METHODS FOR IDENTIFYING CANCELLOUS BONE SPECIMEN LOCATION AND SIZE FOR THE REDUCED PLATEN COMPRESSION TEST

A Senior Honors Thesis

By

KYLE RAY COWEN

Submitted to the Office of Honors Programs
& Academic Scholarships
Texas A&M University
In partial fulfillment of the requirements of the

UNIVERSITY UNDERGRADUATE
RESEARCH FELLOWS

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Group: Engineering

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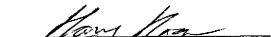
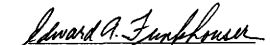
Approved as to the style and content by:



Harry Hogan
(Fellows Advisor)



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ABSTRACT

Methods for Identifying Cancellous Bone Specimen Location and Size for the Reduced Platen Compression Test. (April 14, 2000)

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The skeleton functions as a vital part of our everyday existence and acts as a framework for the body to provide movement, resist the forces of gravity, and protect vital organs. Skeletal research studies the effect of disease, lifestyle, and stimuli on the skeleton and its ability to perform these everyday functions. The current state of bone testing is focused on understanding the mechanical properties of bone through use of traditional mechanical testing procedures such as three point bending, torsion, and compression testing. The traditional method of compression testing involves compressing a bone specimen between two parallel platens to failure or until a desired displacement is obtained. This method is useful for studying the properties of the entire bone sample. Bone can be catagorized into two major types: cortical bone and cancellous bone. Current compression testing techniques do not allow the properties of cancellous bone to be determined. The Reduced Platen Compression Test attempts to improve the traditional compression test to allow cancellous bone to be tested while the outer cortical shell remains on the specimen by using smaller diameter platens to compress only the inner cancellous area of the specimen. The RPC is relatively new and several questions

still remain as to the correct method for identifying the location and size of the test specimen. Rat femurs used in preliminary RPC Testing were analyzed to determine the best method for locating and sizing the test specimen. X-rays of approximately 120 rat femurs were studied to see if a standard location and size could be defined for the RPC test specimen. The results indicate that the rat femur develops too inconsistently for a standard length or percentage of the overall length to be used to define the location. The best method for locating the specimen is to identify the location of the distal end of the epiphyseal growth plate and take the specimen just below that location. The results also indicate that the best method for defining the specimen thickness is to average the largest and smallest overall bone lengths in the test group and use a reference thickness of 2 millimeters as a percentage of this average length. This percentage of the overall average length then defines the specimen thickness for each individual bone.

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CHAPTER I.
BACKGROUND

1.1 Motivation for Mechanical Testing of Bone

Bone is the foundation and structural support of the skeletal system. It is an internal framework that provides strength and aides the body in supporting itself against the forces of gravity and producing limb movement. The ability of the skeletal system to perform these functions is integral in our everyday lives. Understanding how age, disease, and lifestyles affect the skeletal system, scientists and doctors can developed better treatments and cures for debilitating diseases such as osteoporosis and predict the effects of long term exposure to nicotine, alcohol, and other environmental conditions. Mechanical testing of bones is widely used to explore how the skeleton reacts to disease and changes in lifestyle. The most widely used vehicle to explore the effects of diseases such as osteoporosis and their possible treatments is through compositional and structural studies on bone tissue (Ruhmann, 1998). Traditional mechanical testing has long been used as the method for determining the mechanical properties of bone tissue. Initially, the focus of many of these studies was to simply understand the mechanisms by which bone fractured and the loads at which this occurred. As early as 1884, the scientist Julius Wolff tested the mechanical strength of bone in an attempt to understand these mechanisms (Engesaeter et al., 1978). In recent years, the mechanical properties of bone have been examined through a variety of testing procedures such as torsion, tension, compression, and three point bending.

This thesis follows the style of the *Journal of Biomechanics*

1.2 Structure of Bone

The first major step towards understanding and describing the structure and functions of bone came with the invention of the compound microscope in the 17th century (Martin and Burr, 1989). With the microscope, scientists were able to make observations of an extensive canal system running longitudinally and transversely through the structure of bone. With these observations came the understanding that bone is actually a porous structure and porosity varies depending upon anatomical location (Martin and Burr, 1989). In recent years, research stemming from these foundational observations have continued the journey towards understanding the complete morphology and functions of bone.

In general, bone can be though of as a composite material containing primarily collagen fibers and a rigid crystalline matrix (Ruhmann, 1998). The exact composition of bone varies depending on the age and sex of the animal, the specific location within the skeleton, and the area within the individual bone in question. Bone structure in mammals can be categorized into two basic types: cancellous (trabecular or spongy) bone and cortical (compact) bone. Cortical bone is highly organized in structure, and is found in highest concentration in the mid-shaft (diaphyseal) region of long bones like the tibia and femur. Cancellous bone is highly porous with as much as a fifty percent pore volume and more randomly organized when compared to cortical bone. In long bones, it is primarily located in the ends of the bone (metaphyseal region) and along the lining of the marrow cavity in the diaphyseal region (Cowin, 1989; Parks and Lakes, 1992). Cancellous bone is also found in large concentrations in the vertebral bodies where it is surrounded by a thin cortical wall. Cancellous bone structure consists of three dimensional branches or bony trabeculae interspersed by bone marrow. It is important to note the high porosity of

cancellous bone compared to cortical bone in reference to the rate of growth of the different tissues. Cancellous bone is reproduced at a higher rate and more often than cortical bone due to the large surface area associated with cancellous tissue.

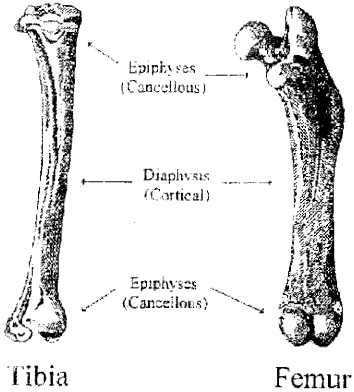

Figure 1.1 Rat tibia and femur. The mid-shaft (or diaphysis) region is composed largely of cortical bone. The ends of the bone are composed of a cortical shell enclosing a cancellous interior.

1.3 Function of Bone

The function of bone tissue can be divided into four main areas: protection of vital structures, hematopoiesis, mineral homeostasis, and structural support (Ruhmann, 1998). The protection of vital of vital structures is one of the more obvious functions of bone. Bone serving in this role is most readily found in the rib cage, the skull, and the vertebral column. Grossly, this type of bone is constructed of two cortical layers seperated by a region of cancellous bone (Martin and Burr, 1989). This construction allows the bone to be both hard and resistive to damage through the cortical layers, and energy absorbent through the interior cancellous layer (Martin and Burr, 1989).

Hematopoiesis, a metabolic function, is a less obvious role as compared to the more structural functions. Hematopoiesis involves the production of red blood cells and is important in the long term control and balance of the body's calcium supply (Ruhmann, 1998).

Another function of bone is mineral homeostasis which is the supply of calcium needed for nerve conduction, muscle contraction, blood clot formation, and cell secretion (Cowin, 1989). Bone contains 99% of the body's total calcium and phosphorous supply and thus is the major repository for these minerals (Martin and Burr, 1989).

The function of bone as a load bearing structure is by far the most widely studied role of bone (Martin and Burr, 1989). In this capacity, bone serves as a mechanical support against the forces of gravity, and as a rigid lever system operated by muscles to perform locomotion or even the simple action of using your hands to turn a page.

1.4 Use of Rats in Mechanical Testing of Bones

The rat has been widely accepted for years as a model for skeletal research. The anatomy of the rat closely resembles that of a human, and reacts comparably to various diseases and stimuli. Rats are relatively inexpensive for research when compared to larger animals, and their lifestyles can be easily manipulated and controlled for specific studies. The small size of the rat anatomy is a challenging problem that is encountered, though, when using rats in biological research.

1.5 Current State of Mechanical Testing of Cancellous Bone

Methods for evaluating the strength of cancellous bone have been limited to compression testing of vertebrae or a more complicated method in which a compressive load is applied to the femoral head causing bending in the femoral neck (Hou et al., 1990). In either case, these methods generally test specimens containing the exterior cortical shell and therefore do not give a true measure of cancellous bone strength. One notable exception to this is a study by Demetropoulos et al., (1993) in which isolated cancellous bone samples were cored from vertebrae and tested in compression. However, useful or conclusive results were not presented in this article, so there is still a need for a more comprehensive investigation into methods for testing cancellous bone of the rat (Ruhmann, 1998). The necessity lies in the fact that the structural properties of cancellous bone vary for different anatomical regions (Turner and Burr, 1993). As previously stated, current methods for testing the compressive strength of cancellous bone involves testing slice or whole specimens usually with the exterior cortical shell intact. For the majority of tests using this technique, the objective was to draw some conclusion on the mechanical properties of cancellous bone. However, because the

specimens contain the exterior cortical shell, the results are not a true reflection of cancellous bone strength, but more so, reveal the composite strength of cortical and cancellous bone. The actual testing follows that of traditional compression testing with the exception that specimens are kept moist with Ringer's solution to maintain a hydrated condition. Hydration helps maintain the bone's in vivo properties (Turner and Burr, 1993). The procedure for compression testing involves placing a specimen between two flat surfaced loading platens and applying a uniaxial compressive load to the specimen until failure or a desired displacement has been reached. The main drawback with this method of testing is that for the majority of specimens, machining is necessary to produce plano-parallel ends necessary for loading. Machining of the specimens may cause boundary errors and also produce loading faces that are not perfectly plano-parallel which can cause some error during testing (Ruhmann, 1998).

1.6 Handling and Testing of Specimens

For most mechanical testing of materials, it is important to use specimens that reflect, or closely parallel, the true or working properties of the material. Thus, specimens should not be handled because oils could be passed from the hands of the experimenter to the specimen which could alter the mechanical properties. In general, for mechanical testing of materials, it is important to use specimens that have not been damaged with notches, chips, or other such conditions which could alter the mechanical properties. When testing bone specimens, it is important through the time of removal through mechanical testing, to keep the bone as similar to its in vivo conditions as possible. Thus, specimen preservation, hydration, and temperature become important (Turner and Burr, 1993). The most commonly used method for preservation prior to testing is to wrap the

specimen in gauze wetted with Ringers solution and freeze it at -20°C (Turner and Burr, 1993). While the specimen is "in limbo" between removal and freezing, or freezing and testing, it is important to keep the bone moist with Ringers solution. This is important because as the bone dries, the mechanical properties change. Considering temperature, as with most biological materials, bone's mechanical properties are influenced by the surrounding temperatures (Ruhmann, 1998). Thus, mechanical testing should ideally be performed at 37°C (Turner and Burr, 1993) However, this temperature level is often difficult to attain in most material testing facilities without complex modification of the testing set up, so a standard testing temperature of 23°C (or room temperature) is acceptable

1.7 Reduced Platen Compression Test

1.7a RPC Test Idea

The difficulties associated with separating cancellous bone tissue from the surrounding cortical wall have recently led researchers at Texas A&M to develop a version of the traditional compression test that attempts to isolate the properties of the inner cancellous bone material while still surrounded by the cortical shell. The Reduced Platen Compression Test, RPC Test, as it is called is designed to load only the inner cancellous tissue in compression. This is accomplished by using platens which are smaller in diameter than the cortical wall to load the specimen.

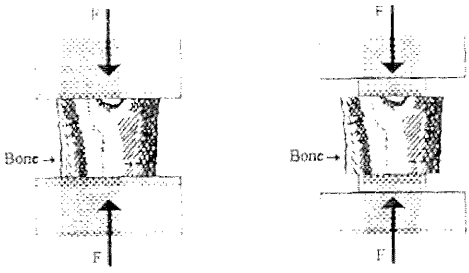

Figure 1.2 Compression Testing: (A) traditional compression testing, (B) Reduced Platen Compression Test method

1.7b Bone Removal and Preservation

The femur and tibia from both hind legs of the rats in the test groups were collected at necropsy. The bones were carefully cleaned of adhering soft tissue, wrapped separately in gauze wetted with Ringer's solution, sealed in plastic bags, and frozen at -20°C. It should be noted that such freezing and preservation techniques have been shown to have minimal effect on the mechanical properties of bone (Pelker, 1984).

1.7c Measurement of Bone Length, Thickness, and Diameters

1.7c.1 Digital Micrometer Method

The maximum length of the femur and tibia were measured in millimeters to the second decimal place using a digital micrometer. For the femur, length was measured as the distance between the top of the trochanter and the ridge between the end of the

medial-lateral condyle. For the tibia, length was measured as the distance between the top of the medial-lateral condyle and the end of the medial-lateral malleolus.

The outer diameters in the diaphysis region of the tibia and femur were also measured in millimeters to the second decimal place using a digital micrometer. Measurements were taken in anterior-posterior and medial-lateral directions.

1.7c.2 Contact Radiograph Method

Two sets of contact radiographs were taken of the whole bones. The first set was taken with the bones oriented so that their posterior aspect was facing down. The second set was taken with the bones oriented so that their lateral aspect was facing down. X-rays were taken on Kodak X-Omat TL Film (Eastman Kodak Company, Rochester, NY) using a General Electric Industrial Radiograph Machine (Lexington, MA) set to 25 kV and 1 mA. The focal film distance (FFD) was set at 30 inches and the exposure time was 85 seconds. The developed x-rays were then scanned into a PC for image analysis using SigmaScan/Image Software (AISN Software Inc., Jandel Scientific Software, San Rafael, CA). For the femurs, the total length and the midpoint along the long axis of the bone were determined. Once the midpoint was determined, a method developed by Warren et al., (unpublished) was used to determine the internal and external diameters for each radiograph. Using this method, a line was drawn one pixel wide through the bone midpoint perpendicular to the diaphyseal long axis. The plot of pixel intensity along this line has two distinct peaks associated with the edges of the marrow cavity. The distance between these two peaks was taken as the marrow cavity diameter (internal diameter)(Warren et al., unpublished). For the external diameter, the diameter was taken as the distance between the two pixels in the pixel intensity plot where the pixel

intensities first exceed the mean background threshold (Warren et al., unpublished). This was done for both the anterior-posterior and medial-lateral radiographs for each bone.

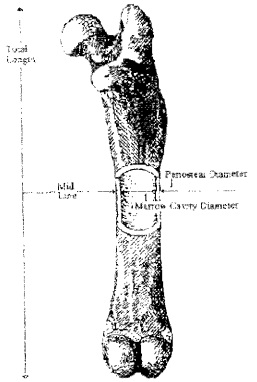

Figure 1.3 Dimensions for femur and tibia measured using SigmaScan/Image Software

1.7d RPC Specimen Extraction

Slice specimens were taken from the proximal tibia and femurs. The desird location of each specimen was determined from the developed x-rays using SigmaScan/Image Software by visually identifying a section 2 mm long lying below the epiphyseal growth plate and containing a maximum amount of cancellous bone material. In an attempt to standardize the locations, the total length of each bone was measured, and the length from the distal end of the bone to the distal extent of the specimen target region was measured as well. The ratio of these two lengths was calculated for each

specimen and averaged to obtain a final value. This average value was then used to locate each specimen (Ruhmann, Hogan and Sampson, 1997). Specimens were cut perpendicular to the long axis of the bone to a length of two millimeters using a low speed diamond blade wafering saw (Buehler LTD, Lake Bluff, IL) under constant irrigation with Ringers solution. Additional x-rays were then taken of each slice to give images of the cross-sections from which geometric and area calculations were made. The slice x-rays were taken on Kodak X-Omat TL Film using a General Electric Industrial Radiograph Machine set to 20 kV and 1 mA. The focal film distance (FFD) was set at 30 inches. In order to determine the optimal exposure time, a series of x-rays with exposure times ranging from 20 to 85 seconds with a 5 second increment were taken. From this set, exposure times of 55 and 30 seconds were chosen as the optimal exposure lengths.

1.7e Compression Testing

Each of the slice specimens was tested in quasi-static compression on a MTS model # 312.31S servo-hydraulic testing machine (Minneapolis, MN) with a displacement rate of 0.51 mm/min. Specimens were loaded through two axially aligned, cylindrical, plano-parallel platens which contacted only the central cancellous region of the bone (Ruhmann, Hogan and Sampson. 1997). A platen diameter of 3 mm was chosen to provide a loaded area well within the endocortical perimeter of all specimens.

CHAPTER II
DATA ANALYSIS AND RESULTS

2.1 Motivation

The advent of the RPC Test method for analysis of cancellous bone properties in rats may lead to significant findings in skeletal research. However, the method is new and some questions still exist as to the best method for conducting the test. Results presented from earlier research using this test method contained a high degree of variability in the results (Ruhmann, 1998). Standard deviations of up to sixty percent of the mean were common.

2.2 Objective

The objective of this research is to attempt to develop test standards for the RPC Test and to try to reduce or identify causes of variability in the results by implementing these test standards.

2.3 Animals

Rats from two previous study groups will be used throughout this research to gather and analyze data for the RPC Test. Group 1 consists of femurs extracted from 69 virgin female Spraque-Dawley rats used to study the effects of osteoporosis on cancellous bone properties. The RPC Test was used on Group 1 for comparison with the traditional method of compression testing. The data gathered from Group 1 will be used to analyze the test methods used in the RPC Test for consistency and possible sources of error. This information will then be used to develop better RPC Test methods. Group 2 consists of femurs extracted from 60 rats used in an alcohol/OVX study. Data gathered from the

bone and SigmaScan/Image measurements of Group 2 will be used to refine the recommended test procedures developed from Group 1. These new test procedures will be implemented as the RPC Test is used on the femurs in Group 2.

2.4 Location of the RPC Test Specimen

The first objective of this study was to develop a method for consistently identifying the location of the RPC Test specimen within the femur. In previous tests, SigmaScan/Image was used to measure the distance from the distal end of the femur to the distal extent of the epiphyseal growth plate. The distance was averaged for the entire test group, and this average was used to locate how far from the distal end of the femur the RPC specimen would be taken. The data and SigmaScan images taken from Group 1 and Group 2 were used to determine if this method is valid, or if a better method should be developed.

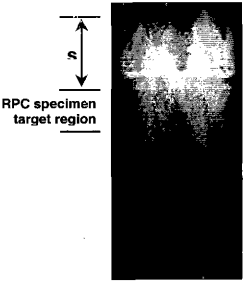

Figure 2.1 Location, S, of the RPC Test Specimen and target region location for maximum cancellous bone density.

Table 2.1 Overall femur lengths and distances from the distal end to the distal extent of the epiphyseal growth plate for Group 1

	Femur Length (mm)	Distance to Growth Plate
1A - Avg.	36.83	4.60
STD	0.97	0.69
%COV	2.63	15.03
2A - Avg.	37.49	5.17
STD	1.31	0.31
%COV	3.49	6.07
3A - Avg.	37.56	4.88
STD	0.70	0.29
%COV	1.87	6.03
1P - Avg.	37.35	4.68
STD	1.28	0.26
%COV	3.42	5.58
2P - Avg.	37.38	4.60
STD	1.11	0.24
%COV	2.96	5.22
3P - Avg.	37.56	4.98
STD	0.68	0.63
%COV	1.81	12.70
1C - Avg.	37.56	4.62
STD	0.92	0.16
%COV	2.44	3.46
3C - Avg.	38.09	5.13
STD	1.10	0.75
%COV	2.90	14.55
ADLB - Avg.	37.52	4.91
STD	1.26	0.23
%COV	3.36	4.66

STD- Standard Deviation
% COV – Standard deviation as a percentage of the mean

Table 2.1 Shows the average femur lengths for the individual test sets in Group1 and the distances from the distal end of the femur to the distal extent of the epiphyseal growth plate. These results seem to indicate that the distance from the distal end of the

femur to the distal extent of the epiphyseal growth plate vary between individual test sets to a large enough extent that a single average distance for all specimens is incorrect.

One hypothesis for a better method of determining the location of the RPC Test specimen was to use a standard percentage of the overall femur length to define the distance from the distal end of the femur that the RPC specimen would be located. Images of the rat femurs from SigmaScan were used to determine the location of the growth plate in each femur. These distances were then converted to a percentage of the overall femur length.

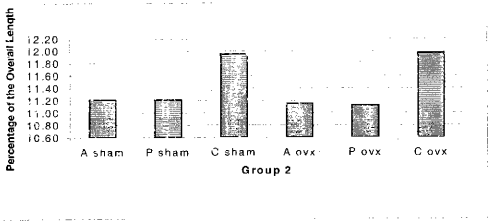

Figure 2.2 RPC specimen location as a percentage of the overall length of the rat femur

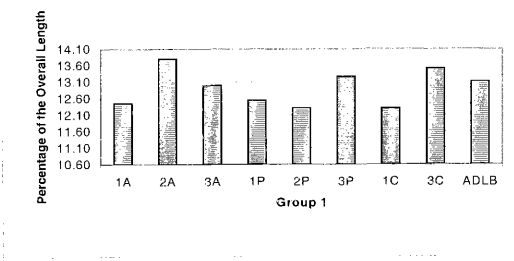

Figure 2.3 Average specimen location as a percentage of the overall length.

Figures 2.2 and 2.3 show the average RPC specimen locations as a percentage of the overall length. These results show that the anatomical dimensions of the rat femurs are too inconsistent between individual test sets to define a standard percentage of the length as the location of the RPC Test specimen. For the compression test to be accurate, the test specimen must not contain any portion of the epiphyseal growth plate. The growth plate is much more dense than the cancellous bone and would therefore give invalid results. Therefore, care must be taken to avoid locating the test specimen to close to the distal end of the femur. However, the most dense regions of cancellous bone material are located just below the epiphyseal growth plate. Often these areas are very small. Therefore, the specimen location cannot be too far beyond the growth plate, or an insufficient amount of cancellous bone material will be in the specimen. A conservative value for the percentage could be used to ensure that the growth plate is always missed, but then one cannot guarantee a sufficient amount of cancellous bone material will be left in the specimen.

2.5 Size of the RPC Test Specimen

The second objective of this study was to determine a standard for sizing the RPC Test specimen. In previous tests, a 2 mm thickness was arbitrarily chosen as the size of the RPC Test specimen. This method periodically resulted in specimens with voids in the center where no cancellous bone was present. Again, the SigmaScan images for Groups 1 and 2 were evaluated to identify the ideal test specimen size, and a possible method for standardizing this size.

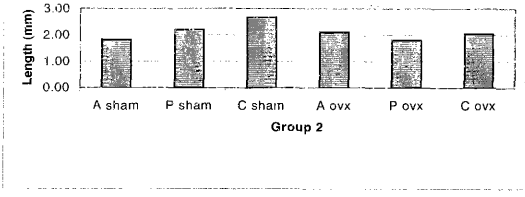

Figure 2.4 Average ideal specimen length from SigmaScan

Figure 2.4 shows the ideal locations for the RPC Test specimens in Group 2. The ideal lengths were determined by identifying the location where the epiphyseal growth plate ended, and measuring the thickness of the portion below the growth plate that contained dense cancellous bone material. This method is admittedly subjective and completely dependent upon the quality of the contact radiographs.

A hypothesis for standardizing the size of the RPC specimen was to define the ideal thickness as a percentage of the overall length. Figure 2.5 shows the results from the data gathered from Group 2.

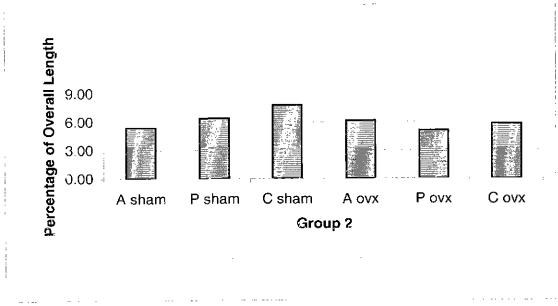

Figure 2.5 Ideal specimen size as a percentage of the overall length

Again, the data seems to suggest that a single standard percentage of the overall length would not be acceptable in defining the size of the RPC Test specimen.

A second hypothesis for defining the size of the RPC specimen was also studied. The average overall femur lengths for each test set in Group 2 were measured using SigmaScan. A 2 mm length was then calculated as a percentage of the largest and smallest average femur lengths. These two average percentages were then averaged to give one percentage of the overall length. This percentage was then used to define the thickness of each RPC Test specimen. The percentage of the overall length used to define the specimen thickness would be calculated for each different test set. A standard percentage for all RPC Test specimens would not be used. The results indicate that the

thicknesses resulting from this method closely parallel the ideal thicknesses found from analysis of the SigmaScan images.

CHAPTER III.
CONCLUSIONS AND RECOMMENDATIONS

3.1 Conclusions for the RPC Test Specimen Location

The conclusion obtained from analysis of the results gathered from Groups 1 and 2 suggest that use of a standard percentage of the overall length to define the location of the distal end of the RPC Test specimen is not possible. A conservative percentage could be used to ensure that the location of the distal end would be below the distal extent of the epiphyseal growth plate; however, in many specimens, sufficient cancellous bone material would not be present because the RPC specimen location would be too far below the area of dense cancellous bone. The best method for locating the test specimen is to identify the distance of the distal end of the growth plate from the distal end of the femur using SigmaScan. This analysis must be done on an individual bone basis. This method is tedious and time consuming, but it is the only way to ensure that the specimen will be located below the growth plate and contain sufficient cancellous material.

3.2 Conclusions for the RPC Test Specimen Thickness

The data gathered from analysis of Groups 1 and 2 suggest that a standard 2 mm thick specimen is not a good standard. The SigmaScan images show that there is not always 2 mm of cancellous material present. The method determined for sizing the test specimens is to a percentage of the overall length for each individual test set. This percentage is found by taking the longest and shortest overall femur lengths and defining 2 mm as a percentage of each length. These two percentages are then averaged to give one percentage of the overall length that is used to define the thickness of each individual test specimen.

3.3 Recommendations

Other areas of the RPC Test should be analyzed to determine correct procedures. One specific area that attention should be given to is the sizing of the platens used to load the specimens in compression. Care must be taken to accurately size each platen. If the platen is too large, the edge of the platen will be too close to the inner diameter of the cortical wall. This will cause shearing of the cancellous bone along the cortical wall. Also, if the platen diameter is too small, the effective loading area will be too small, and loading will be much more like an indention test than a compression test. Furthermore, platen shape should be tested to determine if a shape other than circular is ideal for loading the cancellous area.

One final area that needs to be improved is the consistency of quality of the contact radiographs. Much of the identification and sizing of the RPC Test specimens is dependent upon analysis of the scanned x-ray images. Improper exposure time or quality of the x-rays inhibits the researcher from accurately identifying the location of the epiphyseal growth plate and area of dense cancellous bone material.

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VITA

Kyle Ray Cowen was born in Amarillo, Texas on February 17, 1977 to Alvin and Carol Cowen. Kyle is the youngest of two children, the oldest being his sister Karis who was born on Decemder 2, 1973. Kyle grew up in Amarillo, TX., and attended Amarillo High School, where he graduated in May 1995. Kyle went on to attend Texas A&M University in College Station, TX. Kyle will graduate in May of 2000 with a Bachelor's of Science in Mechanical Engineering. In September of 2000, Kyle when begin work at Deloitte Consulting in Dallas, TX. as a Strategic Management and Technology Consultant fir the Texas Practice. It is in Dallas that Kyle will begin the remainder of a long and fruitful life.

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