TRABECULAR BONE MECHANICAL PROPERTIES AND FRACTAL DIMENSION

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ABSTRACT

Countermeasures for reducing bone loss and muscle atrophy due to extended exposure to the microgravity environment of space are continuing to be developed and improved. An important component of this effort is finite element modeling of the lower extremity and spinal column. These models will permit analysis and evaluation specific to each individual and thereby provide more efficient and effective exercise protocols. In-flight countermeasures and post-flight rehabilitation can then be customized and targeted on a case-by-case basis. Recent Summer Faculty Fellowship participants have focused upon finite element mesh generation, muscle force estimation, and fractal calculations of trabecular bone microstructure. Methods have been developed for generating the three-dimensional geometry of the femur from serial section magnetic resonance images (MRI). The use of MRI as an imaging modality avoids excessive exposure to radiation associated with X-ray based methods. These images can also detect trabecular bone microstructure and architecture. The goal of the current research is to determine the degree to which the fractal dimension of trabecular architecture can be used to predict the mechanical properties of trabecular bone tissue. The elastic modulus and the ultimate strength (or strain) can then be estimated from non-invasive, non-radiating imaging and incorporated into the finite element models to more accurately represent the bone tissue of each individual of interest. Trabecular bone specimens from the proximal tibia are being studied in this first phase of the work. Detailed protocols and procedures have been developed for carrying test specimens through all of the steps of a multi-faceted test program. The test program begins with MRI and X-ray imaging of the whole bones before excising a smaller workpiece from the proximal tibia region. High resolution MRI scans are then made and the piece further cut into slabs (roughly 1 cm thick). The slabs are X-rayed again and also scanned using dual-energy X-ray absorptiometry (DEXA). Cube specimens are then cut from the slabs and tested mechanically in compression. Correlations between mechanical properties and fractal dimension will then be examined to assess and quantify the predictive capability of the fractal calculations.
INTRODUCTION

As plans and progress continue toward establishing a permanent space station, and ultimately resuming spaceflight beyond the earth’s orbit, coping with the physical and psychological demands placed upon the astronauts continues to pose a significant challenge. The musculoskeletal system in particular can undergo dramatic changes from extended exposure to the weightless environment associated with long duration spaceflight. In addressing these issues, efforts centered at the Space Biomedical Research Institute of the Johnson Space Center are continuing to focus on developing improved techniques for monitoring musculoskeletal condition and applying effective countermeasures to minimize losses and maintain adequate function. An important component of this broad-based effort is aimed specifically at developing computer models that can be customized to individuals on a case-by-case basis. Much of the initial work deals primarily with the lower limb because of its prominence in overall skeletal function. Recent Summer Faculty Fellowship participants have devised methods for non-invasively determining the size and shape of the main load-bearing bones, such as the femur and tibia (Todd, 1994), and also estimating muscle forces and joint kinematics during exercise (Figueroa, 1995). The bone dimensions and geometry are derived from magnetic resonance images (MRI) since this avoids exposure to ionizing radiation as is customary with X-ray and similar methods. Finite element models of the femur can be constructed from the geometry data and the applied loads derived from physiological muscle force data. The goal is to use such models to predict the stresses and strains within the bone, and from them identify and assess regions of weakness and potential fracture risk. This detailed and quantitative insight will allow individualized evaluation of skeletal condition (pre-, post-, and in-flight) and prescription of exercise protocols for in-flight countermeasures as well as post-flight rehabilitation. The next major step in developing this capability is to determine the material properties of the bone tissue within the bones. With methods available to generate the geometry and loads, a complete and more accurate model also requires information on the material properties of the bone or bones of interest. A major challenge at this point is to devise a way to estimate these properties from MRI data, which is a process essentially unexplored to date. The goal of the current research is therefore to evaluate the usefulness of estimating mechanical properties of trabecular bone tissue from MRI data. The study is limited to trabecular bone (as opposed to cortical bone) because MRI signals do not detect cortical bone, but this is not a severe limitation since trabecular bone is much more adaptive to changes in loading and is also more susceptible to fracture risk. The specific parameter being used to quantify trabecular bone microstructure is the fractal dimension. This quantity is calculated from the MRI following the methods developed by Acharya (1995), another recent Summer Faculty Fellowship program participant. Thus, the specific aim of the current research is to correlate mechanical properties of trabecular bone tissue with fractal dimension. The properties are measured from a series of mechanical tests on in vitro specimens taken from bones that have first been scanned with the MRI. This will allow direct correlation of the properties and fractal dimension from the same specific sample of tissue.
PROTOCOL AND PROCEDURES

Overview

Much of the time and effort during the summer research period has been spent formulating and refining the detailed research plan. Protocol details and various test procedure options for executing the research have been evaluated. The research involves collaboration between several different laboratories and personnel so extensive coordination is essential. Identifying all of the necessary tasks and the appropriate order and procedures has been a major focus. The main steps in the overall process are:

1. Acquire human tibia specimens and prepare for "whole bone" MRI scanning.
   (at University of Texas Medical School, Orthopedics Biomechanics Laboratory)

2. MRI scan proximal portion of whole tibia using clinically available resolution.
   (at Baylor College of Medicine/Methodist Hospital, Medical Physics)

3. Cut section beneath tibial plateau (3-4 cm long) and prepare for "high-resolution" MRI.
   (at University of Texas Medical School, Orthopedics Biomechanics Laboratory)

4. MRI scan excised piece of proximal tibia to image trabecular architecture.
   (at Baylor College of Medicine/Methodist Hospital, Medical Physics)

5. Slice proximal tibia piece into slabs approximately 1 cm thick.
   (at University of Texas Medical School, Orthopedics Biomechanics Laboratory)

6. Scan slabs for bone mineral density using dual energy X-ray absorptiometry (DEXA).
   (at Baylor College of Medicine/Methodist Hospital, Medical Physics)

7. X-ray slabs to provide alternate images of trabecular architecture.
   (at University of Texas Medical School, Orthopedics Biomechanics Laboratory)

8. Cut slabs into cubes for mechanical testing.
   (at UT Med School and/or Texas A&M University)

9. Conduct mechanical tests and calculate mechanical properties of interest.
   (at UT Med School and/or Texas A&M University)

10. Determine wet and dry densities and ash weights.
    (at UT Med School and/or Texas A&M University)

11. Calculate fractal dimension from MRI and X-ray images.
    (at State University of New York at Buffalo, Biomedical Imaging Group)
Once the experimental tests are completed and the data analyzed, correlations will be examined between mechanical properties and other parameters. The mechanical properties of interest are the elastic moduli (in all 3 orthogonal directions) and the ultimate stress, ultimate strain, and energy absorbed along the primary axis of \textit{in vivo} loading (i.e. the superior-inferior direction). The elastic moduli are measures of material stiffness while the other quantities are measures of strength or "ductility". The main microstructural parameter is the fractal dimension of the trabecular architecture. The fractal dimension is a novel quantity not commonly used for such purposes but recent studies have shown it to be a unique measure in distinguishing between normal and osteoporotic bone (Ruttimann \textit{et al.}, 1992; Majumdar \textit{et al.}, 1993; Weinstein and Majumdar, 1994). This suggests that the fractal dimension may likewise be a promising parameter for predicting mechanical properties. Addressing this question is therefore a major goal of the current research. For completeness and reference with other studies, additional microstructural measures to be included in correlation studies are the wet and dry densities and the ash weights.

As mentioned previously and indicated in the above outline, the research is being carried out at several different laboratories. The Orthopedic Biomechanics Laboratory at the University of Texas Medical School is under the direction of Dr. Timothy Harrigan and also staffed by Dr. Catherine Ambrose and Ms. Frances Biegler. This facility provides the source for fresh human bones and also is equipped for specimen cutting and mechanical testing. A recently funded collaborative research project between the UT Medical School, the Space Biomedical Research Institute at NASA Johnson Space Center, and the Baylor College of Medicine provides the focal point and programmatic framework for the current research. Drs. Linda Shackelford and Laurie Webster oversee the NASA participation providing guidance and direction on scientific issues as well as maintaining the relevance of the work to NASA programs and mission. Drs. Adrian LeBlanc, Harlan Evans, and Chen Lin of the Baylor College of Medicine are responsible for all MRI scanning and image preparation. Fractal analysis is being conducted by Dr. Raj Acharya of the Biomedical Imaging Group at the State University of New York at Buffalo. As a result of participation in the Summer Faculty Fellowship program the tasks associated with specimen preparation, mechanical testing, and post-test measurement of physical properties will likely be shared between the UT Medical School and facilities at Texas A&M University.

More detailed descriptions of the research activities are outlined below. The overall project can be roughly divided into four phases based upon the size and nature of the bone specimens being examined. The first phase involves acquisition, preparation, and imaging of whole bone human tibia specimens. The second phase deals with preparation and imaging of a 3-4 cm section excised from the proximal tibia just beneath the tibial plateau. The third phase involves further cutting the specimen into slabs roughly 1 cm thick and imaging each slab. The fourth and final phase involves cutting the slabs into 1 cm cubes, mechanically testing the cubes, and analyzing the physical properties of the tissue in each cube specimen.
Phase 1 — Whole Tibia Procedures

Whole tibia bones are acquired through the University of Texas Medical School and prepared for imaging in the Orthopedic Biomechanics Laboratory. Each tibia is cleaned of soft tissue and stored frozen. The bone is first X-rayed to ensure that it is free of disease or otherwise not suitable to be included in the study. For proper MRI scanning, each bone must be mounted in a container such that the bone is immersed in 'doped' water. A container for this purpose was fabricated at NASA/JSC from 1/4" thick plexiglass plate material. The box assembly with a tibia mounted is depicted in Figure 1. The plexiglass box is 4.5" wide, 4" tall, 16.5" long, and open at the top. A lid was made to cover the box to minimize splashing or leakage of the fluid during handling. An insert piece was also made to actually hold the bone. This permits easy placement and removal of the bone from the box. It was also intended to provide easy removal of the bone after it is embedded in plastic subsequent to MRI scanning. The insert was constructed from 1/8" plexiglass and made to fit just inside the walls of the box like a thin liner. Holes (1/4" diameter) were drilled in the long sides of the insert for holding rods inserted transversely through the bone. Two transverse holes were drilled diametricallly through the bone near the proximal and distal bounds of the diaphysis region. The holes are 1/4" in diameter and lie in the medial/lateral plane. Plexiglass rods were inserted through these holes and then mounted in the holes in the insert liner.

Figure 1.— Schematic drawing of container for whole bone MRI scanning.
The final preparation for imaging is to mark the boundaries of the proximal section from which mechanical testing specimens will be cut. This section should typically be roughly 3 to 4 cm long and located just beneath the tibial plateau. Landmarks must also be created to provide reference marks for locating tissue regions within the plane of each image slice. Four shallow "grooves" in the outer surface will be made for this purpose. These grooves will run axially along the length of the bone and will be cut with a Dremel tool to a depth of 1 to 2 mm. The grooves will be located along the medial, lateral, anterior, and posterior aspects of the periosteal surface of the bone cortex. The grooves will appear as notches in each planar transverse image. The intersection of lines drawn connecting the medial/lateral notches and the anterior/posterior notches will thereby form a systematically defined origin for coordinate locations within the plane of each image.

After the bone is properly marked and mounted in its container it is transferred to the Baylor College of Medicine for MRI scanning. The whole bone scans are made using a knee resonator coil, which has an inside diameter of 20.5 cm and an axial length of 26.5 cm. Axial scans (i.e. transverse to the bone axis) were made of the 3 to 4 cm region identified in the proximal portion with no gap between adjacent scans and with a scan thickness of 2 mm. These scans actually required multiple images to permit the determination of more detailed parameters such as T1, T2, and T2* in addition to the more routine spin-echo signals. The in-plane resolution of these images is approximately 0.5 mm. Additional spin-echo scans are made at 2 to 3 cm intervals moving proximally into the diaphysis region of the bone. These will be used for further studies on constructing finite element models from MRI data. In addition, five roughly evenly spaced coronal scans (i.e. parallel to the bone axis in the medial/lateral plane) were also made in order to register the axial position of the axial scans.

Following the MRI scanning, the container was emptied of water and returned with the bone still mounted to the UT Medical School. The container was then filled with polyester resin to completely embed the bone in plastic. A plastic sheet liner was placed in the container first to facilitate removal after the plastic hardened. The embedding procedure was intended to provide orthogonal reference surfaces for the whole assembly to guide subsequent cutting operations and also for defining common coordinate systems for locating positions within images and within the physical bone slab specimens. This particular procedure is currently being reviewed in lieu of using the "grooves" described above for the same purpose. Further preliminary studies are underway to address this.

**Phase 2 – Proximal Tibia Section Procedures**

In order to obtain images with high enough resolution to depict the microstructural architecture within trabecular bone tissue the size of the bone sample to be imaged must be reduced. The MRI facilities at Baylor College of Medicine are again used for this imaging. An orbit coil is used for high resolution MRI but its chamber is only 8 cm in
diameter. Thus, a smaller section from the proximal portion of the tibia was cut using a band saw at the UT Medical School. This piece was roughly 3.5 cm long with the first cut 1-2 cm below the tibial plateau (see Fig. 2). Before making these cuts, care must be taken to document the axial location of the cuts relative to a landmark that can be identified in the MRI. Two choices are apparent: the proximal-most tip of the articular surface, or the location of the proximal transverse rod upon which the bone is mounted. The first tibia that was used in preliminary tests was rather large and had to be trimmed significantly on the medial and lateral aspects as well in order to fit within the orbit coil. This also meant cutting away essentially all of the surrounding plastic in which the bone had been embedded. The large size of the bone also permitted cutting an additional 3.5 cm section below (or distal to) the first. This piece was small enough to fit within the orbit coil without further trimming. The in-plane resolution of the orbit coil is 0.125 microns for a scan thickness of 3 mm. Axial scans were made for the entire length of the piece with no gap between successive scans. Similarly, coronal scans were made of the same piece with no gap between scans. These scans were initially made on both pieces with the specimens simply wrapped with paraffin sheets. Upon reviewing the images, however, artifacts due to air pockets were observed, particularly in the central portions of the pieces where the porosity is high. Thus, the protocol has been revised to have the pieces fully hydrated before scanning and placed in a container of water during scanning. Placing the specimens in water will also ensure that the reference notches on the external surface of the cortical wall are clearly imaged.

Figure 2.- Region cut from proximal tibia.
Phase 3 – Transverse Slab Procedures

The next phase begins with cutting the 3 to 4 cm pieces into slabs roughly 1 cm thick or slightly thicker. The goal is to get 3 slabs from each piece. A band saw at the University of Texas Medical School was used for cutting the preliminary pieces examined thus far. Using a band saw makes it difficult to maintain precisely straight, flat, and parallel cutting surfaces and this must be considered in subsequent cutting operations for making cube specimens. Proper marking and/or measuring must be included at this stage in order to maintain the axial position of the cut faces relative to coordinate landmarks. The width of material removed by the saw must also be accounted for in this process. The next two steps involve imaging each separate slab and the order in which they are carried out is not critical. An X-ray image of each slab will be made by contact radiography at the UT Medical School. These X-rays will produce detailed images of the trabecular structure from which alternate calculations of fractal parameters can be made. This will provide a direct comparison of the image quality and fractal parameters between images derived from X-ray and those from MRI. The slabs will also be imaged using dual-energy X-ray absorptiometry (DEXA) scanning techniques at the Baylor College of Medicine. DEXA scans are available clinically and give measures of bone mineral density (BMD) and bone mineral content (BMC). The BMD and BMC values can be determined for regions of interest defined graphically on the image. Thus, with proper documentation of coordinates, BMD and BMC can be calculated for the same specific tissue regions from which the cube specimens for mechanical testing were ultimately cut.

Phase 4 – Cube Specimen Procedures

The final phase of the protocol begins with cutting each slab into a series of roughly cube shaped specimens for mechanical testing. Considerable time and effort has been spent in developing and refining these procedures but only the highlights will be summarized here. First, each slab must be marked in some manner to identify which flat surfaces are proximal (or superior) and/or distal (or inferior) and which reference notches are medial/lateral and anterior/posterior. These markings should preserve these identifications to the degree possible throughout the process of being cut into the cube specimens. A Buehler Isomet low speed diamond blade wafering saw is used for this cutting. The saw used during the summer research period was located at the University of Texas Medical School, but one is also available at Texas A&M University. A series of parallel cuts are made to cut the slab first into "bars" as indicated by the long-dashed lines in Figure 3. Two blades were gang-mounted approximately 1 cm apart to create two parallel cuts with each cutting operation. A custom gripper was made to hold the slab during this process to allow 3 or 4 such bars to be cut without re-gripping the workpiece. The precision of these cuts will routinely produce surfaces parallel to well within required tolerances for opposite faces of the cube specimens. The surfaces of the bars formed from the band saw will not be parallel enough, however, so each bar must be rotated 90 degrees
and cut to face off one of the two surfaces cut with the band saw. Each bar is next cut transverse to its long axis to yield a set of cube specimens (these cuts are indicated by the short-dashed lines in Fig. 3). Each specimen must be properly marked to identify its orientation and coordinate location within the slab cross-section. A unique requirement of the current work is the need to cut slabs before making cross-cuts to produce cubes. This is necessary to allow DEXA and X-ray imaging as described previously and thereby precludes using a milling machine with blade cutters (as used by Ciarelli et al., 1991, for example). The detailed steps and procedures in the cutting process have been developed with the assistance of a volunteer undergraduate research assistance and are outlined in a separate document resulting from that work (Brandt, 1995).

Cube specimens will be stored wet and frozen until time for mechanical testing. Mechanical tests will be conducted in compression under displacement control at a rate of 0.01s⁻¹. Each test specimen will be loaded between two flat platens lubricated to eliminate lateral constraint due to friction. One of the platens will be articulated to self-align and reduce non-uniform loading from imperfectly parallel specimen surfaces. A shallow (1-2 mm deep) recessed area will be machined in the platens to facilitate placement of the specimens centered along the machine loading axis. Each specimen will be tested nondestructively along each of its 3 orthogonal axes (i.e., superior/inferior, medial/lateral,
anterior/posterior) following procedures similar to those of Goulet et al. (1994), Keller (1994), and Linde et al. (1992). This will permit calculation of elastic moduli for each direction. A final destructive test to failure will then be conducted along the primary anatomical axis of loading (superior/inferior for proximal tibial bone). Quantities such as the ultimate strength, ultimate strain, and energy absorbed to failure can then be calculated for this direction of loading. Additional details to be determined through preliminary testing and further study include whether to use preconditioning cyclic loading, which particular testing machine to use, and whether to use extensometers for surface strain measurement. Using extensometers will likely require more elaborate design of the platens in order to provide adequate clearance to prevent the extensometers from interfering with the platens during testing. Numerous studies have addressed the issue of end effects (Allard & Ashman, 1991; Aspden, 1990; Harrigan et al., 1988; Keaveny et al., 1993; Linde et al., 1992; Simmons & Hipp, 1995; Zhu et al., 1994), but no simple solution has been developed to date, including using extensometers. Thus, applying an extensometer will definitely provide more insight into the details of each test, but it will not totally mitigate the problems associated with end effects. Testing machines are available at the University of Texas Medical School and also at Texas A&M University. Facilities are also available at Texas A&M for determining the wet and dry densities and ash weights. The wet density is simply the wet weight of the specimen (in g), which can be measured before or after testing, divided by the volume of the specimen (in cm³) as calculated from caliper measurements of physical dimensions. The dry density is the weight of the specimen (in g) taken after drying in an oven at 100°C for 24 hours. The specimen is then ashed in an oven at 500°C for 48 hours and weighed. The ratio of ash weight (in g) to dry weight (in g) is commonly expressed as a percentage and termed the "ash weight percent", or sometimes even just "ash weight". An ash density can also be calculated by dividing the ash weight (in g) by the volume (in cm³).

SUMMARY

Much progress has been made during the summer research period in defining the relevant requirements, evaluating available options, and establishing detailed procedures for conducting the tests and analyses required for this complex, multi-disciplinary, multi-institution collaborative research effort. Extensive study of the literature has been combined with preliminary tests as needed to address the major issues encountered. Four tibia (2 sets of paired) have been acquired and mounted in the removable insert pieces for whole bone MRI scanning. One has already been scanned and used for the preliminary studies. As the preliminary work progressed through mechanical testing the images from the MRI scans are being reviewed and analyzed by Dr. Acharya. When all details of the protocol are approved and agreed upon by all investigators, the other three tibia will be tested. Correlation studies will then be conducted to establish the relationships between the measured mechanical properties and the various independent variables (fractal dimension, wet/dry/ash densities, BMD/ BMC, T2*, etc.).
REFERENCES


