The Use of Radiographs, Dual-energy X-ray Absorptiometry, Quantitative Computed Tomography and Micro-computed Tomography to Determine Local Cancellous Bone Quality in the Canine Proximal Femur

A Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

Katy Townsend BVSc(hons)
Graduate Program in Comparative and Veterinary Medicine

The Ohio State University

2012

Thesis Examination Committee:

Dr. Matthew Allen, Advisor
Dr. Richard Hart
Dr. Valerie Samii
Dr. Jonathan Dyce
Abstract

Proximal femoral bone quality and quantity are two important variables to provide long-term stability in cementless total hip arthroplasty (THA). The goal of this study is to determine the feasibility of using non-invasive imaging data to predict the quality and quantity of cancellous bone in the canine proximal femur and directly compare this with bone microstructure and mechanical analysis. Twelve cadaveric canine femora were used. Radiographs were taken, along with DEXA scans and quantitative CT. Bone Mineral Content (BMC) and Bone Mineral Density (BMD) were measured from DEXA scans and CT, and radiographs were scored. Cores of cancellous bone were harvested from the proximal femur. High-resolution micro computed tomography (uCT) was performed to assess bone microstructure (fractional bone volume BV/TV, polar moment of inertia). Direct mechanical testing of the bone core was performed to assess bone strength (break load, stiffness, energy at break). Pearson correlation was used to compare the biomechanical parameters with the imaging modalities. uCT values (BV/TV and AMI) had the best predictor of mechanical properties of cancellous bone in the proximal femur, while clinical imaging modalities such as radiographs, DEXA BMD and BMC and qCT had only mild correlation with predicting bone strength. uCT has the best predictor of bone strength, suggesting that bone architecture plays a significant role in
determining bone strength. CT, DEXA and radiographs all showed only mild correlation of bone strength, indicating that clinical parameters are not useful in predicting proximal femoral bone strength.
I would like to acknowledge The Ohio State University Canine Intramural Fund for the financial support of this project. I would also like to sincerely thank Dr. Matthew Allen for being a wonderful mentor, for his guidance in both research and life, without him, this project would not have been possible. Thanks to my committee members Dr Richard Hart, Dr Valerie Samii and Dr. Jonathan Dyce, along with Dr Tatiana Motta and Garret Noble who have provided insight and guidance. I would also like to thank the small animal surgery faculty members, who have taught me so much. Finally, I would like to thank the most important person in my life, my husband Duncan, for his unconditional love and support through my residency.
Dedications

Dedicated to my parents, Murray and Judy, for teaching me how important education is, and who developed my passion for learning from a young age, and my husband, Duncan, who makes my life complete.
Vita

2004 ......................................................... BVSc(hons), The University
                                          of Sydney

2005-2006 ................................................ Internship, Small animal
                                          medicine and surgery, The
                                          Animal Health Trust

2006-2007 ................................................ Orthopedic research
                                          Fellow, The Ohio State
                                          University

2007-2008 ................................................ Comparative Orthopedic
                                          Research Fellow, State
                                          University New York
                                          (SUNY)

2008-2009 ................................................ Orthopedic Research
                                          Fellow, The Ohio State
                                          University

2009 to present .......................................... Residency, Small Animal
                                          Surgery, Department of
                                          Clinical Sciences, The Ohio
                                          State University
Publications


Book Chapter:

Fields of Study

Major Field: Comparative and Veterinary Medicine
Specialty: Small Animal Surgery
Table of contents

Abstract ...........................................................................................................ii
Acknowledgements ......................................................................................iv
Vita ..............................................................................................................vi
List of Tables ................................................................................................ix
List of Figures ...........................................................................................xi
Chapter 1: Introduction .................................................................................1
Chapter 2: Methods ......................................................................................7
Chapter 3: Results ......................................................................................22
Chapter 4: Discussion .................................................................................30
References ....................................................................................................42
List of Tables

**Table 1:** Micro-CT parameters trabecular thickness (mm), trabecular number (#/mm), bone volume/total volume (%) and polar moment of inertia (mm⁴) with mean, standard deviation and range

**Table 2:** Biomechanical data with mechanical properties of break Load (N), stiffness (N/mm) and energy at break (Nmm)

**Table 3:** Correlation coefficients between the different imaging parameters – radiographic score (RAD\_SCORE), mean gray value (RAD\_MGV), bone mineral density measured by DEXA (BMD\_DEXA), bone mineral density measured by QCT (BMD\_QCT), bone volume/total volume (BV/TV) and polar moment of inertia (PMI). Asterisk indicates statistical significance at p<0.05

**Table 4:** Correlations coefficients of the imaging modalities (bone volume/total volume, polar moment moment of inertia, bone mineral content by DEXA (BMC\_DEXA) bone mineral content by DEXA (BMD\_DEXA), radiographic score (RAD\_SCORE) and BMD measured by QCT (BMD\_QCT) compared with the break load, stiffness and energy to
failure as determined by direct mechanical testing. Asterisk indicates statistical significance at p<0.05.
List of Figures

Figure 1: Craniocaudal radiographic projection showing the 1.5cm² area in the proximal femur where the Mean Grey Value (RADMGV) was obtained..........................10

Figure 2: Axial image of three femurs through the femoral head and neck region. Noted the five varied densities of the dipotassium phosphate phantom directly underlying the femurs.................................................................12

Figure 3: Dual Energy X-Ray Absorptiometry scan showing the core region marked 1 and the proximal femoral region marked 2.................................................................14

Figure 4: A femoral head and neck ostectomy was performed...............................16

Figure 5: A 5mm by 10mm cored sample was obtained from the proximal femur using cored cylindrical device.................................................................17

Figure 6: The cored sample was lavaged to remove marrow, and potted into custom made cylindrical end caps.................................................................18
Figure 7: The samples were mechanically tested using a load displacement algorithm at 1mm/minute until failure.

Figure 8: Cranio-caudal radiographic projection (A) of the dog with poor bone quality. A cored sample was unable to be extracted from this femur. For comparison, a femur from a dog with average bone quality is shown in (B).
Chapter 1: Introduction

Total hip arthroplasty (THA) is used clinically in canine patients to treat osteoarthritis and other painful conditions of the coxofemoral joint. Cementless THA has been developed and gained in popularity to reduce the complications associated with cemented fixation of the implants and to increase the longevity of the implants. Potential complications of cemented THA include cement mantle failure, infection, aseptic loosening or failure of stability at either the implant-cement interface or the cement-bone interface (1). Long-term stability of cementless implants is due to bone ongrowth to the implant or ingrowth into the porous coating. Long-term stability requires an initial stability period that is achieved through the mechanical interlock of the implant with the surrounding bone. Cementless implants are placed in animals with good bone strength, as the bone immediately surrounding the implant is responsible for the initial fixation and ultimately the long-term fixation of the implant is provided by new bone ingrowth into the surface of the implant. If stability is not achieved in cementless implants, complications such as implant subsidence, fracture, fibrous fixation of implant, luxation, increased risk of infection.

The initial stability of cementless implants is paramount to success. Several factors have been studied to determine which variables contribute to success of initial stability and include initial fit and size of the implant, whole bone geometry and level of osteotomy. One previous report on experimental cementless porous
coated anatomical (PCA) THA recommended that the midpoint of femoral stem should fill greater than 85% of the marrow cavity to prevent subsidence (2). This study also determined that femoral morphology played an important role in femoral stem subsidence. Femoral morphology was defined using a “canal flare index”. Animals with a canal flare index of < 1.8, known as a “stovepipe” morphology due to it being a relatively straight shaped femur, were six times more likely to have implant subsidence than femora with a canal flare index of 1.8-2.5 (2). A mechanical cadaveric study assessing level of osteotomy and implant size on initial stability showed that implants with a larger percentage canal fill had a higher yield load prior to subsidence, than implants with a smaller percentage canal fill (3). A more proximal femoral osteotomy retaining femoral neck provided more initial stability to axial load than a more distally placed femoral osteotomy. This study concluded that when performing a cementless THA, a more proximal femoral osteotomy along with a larger femoral implant should be used to increase initial implant stability (3).

In humans, femoral prosthesis loosening can be predicted by mechanical bone quality at the time of surgery (4). Decreased femoral strength and axial stiffness resulted in increased radiolucent lines present around the femoral prosthesis at seven years postoperatively (4). Low mechanical values were also associated with an increased risk incidence of stem deviation. In this study, BMD did not correlate with stem loosening or malorientation of the femoral prosthesis (4). A finite element study showed that a 65% increase in maximum micromotion of a
femoral prosthesis when there was a 40% reduction in cancellous bone stiffness (5).

Proximal femoral bone quantity and bone quality are two important variables to provide initial stability in cementless THA. Bone quantity refers to the total amount of bone in a structure. Bone quality refers to the sum total of characteristics that influence the bones resistance to fracture (6). This includes all of the geometric and material factors that contribute to fracture resistance (7). Geometric factors include the shape of the whole bone, along with the microscopic architecture. Material factors include the individual material factors of the substrates that make up bone such as collagen and mineral (7). Bone strength combines bone quality and quantity. If bone quality and thus strength is considered poor, cemented femoral implants may be used since they allow for immediate stability.

No one method is able to reliably predict the strength of bone (8). Bone quantity of the proximal femur can be measured through a variety of imaging methods to include radiographs, dual energy X-ray absorptiometry (DEXA), quantitative computed tomography (qCT) and micro-computed tomography (uCT) (7, 9, 9-12). When combined with mechanical testing of bone, bone quality can be interpreted. Routinely in canine THA patients, bone quality is subjectively measured with radiographs only.
In the human literature, radiographic interpretation of bone quality of the proximal femur is related to the groupings of trabeculae referred to as primary and secondary compressive groups and primary and secondary tensile groups (12). Changes of these trabecular patterns are grouped into six grades representing increasing degree of bone loss (13). These grades are used to describe the extent of osteoporosis and have been shown to be useful in predicting fracture risk (13). The Singh index is clinically established for semi quantitative evaluation of bone quality in the proximal femur. There is variability in the literature regarding the predictive capability and reliability of the Singh Index (4, 14-16). The poor correlation is thought to be due to the fact that radiography only detects decreases in bone opacity when bone density has been reduced by 30-40% (17). Another radiographic scoring system is the Dorr classification (18). This method classifies bone quality into Type A, B and C, based on findings in both the anterior and lateral projections. Parameters assessed in this method include thickness of the cortices, the shape of the medullary canal, and the width of the canal at the diaphyseal part (18). These femur were also assessed by histomorphometry. There is no similar scoring system available in the canine femur, although similar trabecular patterns are seen on canine radiographs. Subjective evaluations to assess bone quality in the proximal femur are used as a guide whether a cemented or cementless femoral implant will be placed in a patient undergoing a THA. Mean gray value (MGV) has been reported
to be an accurate way of assessing BMD (19). MGV method of analysis is based on gray-level analysis using a computer assisted image analysis program.

DEXA is a quantitative imaging modality that provides an objective mean for evaluation small changes in bone mineralization. DEXA is recognized as the reference method to measure bone mineral density (BMD) with acceptable accuracy errors and good precision and reproducibility (20). The main use in humans is to measure osteoporosis, where it is the gold standard for measuring BMD in the proximal femur (20). DEXA scanners use photos of two different energies, which are impeded differently by bone mineral, lipid and lean tissue. Bone mineral content (BMC) corresponds to the 60% of hydrated bone tissue that is mineral and correlates well with bone ash; BMD is BMC divided by the area computed to be bone, expressed as grams per square centimeter. BMD values are derived from a two-dimensional view of a three-dimensional object, requiring that the third assumption (thickness) is relatively constant. Proprietary algorithms are then used to calculate the type and quantity of each tissue. DEXA imaging has been used in a variety of veterinary applications involving fracture healing characteristics (21-26), disuse osteopenia (27), fragmented medial coronoid process (21), canine Legg-Calve-Perthes disease (28) and total joint arthroplasty (25). The use of DEXA to specifically assess bone quality in the proximal femur before total hip replacement has not been performed in dogs.
Quantitative computed tomography (QCT) is a method that can be used to quantitate the BMD of bone (29). Accuracy for single-energy qCT is between 5% and 15% (29). Dual-energy QCT improves accuracy, however, the disadvantages of longer scanning times and higher radiation doses are not practical (29). CT utilizes x-rays and provides a grayscale image based on the linear x-ray attenuation of the tissues through which the x-ray beam passes. CT scans are routinely calibrated to the x-ray attenuation of water and expressed in Hounsfield units (HU), with water being assigned a HU value of zero. Areas of high atomic number materials such as bone absorb more x-rays, have a higher HU number and appear white on the image (29). Generation of the CT image is a two-step process of initial scan data acquisition and then tomographic reconstruction by a mathematical process of calculating the image from the acquired data. To transform HU into BMD equivalents (mg/cm³), an appropriate bone mineral phantom is used. Calibration phantoms contain various concentrations of material with similar x-ray attenuation characteristics to bone. Examples of commonly used phantoms include dipotassium phosphate, K₂HPO₄, or mixtures of hydroxyapatite and epoxy. QCT has been used in veterinary research to assess BMD and was deemed precise to 1.2-4.7%, with an accuracy of 1.3-7.5% (30). The main advantage of assessment of BMD from QCT when compared with DEXA scans is that QCT provides true volumetric BMD compared with the areal BMD provided by DEXA scans. This means that QCT is not size or shape-dependent. QCT is also able to separately measure cortical and cancellous BMD. Disadvantages associated with QCT include increased radiation dose, reduced accessibility,
increased cost, lack of commercial analysis packages, and the requirement for greater technical proficiency for both scan acquisition and analysis.

Micro-computed tomography (uCT) is a radiographic technique that creates images with very high spatial resolution (12). The systems can obtain three-dimensional geometry to clearly visualize individual trabeculae, allowing an analysis of the trabecular network. Parameters that are routinely assessed with micro-CT include trabecular number, thickness and spacing, along with the trabecular bone volume as a percentage of total tissue volume (i.e. fractional bone volume). The microarchitecture of the bone plays a large role in the strength of bone in human proximal femora (12).

Mechanical testing is used to directly measure the strength of bone. Changes in bone structure and composition can manifest in different ways, so a panel of mechanical indicators are typically used to characterize the properties of the bone specimen. In the context of THA, the most useful parameters are the stiffness of the bone-implant construct, the load at which it fails, and the energy required to break the bone-implant interface (or the periprosthetic bone). Bone is anisotropic, meaning that the material properties vary according to the prevailing loading direction, so it is critically important to control specimen geometry and alignment. Imaging modalities are good for predicting bone strength, but it is the mechanical properties of the bone and the implant-bone interface that determines whether an
implant-bone construct survives under loading. Ultimately, failure of bone in bone-implant interface occurs when the weight bearing capacity is exceeded. Understanding the mechanisms and the locations at which failures occur is critically important in canines undergoing THA - if too much load is placed on the implant and femur after surgery, catastrophic periprosthetic fracture can occur. This knowledge can be used to influence implant design, surgical technique and post-operative rehabilitation with the goal of reducing intra- and post-operative complications following THA.

This study had two overarching aims. The first was to determine the utility of currently available “whole bone” imaging modalities (plain radiography, DEXA and QCT) in describing cancellous bone quality in the proximal femur. The second was to determine the strength of the cancellous bone by direct mechanical testing of a bone core from the proximal femur, and to determine whether high-resolution imaging (by micro-CT) was better than standard imaging modalities in predicting the strength of this bone specimen. Ultimately, the goal of this research is to develop improved non-invasive methods for predicting bone strength in the proximal femur so that surgeons can make more informed decisions when choosing between cemented versus cementless femoral fixation.
Chapter 2: Materials and Methods

12 paired femurs were harvested from skeletally mature dogs >20kg euthanized for reasons unrelated to this study. This study was reviewed and approved by the local IACUC. The femurs were stripped of soft tissue attachments and frozen at -20 degrees Celsius until use.

Plain Radiography

Femurs were digitally radiographed in a cranio-caudal projection to ensure skeletal normality. Radiographs were taken by means of an x-ray unit (Sedecal A6276-03, Buffalo Grove, Il) operating at 4.0mAS, 70kVP, with a focus distance of 100cm, with a 10cm radiopaque marker. Radiographs were examined using an image analysis program (eFilm Workstation 2.1, Merge Healthcare, Milwaukee, WI). A board certified surgeon evaluated the radiographs scoring them from 0-10 on a visual analog scale, with 5 considered normal bone, 0 considered poor bone quality and 10 being sclerotic bone. The surgeon scored them twice on separate occasions and the average of the score (RAD$\text{SCORE}$) was used for the study. Mean gray scales (RAD$\text{MGV}$) were assessed by using an oval area (1.5cm$^2$) in the proximal femur using an image analysis program (eFilm Workstation 2.1, Merge Healthcare, Milwaukee, WI) (Figure 1).
Figure 1: Craniocaudal radiographic projection showing the 1.5cm² area in the proximal femur where the Mean Grey Value was obtained.
Computed Tomography

Computed tomography scans were then performed using a multi-detector (8-slice) CT scanner (GE Lightspeed; GE Healthcare, Milwaukee, WI). The femurs were scanned in axial slices of 0.625mm and a dipotassium phosphate phantom was used to calibrate for subsequent bone mineral density measurement (Model 13002 CT calibration phantom, Mindways Software Inc. San Francisco, CA) (Figure 2). The images were reconstructed into three dimensions and analyzed in an image analysis program to assess bone mineral density. A 10mm by 5mm diameter core was extracted digitally mimicking the region that the core was to be taken, and the BMD of the core was performed by comparison to the density phantom. For convenience, the BMD determined by QCT will be referred to as $\text{BMD}_{\text{QCT}}$ from this point forward.
Figure 2: Axial image of three femurs through the femoral head and neck region. Noted the five varied densities of the dipotassium phosphate phantom directly underlying the femurs.
Dual-energy X-ray Absorptiometry (DEXA)

DEXA was performed on the proximal femur using a GE Lunar Prodigy scanner (GE Healthcare, Milwaukee, WI). The femurs were placed on a jig to ensure proper positioning and submerged in 7cm of water to mimic soft tissue covering. A dual femur protocol was performed. One pair was scanned 10 times for reproducibility and consistency in scan acquisition. The scans were analyzed by creating a custom region of interest 10mm by 5mm that was positioned where the bone core was going to be removed from. Another region was used outlining the proximal area of the femur, not to include the cortical bone (Figure 3). One scan was analyzed 10 times to determine accuracy of the region of interest. The outputs from these scans were bone mineral density in grams per cubic centimeter and bone mineral content in grams; for convenience, these will be referred to as BMD$_{\text{DEXA}}$ and BMC$_{\text{DEXA}}$ respectively.
**Figure 3:** Dual energy X-ray absorptiometry scan showing the regions of interest used to measure the bone in two locations within the proximal femur: ROI#1 represents the location from which the bone core was subsequently removed, while ROI#2 represents a more global assessment of the cancellous bone in the proximal femur.
Harvesting of a Bone Core from the Proximal Femur

A femoral head ostectomy of the right femur was performed (Figure 4). A 5mm internal diameter trephine burr (MT – 00500, MIS USA, NJ) was used in a battery powered drill (Stryker System 5, Stryker, Kalamazoo, MI) to core out a 5mm diameter by 10m length of bone core axially from the proximal femur (Figure 5). The cored sample was frozen until further use. The core was rinsed with saline using a commercial lavage system (WaterPik Inc., Fort Collins, CO) to remove bone marrow and blood, then secured to custom polyethylene end caps using an adhesive (Staples Super Glue, Staples Inc., Farmingham MA) (Figure 6).
Figure 4: A femoral head and neck ostectomy was performed.
**Figure 5:** A 5mm by 10mm cored sample was obtained from the proximal femur using cored cylindrical device.
Figure 6: The cored sample was lavaged to remove marrow, and potted into custom made cylindrical end caps.
Micro-computed Tomography

The potted bone samples were then submerged with a saline in a polyethylene plastic container and scanned at a nominal resolution of 13.3um using a bench top micro-CT scanner (Model 1172; Skyscan, Aartselaar, Belgium). Images were reconstructed using commercial software (NRecon; Skyscan, Aartselaar, Belgium) and the following 3-dimensional microarchitectural parameters calculated using proprietary software (CTAn; Skyscan, Aartselaar, Belgium): trabecular bone volume as a fraction of total tissue volume (BV/TV, %), trabecular thickness (mm), trabecular number (#/mm) and polar moment of inertia (PMI, mm$^4$), a measure of a structure’s ability to resist torsion.

Mechanical Testing

The cores were then axially tested in a screw-driven MTS machine under displacement control at 1mm per minute, to failure, which was defined as greater than 5mm of axial crushing, a maximum of 250N of applied load or a strain difference on 50%. Parameters evaluated were yield load, stiffness, failure load, energy to yield and energy to failure. Stiffness was calculated from the slope of the stress-strain curve, energy to failure of maximum strength was the first local maximum of the stress strain-curve. The energy absorbed was the area under the curve (Figure 7).
**Figure 7:** The samples were mechanically tested using a load displacement algorithm at 1mm/minute until failure.
**Statistical analysis:**

Descriptive statistics were used to assess each imaging modality and properties. Pearson Correlation co-efficients were used to assess the predictive value of imaging modality against the mechanical data testing set along with comparisons for assessing BMD between imaging modalities.
Chapter 3: Results

Plain Radiography

The median bone quality radiographic score, $\text{RAD}_{\text{SCORE}}$, was 5 with a mean ($\pm$ SD) of 4.66 ($\pm$ 0.88) (range 4-6). The mean ($\pm$ SD) $\text{RAD}_{\text{MGV}}$ was 2037 ($\pm$ 196) (range 1643-2276). The correlation between the two methods of interpreting radiograph was strong and statistically significant (0.78; $p<0.05$).

DEXA analysis:

BMD was analyzed on the image 10 times on one specimen to assess reproducibility. The coefficient of variance, a measure of reproducibility, was 0.93%. DEXA is therefore considered a highly reproducible analytical method for assessing BMD.

There was strong correlation between the BMD of the proximal femur (ROI #2 in Figure 3) when compared to that of the region from which the core was removed (ROI #1 in Figure 3) ($r = 0.83; p<0.05$). The mean BMD for the region of interest within the cancellous region of the proximal femur was 0.659 g/cm$^3$ (+/- 0.08, 0.568-0.766) and the mean BMD for the core was 0.56 g/cm$^3$ (+/-0.07, 0.462 - 0.663). The mean for the BMC was 0.283g (+/-0.039, 0.23-0.33).
Quantitative CT:

The mean BMD from the area derived from the core was 201, with a standard deviation of 32.5. The range was from 158 to 266.

Core Harvesting and Micro-CT Analysis

It was possible to obtain cores from 11 of the 12 specimens. One dog was found to have extremely poor quality bone in the proximal femur (Figure 8) and an intact core could not be removed from either femur. The radiographic score for this dog was a 2/10 and the DEXA score was also low (0.423g/cm³).

The microstructural and mechanical testing parameters determined from these bone cores are described in Tables 1 and 2 respectively.
<table>
<thead>
<tr>
<th>Property</th>
<th>Mean (+/- SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trabecular thickness (mm)</td>
<td>0.55 (0.009)</td>
<td>0.042-0.078</td>
</tr>
<tr>
<td>Trabecular number (#/mm)</td>
<td>1.178 (0.0511)</td>
<td>0.374 – 2.120</td>
</tr>
<tr>
<td>Bone volume/Total volume (%)</td>
<td>6.765 (3.889)</td>
<td>3.829 – 15.095</td>
</tr>
<tr>
<td>Area Moment of inertia (mm$^4$)</td>
<td>1.672 (-0.0993)</td>
<td>0.431 – 3.722</td>
</tr>
</tbody>
</table>

**Table 1:** Micro-CT parameters trabecular thickness (mm), trabecular number (#/mm), Bone volume/Total Volume (%) and Area Moment of Inertia (mm$^4$) with mean, standard deviation and range
**Figure 8:** Cranio-caudal radiographic projection (A) of the dog with poor bone quality. A cored sample was unable to be extracted from this femur. For comparison, a femur from a dog with average bone quality is shown in (B).
<table>
<thead>
<tr>
<th>Property</th>
<th>Mean (+/- SD)</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Break Load (N)</td>
<td>27.98 (27.13)</td>
<td>3.99 – 93.75</td>
</tr>
<tr>
<td>Stiffness (N/mm)</td>
<td>85.30 (75.28)</td>
<td>1.3 – 221.6</td>
</tr>
<tr>
<td>Energy at Break (Nmm)</td>
<td>15.37 (16.37)</td>
<td>0.424 – 53.79</td>
</tr>
</tbody>
</table>

**Table 2:** Biomechanical data with mechanical properties of Break Load (N), Stiffness (N/mm) and Energy at Break (Nmm)

**Correlation Between Imaging Parameters:**

There was mild to excellent correlation between the varied imaging parameters. Unsurprisingly, the two plain radiography measures had good correlation. BMD$_{DEXA}$ had a better correlation with the BMD$_{QCT}$ compared with plain radiography. This seems counterintuitive, as DEXA and plain radiography both assess cortical and cancellous bone, where QCT is able to differentiate between this. The micro-CT parameters had better correlation with BMD$_{QCT}$ and BMD$_{DEXA}$ than with plain radiography. The results are presented in Table 3.
<table>
<thead>
<tr>
<th></th>
<th>RAD_SCORE</th>
<th>RAD_MGV</th>
<th>BMD_DEXA</th>
<th>BMD_QCT</th>
<th>BV/TV</th>
<th>PMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>RAD_SCORE</td>
<td></td>
<td>-0.78*</td>
<td>0.59</td>
<td>0.69*</td>
<td>0.55</td>
<td>0.57</td>
</tr>
<tr>
<td>RAD_MGV</td>
<td>-0.78*</td>
<td></td>
<td>-0.51</td>
<td>-0.84*</td>
<td>-0.58</td>
<td>-0.57</td>
</tr>
<tr>
<td>BMD_DEXA</td>
<td>0.59</td>
<td>-0.51</td>
<td></td>
<td>0.78*</td>
<td>0.56</td>
<td>0.68*</td>
</tr>
<tr>
<td>BMD_QCT</td>
<td>0.69*</td>
<td>-0.84*</td>
<td>0.78*</td>
<td></td>
<td>0.68*</td>
<td>0.67*</td>
</tr>
<tr>
<td>BV/TV</td>
<td>0.55</td>
<td>-0.58</td>
<td>0.68*</td>
<td>0.68*</td>
<td></td>
<td>0.99*</td>
</tr>
<tr>
<td>PMI</td>
<td>0.57</td>
<td>-0.57</td>
<td>0.67*</td>
<td>0.67*</td>
<td>0.99*</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3:** Correlation coefficients between the different imaging parameters – radiographic score (RAD\_SCORE), mean gray value (RAD\_MGV), bone mineral density measured by DEXA (BMD\_DEXA), bone mineral density measured by QCT (BMD\_QCT), bone volume/total volume (BV/TV) and polar moment of inertia (PMI). Asterisk indicates statistical significance at p<0.05
Utility of Imaging in Predicting Mechanical Properties

Micro-CT imaging was best able to predict the mechanical properties. Radiography had good correlation with the mechanical values, along with $\text{BMD}_{\text{DEXA}}$ and BMC. The QCT parameters had poor correlation, even though this method only was assessing the cancellous bone. The results are presented in Table 4.
Table 4 Correlations coefficients of the imaging modalities (bone volume/total volume, polar moment moment of inertia, bone mineral content by DEXA (BMC\textsubscript{DEXA}), bone mineral content by DEXA (BMD\textsubscript{DEXA}), radiographic score and BMD measured by QCT (BMD\textsubscript{QCT}) compared with the break load, stiffness and energy to failure as determined by direct mechanical testing. Asterisk indicates statistical significance at p<0.05.

<table>
<thead>
<tr>
<th></th>
<th>Break load (N)</th>
<th>Stiffness (N/mm)</th>
<th>Energy to Failure (Nmm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BV/TV (%)</td>
<td>0.78*</td>
<td>0.62*</td>
<td>0.78*</td>
</tr>
<tr>
<td>PMI (mm4)</td>
<td>0.80*</td>
<td>0.64*</td>
<td>0.77*</td>
</tr>
<tr>
<td>BMC\textsubscript{DEXA} (g)</td>
<td>0.54*</td>
<td>0.43</td>
<td>0.57*</td>
</tr>
<tr>
<td>BMD\textsubscript{DEXA} (g/cm\textsuperscript{3})</td>
<td>0.54*</td>
<td>0.44</td>
<td>0.54*</td>
</tr>
<tr>
<td>RAD\textsubscript{SCORE}</td>
<td>0.65*</td>
<td>0.44</td>
<td>0.50</td>
</tr>
<tr>
<td>BMD\textsubscript{QCT}</td>
<td>0.48</td>
<td>0.54</td>
<td>0.45</td>
</tr>
</tbody>
</table>
Chapter 4: Discussion

The purpose behind this study was to evaluate non-invasive imaging parameters to assess bone quality and strength. Currently, there are no true objective ways to assess bone quality of the proximal femur before THA. Bone strength in humans is indirectly measured well through imaging studies to include radiographs, DEXA, and qCT. In canine THA cases, subjective evaluation by the surgeon prior to surgery is performed by radiographic evaluation, and then subjectively intra-operative bone evaluation is used. The aim was to correlate the imaging assessment with mechanical strength of bone, to be able to predict successful outcome and changes in surgical technique if needed.

Micro-CT parameters had the best predictability of bone strength and quality of the cancellous bone in the proximal femur. This is not surprising, as the microarchitecture of the cancellous bone should contribute significantly to the strength of the bone. Radiographs had the best clinical ability of predicting bone strength, followed by DEXA and then qCT. This is a surprising result, as quantitative CT has shown to be a better predictor of bone strength in other studies with human femora (31).
**Plain Radiography**

The correlation between the two radiographic scoring systems – an objective mean grey value and a subjective linear scoring system (0-10) – was strong ($r = 0.78$, $p<0.05$). This shows that the scoring system that clinically mimics the way that radiographs are assessed for bone density and quality, is quite objective. The correlation between BMD\textsubscript{DEXA} of the core and radiographic scores was fair ($r=-0.51$ for MGV and $r=0.59$ for radiographic score). The correlation between the radiographic MGV and BMD\textsubscript{CT} was higher ($r=-0.84$, $p<0.05$ for MGV and $r=0.78$, $p<0.05$ for score). This is surprising, as radiographic scores and MGV were expected to be more consistent with BMD\textsubscript{DEXA} due to the inclusion of cortical bone in the DEXA analysis. And others have previously reported that MGV provide an accurate and precise method of predicting BMD compared with DEXA analysis (19). The correlations between radiographic measures of bone density (score and MGV) and uCT parameters (BV/TV and PMI) were not strong. This is likely again due to the radiographic interpretation including cortical bone, and the uCT values being assessed purely on the core obtained.

Radiographic bone quality measured by score (RAD\textsubscript{SCORE}) had a good correlation with peak load, from the mechanical testing values. Radiographic quality, measured with MGV (RAD\textsubscript{MGV}), did not have strong correlation with any of the mechanical testing data. The discrepancy seen is likely due to narrow range that the scored radiographs had (all of the radiographs from this series of dogs scored in
the range 4 to 6). Ideally, the cadaver bones would have a wide range of bone quality and thus radiographic scores. There is a real possibility of sampling bias in a study of this type as bones with very poor cancellous quality may not yield viable bone cores; this happened in one sample in the current study. This dog had bilaterally poor cancellous bone in the proximal femur (Figure 8). The radiographic score for this dog was a 2/10 and the DEXA score was also low (0.423g/cm³). An improved method of bone coring may be required to obtain samples from dogs with extremely poor bone quality.

When comparing the Singh Index to bone quality in human studies, there are inconsistencies in the literature, with many studies describing poor predictive values and high intra-observer variability (4, 13-16). The papers that show a high correlation with Singh Index and bone quality, believe the success using Singh's Index is based on the familiarity of this scoring procedure, and when used often, will have a high predictive index. The scoring of the femurs within this study was performed by a surgeon familiar with assessing bone quality from radiographs in relation to performing total hip arthroplasty. This may explain the unexpectedly high predictive value of plain radiographs in this study.
DEXA:

There low correlation of BMD measured by DEXA when compared to qCT and uCT parameters is likely due to DEXA being an “areal” BMD, and incorporating cortical bone in the analysis, with qCT being true volumetric BMD and uCT including just the cored sample in the analysis.

The low correlation between the $BMD_{\text{DEXA}}$ and mechanical data is not consistent with the majority of human data (20, 32, 33). DEXA is considered the gold standard in measurement of osteoporosis (20, 32, 33). $BMD_{\text{DEXA}}$ is not true BMD as it is “areal” BMD. This is still a two-dimensional imaging technique that incorporates the third dimension or depth into its equation. This means that it is not able to separate cancellous and cortical bone. These two main bone types obviously have very different structure and mechanical properties, as they play two very different roles within the bone.

DEXA scanning has been used previously in a number of clinical and preclinical studies in dogs. It has been used successfully in a fracture gap model and was shown to accurately predict non-union, delayed union and normal union (22, 24, 26). It has also been used to assess BMD after cruciate repair (27). It should be noted that BMD is better applied at serial measurements to assess BMD. In our study, DEXA was only used once to assess BMD and due to the inherent flaws that DEXA has, it is perhaps not surprising that the correlation was rather weak. Two-
dimensional imaging of three-dimensional structures inherently involves some assumptions and, as a result, has potential limitations. DEXA may have stronger correlations when assessing whole bone strength as the cortical bone is also measured, as opposed to mechanically testing just the cancellous bone.

Quantitative CT:

Bone mineral density measured by QCT had moderate correlation when compared with two of the uCT parameters, BV/TV and PMI. This is most likely due to the fact that both methods are capable of measuring cancellous bone in isolation, without the confounding inclusion of the cortical bone envelope. The correlation could potentially have been even better if it has been possible to perfectly coregister the regions of interest that were measured in the QCT and micro-CT.

The low correlation of QCT to mechanical bone testing was surprising. The major advantage of quantitative CT is that the cortical and cancellous bone are able to be separated so just the cancellous BMD is measured. There are accuracy errors associated with QCT, including the inclusion of marrow fat, partial volume averaging and the subjectivity in the selection of threshold values used for analysis (29). In human assessment of proximal femoral bone BMD using QCT, the region typically scanned starts 1-2cm above the femoral head and extends a few centimeter below the lesser trochanter. The principal regions of interest are the femoral neck, the trochanter and the intertrochanteric region. One of the reasons why there was a
poorer correlation than expected in this study could be that there was no way to ensure that the BMD_{qCT} values were measured in the exact location from which the cored specimen was removed. One way to assess the consistency of the area would be to rescan the bone after the core has been removed and to co-register the images, allowing for precise localization of the cored volume.

BMD measured by qCT in human proximal femoral studies show high correlation with strength, energy absorption and elastic modulus of proximal femurs (31). It is considered a reliable and precise method for estimation of cancellous bone properties. Intertrochanteric BMD measured by qCT explains approximately 87% of the variability of strength in the proximal femur of humans (31). We did not see this high correlation with the proximal femurs in canine cadavers. This may represent a Type II error, with a low number of samples being tested. Although not assessed in this study, QCT is superior to DEXA for whole bone strength as it is able to take into account whole bone geometry, which significantly contributes to the whole bones mechanical strength.

*Micro-CT:*

The micro-CT data that were obtained from the cored samples demonstrated the strongest correlation with mechanical parameters measured by direct mechanical testing. This is consistent with the human literature that shows that the architecture of the cancellous bone contributes to increase strength and quality.
The Polar moment of inertia had stronger correlation with peak load (r=0.80, P<0.05) and stiffness (r=0.64, p<0.05) when compared with the BV/TV correlation (r=0.78, p<0.05; r=0.62, p<0.05). The energy to failure showed a similar correlation with BV/TV (r=0.78, p>0.05), than PMI (r=0.77, p<0.05).

Mechanical testing:

There was a wide range and standard deviation for the mechanical testing of the cores. This was reexamined and no outliers were determined, thus were not excluded. There could be many reasons to explain this broad data set. The way that the samples were obtained may have damaged the individual trabeculae of the core, leaving inherent weakness of the cores. Bone strength varies depending on the direction of load. The cored sample, may have been in slightly different directions, in different femora, causing the range to be large.

Potential Limitations and Suggested Alternative Approaches

In this study we used an axial load to failure for mechanical testing. The results may have been different if the specimens underwent cyclic loading to failure. The mechanical analysis may have been different if extensometers were placed on the bone. Extensometers are devices that measure strain. This may have been a more reliable way to test for strain (34). Also, pre-conditioning may have helped with reproducibility.
The bones harvested were free of radiographic evidence of orthopedic disease. The values may be different if femurs with orthopedic conditions were used. In dogs that have hip dysplasia, a common radiographic abnormality is sclerosis and medialization of the proximal medial cortex. This is thought to be in relation changes in stressors of the medial cortex. Findings like this may show that the bone quality of the specimen is high due to sclerosis of that area of the bone.

We extracted the bone from a standardized location, but small deviations from the intended position or extraction of the cored biopsy could not be prevented. This can lead to variation in the mechanical properties, depending on the major trajectories within the specimen. As trabecular bone is known to be anisotropic, the direction of the mechanical testing is important. One specimen that had low bone quality values throughout the radiographic, DEXA and qCT imaging studies was not able to have a core extracted due to such poor bone quality. This meant that we were unable to have as broad of a range as bone quality as we would have liked as the coring extraction was not able to be performed on bones with very poor quality.

The coring of these specimens was initially developed so these cores could be taken out \textit{in vivo} during a total hip arthroplasty. This is the rationale behind the decision to evaluate small bone cores as opposed to the entire proximal femur. The small cores also meant that they were able to have uCT analysis. This does mean the assumption of the core taken is similar to the cancellous bone that is still present.
The coring method will need to be further fine tuned for this to be able to be used in vivo as difficulties were encountered when trying to remove the core, without causing any compressing effects on the core. This was performed on the cadaver femurs by using a separate cut at the distal aspect of the core. This could obviously not be performed \textit{in vivo}. Further work is currently underway to refine the procedures for extracting the bone cores without causing unnecessary damage to adjacent bone.

There are other techniques available for assessing bone quality that were beyond the scope of this study. Microindentation involves the use of a microscopic, rigid indenter that is pushed into the surface of the test specimen under a controlled load (7). The area of resulting impression is estimated optically. The hardness is defined as the force divided by the area of the imprint and characterizes the material's resistance to plastic deformation. Advantages of this type of testing includes the relative ease of testing and the ability to make measurements in multiple locations within tissue (7). Drawbacks of the microindentation technique include the fact that it is limited to measuring a single outcome parameter, tissue hardness, which may not be reflective of clinical outcome.

A torque measuring device (DensiProbe\textsuperscript{TM} - AO Foundation, Davos Switzerland) has been used as an intra-operative objective way of measuring bone strength (35). The intra-operative measurement was then compared with DEXA of
the contra-lateral femur post-operatively. There was reasonable correlation between the two values and it would be interesting to evaluate this device (or a variant of it) in cadaveric canine specimens in order to determine whether it might be useful for intra-operative bone quality assessment.

Quantitative ultrasound is another emerging imaging methods used to assess bone quality. This technique sends ultrasound waves through the bone being measured and measures the velocity and amplitude of the signal. The velocity reflects the material properties of bone, such as elastic modulus and strength, and is decreased in osteoporotic bone (36). This technique is low cost and showed that it could be used to predict calcaneal fractures in an older population, however, the correlation with DEXA is low (37).

An alternative and potentially clinically relevant approach to bone quality measurement in dogs involves the use of modern-generation high-resolution peripheral qCT scanners (7). These scanners have isotropic resolution of approximately 80-um. Their main advantage lies in the potential to measure 3-D microstructural parameters such as BV/TV, trabecular thickness, trabecular separation and trabecular number in a live patient. When combined with an appropriate bone density phantom, BMD values can also be determined in the cancellous bone compartment (7). The major disadvantages of peripheral QCT are that they can only be used for peripheral sites such as the extremities, and they are
extremely expensive (approximately $400,000), limiting their availability within the veterinary field. Radiation exposure from peripheral QCT is also a potential concern, especially for longitudinal studies.

High resolution MRI allows non-ionizing 3D imaging of the trabecular network at peripheral sites (7). The advantage of this technique is its ability to generate 3D images of bone geometry and microarchitecture without ionizing radiation. The disadvantages of this system include the long scan times required for high-resolution images of trabecular bone, along with cost and availability of these scanners.

Finite Element Analysis (FEA) is increasingly being incorporated into non-invasive methods of bone imaging and can improve QCT estimation of bone strength and stiffness in vivo (11). QCT imaging data is converted into “voxel” finite element models following segmentation of cortical and trabecular bone to yield measures of bone strength (11). Individual voxels are assigned material properties according to established density-modulus data. FEA models are already used to predict bone quality in the proximal femur in humans, and these models are used to help design implants and make clinically decisions regarding choosing of the correct implant for specific bone quality (11).
Conclusions:

Microarchitectural analysis is the best predictor of cancellous bone strength. Mechanical strength is a combination of bone quality and bone quantity. The resolution of standard, non-invasive imaging tools is not yet good enough to provide robust predictions of bone strength. Currently, with the human literature reporting increased failed rate of THA with decreased bone quality, bone quantity and ultimately strength, we need to be more pro-active about accurately determining bone quality to predict a successful outcome or to change surgical techniques to compensate for poor bone strength. Pre-operative imaging that would allow us to predict the strength of bone is important for THA implant selection and success of the surgery. A quantitative, objective way of measuring bone strength intra-operatively is also necessary to provide our patients with the best predictors for a successful outcome. Until we are able to find a method of accurately determining bone strength pre-operatively or intra-operatively, we must rely on subjective methods and experience alone.
References:


22. Dorea HC, McLaughlin RM, Cantwell HD, Read R, Armbrust L, Pool R, Roush JK, Boyle C. Evaluation of healing in feline femoral defects filled with cancellous...


