in repeated sampling (1000x) we found the correlation of aBMD to ultimate load to be higher, and the other 50% of the time we found the correlation of hr-pQCT parameters to ultimate load to be higher. We found also that a multiple regression model including volumetric cortical density, polar moment of inertia, cross-sectional moment of inertia, and total cross-sectional area was predictive of the bending stiffness of the bones (\( R^2 = 0.893, p < 0.001 \)). Bootstrap sampling showed that this correlation coefficient is always higher than that of EI-4pt and BMC.
We found also that adding parameters obtained from hr-pQCT to a model containing aBMD alone did not increase the R-squared for the prediction of ultimate load to any important degree (F-test p = 0.688). However, in predicting bending stiffness, we found that parameters obtained from hr-pQCT did increase the R-squared of the model when added to the model with aBMD measured by DXA (F-test p < 0.001). A model containing aBMD, cortical thickness, polar moment of inertia, and cross-sectional moment of inertia was strongly related to bending stiffness (R² = 0.898, p < 0.001).

**DISCUSSION**

We showed that a multiple regression model based on hr-pQCT parameters predicts the failure load of bone as well as aBMD measured by DXA. In addition, we showed that a predictive model based on hr-pQCT parameters predicts the bending stiffness of bone better than models using DXA parameters. This suggests that hr-pQCT is at least as effective as DXA in assessing bone strength. We find that aBMD alone had a higher correlation to ultimate load than individual hr-pQCT parameters. This is likely due to the fact that aBMD is a composite measure reflecting bone's material and structural properties, whereas individual hr-pQCT parameters are each zeroing in on specific properties. As such, our data supports the fact that the mechanical competence of bone is determined by an interplay of many factors (Seeman & Delmas 2006).

In fact, this ability of hr-pQCT to zero in on specific parameters of bone material or geometric competence is one of its unique advantages compared to DXA. In a given region of interest, DXA can only quantitatively make densitometric assessments and cannot discriminate between cortical and cancellous bone (Liu et al. 2007). Neither can it differentiate between changes in tissue density or changes in bone microarchitecture (Nazarian et al. 2009). As such,
DXA cannot be used, for example, to quantitatively differentiate between two patients at risk of a femoral neck fracture, one due to cortical thinning and the other due to trabecular loss, if their aBMD at that site is the same. hr-pQCT, however, allows one to assess in three-dimensions specific changes in bone quality in individuals. This added information can only lead to a better choice of specific preventative or therapeutic options for those at increased risk of fracture. For example, parathyroid hormone (PTH) treatment could be used to increase trabecular structure and density, while a drug treatment like denosumab could be used to increase cortical thickness and cortical density (Deal 2009).

Our results also suggest that hr-pQCT is better in predicting bone strength than QUS. Although QUS has been shown to have the ability to predict fracture risk (Hans & Krieg 2008, Bauer et al. 1997, Gluer et al. 1996, Bauer et al. 1995), we found that SOS had no significant relationships to the mechanical properties of bone. This is likely due to the fact that much evaluation of QUS for fracture prediction has been done with heel devices, measuring mainly metabolically active trabecular bone. In our study, QUS measures of the midshaft of long bones were of little relevance to the overall mechanical competence of the bone. This again highlights the importance of considering whole bone geometry, rather than simply material properties, when assessing whole bone strength.

Our use of the emu model is based primarily on the advantages conferred from using bones of a larger size. The emu tibia, being roughly similar in size to human long bones, is ideal for testing on human-sized clinical devices. In addition, the length of the bones provides greater transferability of results from biomechanical tests. With small animal bones, there may be a greater presence of shear stresses during bending (Turner & Burr 1993). In addition, although it is an avian bone, the emu tibia has more in common to mammalian bones mechanically because
of its evolutionary adaptation to bipedalism (Reed & Brown 2001). The limited range of
densities present in our emu tibiae is admittedly a limitation of this study, but not one that calls
into question the core findings of our study.

In conclusion, this study found that hr-pQCT is able to assess bone strength as well as
DXA and better than QUS. The ability of hr-pQCT to specifically and quantitatively assess the
various determinants of bone strength presents great potential for its future clinical use as a
diagnostic device.
Table 1. Characteristics of the emu bones.

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>1st Quartile</th>
<th>Median</th>
<th>Mean (SD)</th>
<th>3rd Quartile</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Ultimate Load (N)</strong></td>
<td>1505</td>
<td>3013</td>
<td>3510</td>
<td>3526 (1067)</td>
<td>4033</td>
<td>6091</td>
</tr>
<tr>
<td><strong>Bending Stiffness (N/mm)</strong></td>
<td>91.5</td>
<td>112.3</td>
<td>121.2</td>
<td>135.6 (32.7)</td>
<td>156.0d</td>
<td>219.6</td>
</tr>
<tr>
<td><strong>Toughness (mm²)</strong></td>
<td>2368</td>
<td>7002</td>
<td>10064</td>
<td>10876 (6364)</td>
<td>14182</td>
<td>29737</td>
</tr>
<tr>
<td><strong>SOS (m/s)</strong></td>
<td>4163</td>
<td>4391</td>
<td>4451</td>
<td>4467 (127)</td>
<td>4564</td>
<td>4676</td>
</tr>
<tr>
<td><strong>BMC (g)</strong></td>
<td>51.74</td>
<td>70.64</td>
<td>78.14</td>
<td>78.89 (12.27)</td>
<td>86.44</td>
<td>105.53</td>
</tr>
<tr>
<td><strong>aBMD (g/cm²)</strong></td>
<td>0.671</td>
<td>0.937</td>
<td>1.012</td>
<td>1.004 (0.132)</td>
<td>1.089</td>
<td>1.290</td>
</tr>
<tr>
<td><strong>Cortical Thickness (mm)</strong></td>
<td>1.936</td>
<td>3.070</td>
<td>3.322</td>
<td>3.286 (0.494)</td>
<td>3.589</td>
<td>4.418</td>
</tr>
<tr>
<td><strong>Volumetric Cortical Density (g/mm³)</strong></td>
<td>1043</td>
<td>1112</td>
<td>1132</td>
<td>1132 (39)</td>
<td>1165</td>
<td>1190</td>
</tr>
<tr>
<td><strong>Volumetric Total Density (g/mm³)</strong></td>
<td>347.6</td>
<td>507.9</td>
<td>560.5</td>
<td>546.2 (72.7)</td>
<td>594.8</td>
<td>701.6</td>
</tr>
<tr>
<td><strong>Polar Moment of Inertia (mm⁴)</strong></td>
<td>18967</td>
<td>23090</td>
<td>25580</td>
<td>28000 (7051)</td>
<td>32686</td>
<td>47349</td>
</tr>
<tr>
<td><strong>Cross-sectional Moment of Inertia (mm⁴)</strong></td>
<td>11685</td>
<td>14154</td>
<td>16114</td>
<td>17002 (4223)</td>
<td>19760</td>
<td>29783</td>
</tr>
<tr>
<td><strong>Bone Cross-sectional Area (mm²)</strong></td>
<td>145.4</td>
<td>208.6</td>
<td>226.8</td>
<td>225.8 (32.4)</td>
<td>247.1</td>
<td>300.2</td>
</tr>
<tr>
<td><strong>Total Cross-sectional Area (mm²)</strong></td>
<td>368.6</td>
<td>442.8</td>
<td>496.1</td>
<td>489.5 (65.7)</td>
<td>546.8</td>
<td>649.2</td>
</tr>
</tbody>
</table>
Figure 2. Results of correlation analyses between measures of bone mechanical properties obtained in four-point bending tests, and measures of bone quality obtained by DXA, QUS, and hr-pQCT.
Figure 3. Prediction of ultimate load by hr-pQCT parameters is similar to prediction by aBMD measured by DXA. (Left) Multiple regression model built from hr-pQCT parameters was used to predict the ultimate load of bones ($R^2 = 0.588$; $p < 0.001$). (Right) Correlation between aBMD and ultimate load ($R^2 = 0.605$; $p < 0.001$).
APPENDIX A

Decomposition of $R^2$:

We used the methods proposed by Lindeman, Merenda, and Gold (1980) to assess the relative importance of the regressors in the linear model predicting ultimate load from the hr-pQCT parameters cortical thickness and polar moment of inertia (Lindeman et al. 1980). This was done in order to assess each individual variable’s independent contribution to the model. By decomposing the model’s $R^2$ of 0.588, we were able to ascertain the relative importance of each variable in explaining the variance in ultimate load (Figure 4). We found that 42.1% of the variance in ultimate load is due to the effect of cortical thickness, and 16.7% of the variance in ultimate load is due to the effect of polar moment of inertia.

We performed similar analyses to decompose our model predicting cross-sectional bending stiffness from hr-pQCT parameters. We found that the model’s $R^2$ of 0.893 could be decomposed as follows: polar moment of inertia explained 32.3% of the variance in cross-sectional bending stiffness, while cross-sectional moment of inertia, cortical density, and total area explained 22.4%, 17.5%, and 17.0% of the variance, respectively (Figure 5).
Figure 4. Percent of variance in ultimate load explained by each variable in model.

Figure 5. Percent of variance in cross-sectional bending stiffness explained by each variable in model.
Assessing the robustness of the model selection

There is some inherent uncertainty involved around building a regression model from highly correlated predictors. In our case, a few of the parameters measured by hr-pQCT were very strongly correlated (R=0.98 for polar moment of inertia and cross-sectional moment of inertia; R=0.95 for cortical thickness and total density; R=0.91 for polar moment of inertia and total cross-sectional area; R=0.88 for total cross-sectional area and cross-sectional moment of inertia). In many cases where two predictors are highly correlated, one of the predictors can be substituted for the other and including both would not be desirable.

We previously explained our use of backwards stepwise regression to obtain our final model predicting ultimate load from the hr-pQCT parameters cortical thickness and polar moment of inertia. To assess the stability of this model selection given the uncertainty involved, we performed the backwards stepwise procedure on bootstrapped samples 1000 times, and observed the percentage of models that contained each predictor. We used p=0.157 as our measure of significance.

Percent occurrences of the bootstrapped variables are found in Table 2. We found that polar moment of inertia and total cross-sectional area were included in models built from bootstrap samples approximately 77% and 74% of the time, respectively. Cortical thickness was included 68% of the time, while cortical density and total density were included 63% and 66% of the time, respectively. Cross-sectional moment of inertia and cortical area were included 52% and 40% of the time, respectively.

Although polar moment of inertia and total cross-sectional area were most likely to be present in the models, as mentioned above the two parameters are highly correlated, and as such the presence of both predictors in the same is not ideal. Thus, either polar moment of inertia or total area would be present along with another parameter such as cortical thickness. We found in
the stepwise regression on bootstrapped samples that polar moment of inertia and cortical thickness appeared 52% of the time, whereas total area and cortical thickness appeared 47% of the time. Furthermore, models containing polar moment of inertia and cortical thickness alone were present sixteen times as much as models with total area and cortical thickness alone.

The above analysis strongly suggests that despite the inherent uncertainty involved with model building from highly correlated parameters, we can have confidence in the stability of our model selection procedures.

Table 2. Percent Occurrences of bootstrapped variables.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Occurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical Thickness</td>
<td>67.6</td>
</tr>
<tr>
<td>Cortical Density</td>
<td>63.1</td>
</tr>
<tr>
<td>Total Density</td>
<td>65.5</td>
</tr>
<tr>
<td>Polar MOI</td>
<td>77.3</td>
</tr>
<tr>
<td>Cross-sectional MOI</td>
<td>51.7</td>
</tr>
<tr>
<td>Bone Area</td>
<td>39.8</td>
</tr>
<tr>
<td>Total Area</td>
<td>74.2</td>
</tr>
</tbody>
</table>
CHAPTER 4:

The ability of MRTA and hr-pQCT to assess

bone strength in demineralized bones

Idrees Ally, Jeremy Lau, George Tomlinson, Claudia Chan, Jean Zu, Angela Cheung
A single osteoporotic fracture can have severe consequences for health; thus there is a need to reduce the occurrences of fractures. This requires improving the assessment of bone strength in the clinical setting. Two devices that have potential to better assess bone strength than the currently-used DXA are the hr-pQCT and MRTA. We conducted an ex-vivo study of emu bones to examine how hr-pQCT and MRTA compare to DXA in predicting true bone mechanical properties among low-density bones.

DXA and hr-pQCT were used to obtain quantitative information regarding bone material and structural properties from demineralized emu tibiae (n=9). In addition, MRTA was used to obtain a measure of the bones’ cross-sectional bending stiffness. Using 10% formic acid, we then demineralized the bones using methods to simulate endocortical bone loss. We then repeated DXA, hr-pQCT, and MRTA tests, and performed four-point mechanical bending tests to obtain the bones’ true mechanical properties. We used Pearson correlations to examine relationships between measures obtained from DXA, hr-pQCT, and MRTA and those obtained by the bending tests.

We found that total volumetric density as measured by hr-pQCT strongly correlated to both of the mechanical properties: ultimate load (R=0.84) and cross-sectional bending stiffness as measured on the Instron (R=0.85). We found that bending stiffness as measured by MRTA correlated well with ultimate load (R=0.81) and cross-sectional bending stiffness as measured on the Instron (R=0.80). DXA-derived measures of bone quality also showed a moderate relationship to the mechanical properties as measured on the Instron, but not as strong as total volumetric density from hr-pQCT or EI from MRTA.

Our results suggest that cross-sectional bending stiffness obtained non-invasively by MRTA is a useful predictor of both the ultimate load and bending stiffness of demineralized
bones. Our results, taken in light of our previous work, also suggest that hr-pQCT may be of greater utility than DXA in low-density bones due to its ability to tease out the various individual parameters that affect bone strength. Both these tools show great potential as devices that can further aid clinicians in assessing bone strength and preventing fractures.
INTRODUCTION

An important goal in osteoporosis management is the prevention of a first osteoporotic fracture, as it can set off a chain of subsequent fractures that lead to increased deformity, disability, and mortality (Ashe & Khan 2009, Adachi et al. 2001). As fractures result from a reduction in the mechanical integrity of bone, or bone strength, prevention of a first fracture in many cases must be preceded by the accurate non-invasive assessment of bone strength. However, in the clinical setting, no accurate measures of bone strength exist (NIH Consensus Development Panel on Osteoporosis Prevention, Diagnosis, and Therapy 2001). The main tool used for diagnosis of osteoporosis, dual-energy x-ray absorptiometry (DXA), assesses primarily the density of bone material. While an important determinant of fracture risk (Marshall et al. 1996, Stone et al. 2003), bone density alone does not account for all of bone strength (Cheung & Detsky 2008), nor is it enough to fully explain changes in fracture risk after therapy (Harris et al. 1999, Chesnut et al. 2000, Ettinger et al. 1999, Benhamou 2007). Bone strength, rather than being affected simply by bone material properties, is determined also by bone structural and geometric properties (Chavassieux et al. 2007, Augat & Schorlemmer 2006, Seeman & Delmas 2006). It would be useful, then, to develop new devices that can be used to better assess bone strength in the clinical setting.

Previously we have investigated two devices that have demonstrated the potential to provide unique information about bone strength that cannot be obtained by DXA alone. One device, the high-resolution peripheral quantitative computed tomography (hr-pQCT) scanner, is a high-resolution (100 μm) x-ray based 3D imaging device. It can quantitatively provide detailed information about bone material properties such as volumetric density measures. In addition, it can quantitatively assess structural parameters including cortical thickness, trabecular thickness, trabecular separation, and bone cross-sectional area. The ability of this device to measure bone
material and geometric parameters, the determinants of bone strength, indicate its potential usefulness as a clinical tool for the assessment of bone strength.

Another device, the mechanical response tissue analyzer (MRTA) is a vibration-based device that can provide a measure of the cross-sectional bending stiffness (EI) of long bones. Developed by NASA to assess the effect of space travel on astronauts’ bones, the device uses an electromagnetic shaker to deliver, via an impedance head, a low-frequency vibration stimulus (<1600 Hz) to the midpoint of the bone (Djokoto et al. 2004). A signal transducer measures the dynamic response of the bone and a software algorithm is used to calculate the lateral stiffness of the bone, from which EI is computed (Djokoto et al. 2004). This measure is a product of E, the intrinsic stiffness or modulus of the material, and I, the cross-sectional moment of inertia. As such, EI reflects the mechanical competence of the bone, taking into account both the intrinsic mechanical properties of the bone material, as well as the geometry of the bone (Djokoto et al. 2004). EI, then, tells us about the bone’s ability to withstand the bending forces that can ultimately lead to fracture.

The MRTA has previously been shown to be a good indicator of true bone mechanical properties as measured in three-point bending tests (Djokoto et al. 2004, Roberts et al. 1996). In animal studies, EI as measured by MRTA has been found to have a strong correlation to the three-point bending stiffness of the same excised bones (R=0.753 to 0.975) (Roberts et al. 1996, Norrdin et al. 1995, Hutchinson et al. 2001). In addition, EI as measured by MRTA has been found to correlate strongly (R²=0.92) to the ultimate load (the bone’s breaking strength) for the same excised bones tested in a three-point bending setup (Roberts et al. 1996). In clinical populations, MRTA measurements have shown significant amounts of variation between healthy and osteoporotic women. In addition, MRTA has moderate ability to distinguish between subject groups based on age or gender (Kiebzak et al. 1999).
In previous work conducted by our group on untreated emu tibiae (please refer to Chapter 2), we investigated the utility of hr-pQCT and MRTA in predicting true bone mechanical properties, as compared to available clinical devices. We found that hr-pQCT parameters such as cortical thickness and cortical cross-sectional area correlated strongly with ultimate load (R=0.70 for cortical thickness and R=0.74 for bone area), and that a model built from hr-pQCT parameters predicts ultimate load as well as areal bone mineral density (aBMD) measured by DXA (R²=0.588 for hr-pQCT parameters and R²=0.605 for aBMD). We also found that for the untreated emu tibiae, bone cross-sectional bending stiffness (EI) measured by MRTA was moderately related to bone breaking strength (R=0.43). This is of stark difference to the strong relationship (R=0.91) between EI measured by MRTA and ultimate load that we observed in a previous study conducted by our group (unpublished) involving demineralized bones.

Because emu tibiae are generally much more highly mineralized than human bones, we aimed here to examine the relationships in question using bones with densities comparable to human bones. We did this in order to understand the utility of hr-pQCT and the MRTA in assessing bone strength across bones that vary in their densities and structures.

**METHODS**

We obtained fresh-frozen emu legs (male and female, aged 3-5 years) from local farms with most of the musculature already removed. We dissected out the tibia and removed any remaining skin, musculature, and connective tissue, such that we were left with the bare bone. Care was taken to remove the periostuem without damaging the bone surface.

The ends of the bone were then removed. Using a high-speed circular saw, fifteen percent of the length of the tibia was removed from the proximal end, and ten percent removed
from the distal end. Any remaining epiphyseal trabeculae were removed using a drill. Care was taken to ensure that the integrity of the cortical shell was not compromised by the use of the drill. Marrow and marrow fat were flushed out with water, leaving intact a cylindrical shell of cortical bone. A total of nine bones were prepared, then soaked in saline-soaked gauze and stored at -20°C.

Dual-energy X-ray Absorptiometry (DXA) Scans

These nine bones were then scanned using DXA, hr-pQCT, and tested using MRTA. For the DXA scans, we used the Hologic DiscoveryA scanner to obtain measures of bone mineral content (BMC) and areal bone mineral density (aBMD) for a twenty-four centimeter region of interest centered about the midpoint of the bone. We scanned bones once in lumbar spine mode, taking care to ensure identical positioning along the centre of the fan beam to avoid potential artefacts. Bones were scanned while submerged in water that was used to simulate soft tissue.

High-resolution peripheral Quantitative Computed Tomography (hr-pQCT)

We used the Scanco XtremeCT hr-pQCT scanner to obtain detailed information about bone quality. As with the DXA scans, we placed bones in a Plexiglas box filled with water. We positioned the box inside a carbon fiber tube designed to ensure proper placement in the scanner. Bones were scanned once in a scout view to identify the region of interest to be scanned in detail. A small piece of wire was fixed to the bone and used to identify the region of interest on the scout view. Because of size limitations of the hr-pQCT scanner, we could not scan the midpoint of the bone. Instead, we scanned two regions, approximately six centimeters of either side of the midpoint and averaged the values. Measures of volumetric density (cortical and total density), geometric parameters (cortical thickness, cortical and total cross-sectional area), and moment of inertia (cross-sectional and polar) measures were obtained from these scans.
Mechanical Response Tissue Analyzer (MRTA)

We then performed MRTA tests on the bones. Bones were positioned as in a three point bending setup, with the probe positioned at the centre of the bone, which was resting on supports located 12 cm on either side of centre. The probe was coupled to the bone with a thin piece of foam (1 x 2 cm). Scans were performed eight times, with the foam being replaced for each scan.

Demineralization of bones

The nine bones were then demineralized, using a protocol that simulates endocortical demineralization and bone resorption. Ends of the bones were sealed using a plastic dental tray material (SR Ivolen, Ivoclar Vivadent, USA). The proximal ends of the bones were sealed completely while at the distal ends, two small tubes (approximately 5 cm long) were fixed into the seal, to enable us to fill the bones with the demineralizing agent. Bones were filled with 10% formic acid solution (prepared from 88% formic acid, Sigma-Aldrich, Product # 399388), after which the tubes were sealed with Parafilm so as to completely seal the bone. Bones were left to stand vertically to demineralize (first for 2hrs at a time but later for 3hrs at a time), and their position was flipped between fillings to prevent one end of the bone from being more demineralized than the other. In between fillings, bones were emptied and washed out twice with distilled water. During demineralization, bones were soaked with saline (0.9% sodium chloride solution, Baxter). Bones were occasionally scanned using DXA to ascertain their level of demineralization. Three bones were demineralized for a total of 60 hrs, two for 101 hrs, three for 77 hrs, and one for 83 hrs.

Once the desired level of demineralization was reached, fittings at the end of the bone were removed and DXA, hr-pQCT, and MRTA scans were repeated.
Mechanical Tests

In addition, mechanical bending tests were performed in order to obtain information about the bones’ true mechanical properties. Bones were positioned on a four point bending setup, with two lower points at thirteen centimeters on either side of center, and two upper points at either side of six centimeters from center. Bones were loaded in an Instron servo-hydraulic material testing machine (Model 1331) at 0.05 mm/s until failure occurred. To stabilize the bone and to prevent rotation or other movement during loading, we preloaded the bones to 100 N before beginning the tests. We collected force versus displacement data from the tests, from which we obtained the bones’ ultimate load, stiffness, and toughness. Using the equation \( EI = \frac{1}{12d}F(a^2)(3L-4a) \), where ‘L’ is the distance between the two lower points, ‘a’ is the distance between the lower and upper point on each side of centre, and ‘F/d’ is the stiffness measure obtained from the test, we computed the bones’ cross-sectional bending stiffness.

Statistical Analyses

We used Pearson correlations to examine the relationships between measures for mechanical bending tests and measures of bone quality obtained from DXA, hr-pQCT, and MRTA. All analysis was performed using R for Windows version 2.6.2.

RESULTS

Table 3 summarizes some of the parameters measured via DXA, hr-pQCT, MRTA, and mechanical testing. We found from our correlation analysis that total volumetric density as measured by hr-pQCT correlated most strongly to ultimate load (\( R=0.84, p=0.004 \)) and cross-sectional bending stiffness (\( EI-4pt \)) as measured on the Instron (\( R=0.85, p=0.004 \)). We also
found that EI as measured by MRTA correlated well to both ultimate load (R=0.81, p=0.008) and cross-sectional bending stiffness measured on the Instron (R=0.80, p=0.010). Moderate correlations were found between DXA-derived measures of bone quality and ultimate load (R=0.51, p=0.158 for BMC and R=0.49, p=0.180 for aBMD) and EI-4pt measured on the Instron (R=0.56, p=0.118 for BMC and R=0.55, p=0.128 for aBMD). Volumetric cortical density as measured by hr-pQCT was also moderately related to the mechanical properties ultimate load (R=0.39) and EI-4pt (R=0.46). Measures of moment of inertia, area, and cortical thickness had little relationship to either ultimate load or cross-sectional bending stiffness.

**DISCUSSION**

In this study of bones with densities comparable to those of human bones, we found that hr-pQCT parameters reflecting geometry are of little relevance to predicting bone failure load. Instead, it is the measures of volumetric density that determine bone breaking strength. Density, then, seems to be of greater importance in determining bone strength in low density bones.

This is likely due to the fact that the bones were highly demineralized, to the point that many bones had very thin cortical walls. In this case, where the cortices were thinned and the local geometry of the bone could not sufficiently withstand the rigours of bending, the density of the bone material itself was of prime importance. In fact, we observed that even in cases where the overall geometry of the bone seemed to be well-suited to withstand the bending forces being applied, the bone wall would crush under the force. In this case, then, the low density of the bone mineral material determined the bone breaking strength due to its compromised state more so than the overall geometry of the bone.
We also found that EI-MRTA was a useful predictor of both ultimate load (R=0.81) and EI-4pt (R=0.80) in this study of demineralized bones. While in our previous study with untreated bones we found that EI-MRTA was only moderately related to bone breaking strength (R=0.43), our results in this study lend credence to our previous work involving demineralized bones, where we observed a stronger relationship to ultimate load (R=0.91) (unpublished data). These observations suggest that it is likely that the MRTA is better suited to determining bone strength at lower densities, where material properties play a larger role than structural properties in determining strength.

In conclusion, our results bring to the fore the fact that it is not bone density alone that determines bone strength, even though it may be an important determinant of breaking strength in cases of severe bone loss. Our results suggest that hr-pQCT, which is better suited to teasing out the various individual parameters that affect bone strength, is of greater utility than a device like DXA which combines density and geometric information in a single measure of areal density. Our results suggest furthermore that the MRTA has the potential for use as a clinical tool that can assess bone strength at low densities.
Table 3. Characteristics of the demineralized bones.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Minimum</th>
<th>1st Quartile</th>
<th>Median</th>
<th>Mean (SD)</th>
<th>3rd Quartile</th>
<th>Maximum</th>
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<tr>
<td>Ultimate Load (N)</td>
<td>480.9</td>
<td>765.4</td>
<td>923.4</td>
<td>897.9</td>
<td>1051.7</td>
<td>1308.1</td>
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<td>Bending Stiffness (N/mm)</td>
<td>12.59</td>
<td>21.29</td>
<td>26.83</td>
<td>32.28</td>
<td>41.03</td>
<td>71.16</td>
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<tr>
<td>Toughness (mm(^2))</td>
<td>1150</td>
<td>1829</td>
<td>2549</td>
<td>3291</td>
<td>3984</td>
<td>7447</td>
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<tr>
<td>BMC (g)</td>
<td>35.52</td>
<td>37.43</td>
<td>46.62</td>
<td>45.15</td>
<td>47.93</td>
<td>58.81</td>
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<td>aBMD (g/cm(^3))</td>
<td>0.445</td>
<td>0.468</td>
<td>0.578</td>
<td>0.562</td>
<td>0.612</td>
<td>0.755</td>
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<td>Cortical Thickness (mm)</td>
<td>1.483</td>
<td>1.611</td>
<td>1.705</td>
<td>1.802</td>
<td>1.836</td>
<td>2.564</td>
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<tr>
<td>Volumetric Cortical Density (g/mm(^3))</td>
<td>220.6</td>
<td>241.6</td>
<td>279.8</td>
<td>292.1</td>
<td>326.2</td>
<td>434.7</td>
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<td>Volumetric Total Density (g/mm(^3))</td>
<td>880.6</td>
<td>921.8</td>
<td>1038.1</td>
<td>1004.2</td>
<td>1070.3</td>
<td>1119.9</td>
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<td>Polar Moment of Inertia (mm(^4))</td>
<td>15728</td>
<td>17899</td>
<td>18896</td>
<td>19682</td>
<td>21506</td>
<td>24385</td>
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<tr>
<td>Cross-sectional Moment of Inertia (mm(^4))</td>
<td>10387</td>
<td>11639</td>
<td>12238</td>
<td>12557</td>
<td>13124</td>
<td>14806</td>
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<tr>
<td>Bone Cross-sectional Area (mm(^2))</td>
<td>103.0</td>
<td>117.5</td>
<td>127.9</td>
<td>132.1</td>
<td>132.3</td>
<td>186.0</td>
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<tr>
<td>Total Cross-sectional Area (mm(^2))</td>
<td>491.9</td>
<td>503.1</td>
<td>510.4</td>
<td>518.4</td>
<td>527.2</td>
<td>563.6</td>
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DISCUSSION & FUTURE DIRECTIONS

We began with the aim of understanding how measures obtained from hr-pQCT and MRTA reflect true bone strength, as compared to the current tools DXA and QUS. We have shown that in highly mineralized and structurally sound bones, hr-pQCT is at least as effective as DXA in determining bone breaking strength, and in highly demineralized bones, it is of greater utility. We have also argued that one of the unique advantages of hr-pQCT is its ability to assess specific properties of bone, such that it could be used to better understand the reasons behind changes in bone strength where conventional tools cannot. This can be of greater use both in the initiation of therapy and in the monitoring of response to therapy.

We showed also that MRTA is able to assess bone breaking strength and stiffness in demineralized bones. We suggest that this is because material properties of low-density bones, such as its density, play a larger role in determining its overall strength. MRTA, employing vibration that travels through the cortical shell, might be more suited to accounting for material properties and less so for whole bone geometry. This does not mean that MRTA has little potential for future clinical use – it is pertinent to note that the densities of the emu bones are much higher than those of normal human bones, let alone aged human bones that are biomechanically compromised. Given our results, we would expect that MRTA could be used to determine bone breaking strength in human osteoporotic bone.

Our results raised an interesting larger issue regarding the relationship between cortical density and the failure load of bones. Because a density-based approach to assessing bone strength assumes a strong relationship between bone density and bone strength, it was interesting to find that in our non-demineralized bones, geometry played a much larger role in determining
bone strength. For our non-demineralized bones (Chapter 2), we found that while aBMD measured by DXA was strongly correlated to ultimate load, volumetric cortical density measured by hr-pQCT was not, suggesting that cortical density may not be, in and of itself, a strong determinant of failure load. The strong relationship between aBMD and ultimate load with our non-demineralized bones is likely due to the fact that aBMD reflects the size of the bone, a geometric property that affects bone strength (Sievanen 2000). This is supported by our finding of the strong relationship between aBMD and cortical thickness ($R^2 = 0.765, p < 0.001$). In fact, the hr-pQCT parameters that account for bone geometry, such as cortical thickness and bone cross-sectional area show stronger relationships to failure load than the densitometric parameters measured by hr-pQCT, suggesting that perhaps it is bone geometry and not cortical density that plays a larger role in determining bone strength. This is further corroborated by findings made by W. S. Siu et al (2003) in an ex-vivo study with goat femora and humeri (Siu et al. 2003). They found a strong relationship between failure load and aBMD, but a weak one between failure load and volumetric cortical density. As in our study of non-demineralized bones, strong relationships were also found between aBMD and total volumetric density, as well as between failure load and cortical cross-sectional area (Siu et al. 2003). Recent work by D. Liu et al (2007) also showed, in human cadaveric tibiae, that bone geometry is significantly associated with biomechanical properties of bone, but that volumetric cortical density measured by pQCT is not (Liu et al. 2007). These findings support the suggestion based on our work with non-demineralized bones (Chapter 2) that it is primarily the geometry and not density of long bones that ultimately protect them against bending failure.

In fact, it is well known that in age-related bone loss, the mechanical competence of long bones suffering from age-related decreases in bone density is somewhat maintained by geometric compensations, namely a corresponding increase in periosteal apposition (Ahlborg et al. 2003).
Because inertia measures are strongly influenced by the distance of the bone from the center of mass, small increases in periosteal bone mass can make up for loss of endosteal bone, which does not affect bone strength as strongly. hr-pQCT, which can simultaneously measure the loss of cortical density and the increase in cross-sectional area, may be of greater use than DXA in assessing the change in the strength of such bones.

However, in our study of low-density bones, we found that hr-pQCT parameters reflecting geometry were of little relevance to predicting bone failure load. Instead, it is the measures of volumetric density that determined bone breaking strength. Density, then, seems to be of greater importance in determining bone strength in low density bones.

How do we reconcile this? It is most likely that the primary determinant of bone strength is neither geometry in all cases nor density in all cases. Due to the interplay of both geometric and material properties in determining bone strength, ultimately what determines bone strength may depend on the very state of the bones. In highly mineralized, structurally sound bones, we found that the little variation in cortical density values across the bones was not enough to fully account for the variation in bone strength. In that case, the local geometry (such as bone cross-sectional area and cortical thickness) and whole-bone geometry (such as curvature of the bone) played a much greater role in determining bone strength.

The highly demineralized bones, however, had very thin cortical walls. In this case, where the cortices were thinned and the local geometry of the bone could not sufficiently withstand the rigours of bending, the density of the bone material itself was of prime importance. In fact, we observed that even in cases where the overall geometry of the demineralized bone seemed to be well-suited to withstand the bending forces being applied, the bone wall would crush under the applied force, rather than sustaining the load by bending. In this case, then, the
low density of the bone mineral material determined the bone breaking strength due to its compromised state more so than the overall geometry of the bone.

It is reasonable to suggest based on our findings that both density and geometry play an important part in determining bone strength. What ultimately plays a bigger role in any given circumstance depends on the state of the bone. A tool like hr-pQCT which can assess both geometry and density, or a tool like MRTA which measures the stiffness of the bone, would therefore be of greater utility than a device that assesses mainly bone density.

Overall, our work has demonstrated the great potential for the development of hr-pQCT and MRTA as devices for the diagnosis of poor bone health. Our work paves the way for further research on these devices. Clinical studies can be conducted to determine if these tools can distinguish between fracture and non-fracture populations. As a follow-up to that, longitudinal studies looking at the ability of these tools to predict eventual fracture occurrences can and should be performed. In the mean time, further work can be done to optimize the mathematical model used by the MRTA to compute stiffness, and to refine the MRTA system to increase its ease of use.

A few limitations of our study include small sample sizes (for the study of demineralized bones). This stems from the fact that the method we employed to demineralize required long durations of time to reach the desired levels of demineralization. A single larger study with a greater number of demineralized and non-demineralized bones could help address some of the questions we could not specifically address empirically. While our use of emu bones may present some limitations, as mentioned previously we believe these do not undermine the results obtained from this research. Instead, the use of the emu tibia is of specific benefit in a study assessing bone strength.
To conclude, we have shown rather clearly in our ex-vivo study of emu bones that both hr-pQCT and MRTA have the potential to assess bone strength better than the currently available clinical devices. They both confer specific advantages over DXA and QUS such that further work should be performed to develop them for eventual clinical use.
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