OSTEOCYTE MECHANOTRANSDUCTION: CHANGES WITH AGE

A PARAMETRIC FINITE ELEMENT STUDY

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OSTEOCYTE MECHANOTRANSDUCTION: CHANGES WITH AGE

A PARAMETRIC FINITE ELEMENT STUDY

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University of Missouri-Kansas City, 2013

ABSTRACT

The nature of bone is to adapt its structure in response to mechanical loading. When the bone is loaded, it is remodeled to be able to support the increased loads. Bone resorption can occur resulting in bone loss from lack of mechanical loading. Bone adaptation is controlled by: (1) osteoclasts, which resorb bone, (2) osteoblasts, which lay down new bone, and (3) osteocytes, which send signals of bone remodeling. Studies have shown that in the absence of mechanical loading, osteocytes send signals of bone resorption. Understanding the microarchitecture of bone is essential to understanding the mechanotransduction within the osteocyte network. The purpose of this study was to analyze the effect of age on the microarchitecture of murine bone and use the data to conduct a finite element analysis of strains imposed on the lacuna. Ten murine femur bone samples were studied; 5 old (2-years-old), and 5 young (5-months-old). Each sample was resin-embedded, acid etched according to protocol, and imaged. ImageJ was utilized to quantitatively assess changes in the number of canaliculi, the diameter of the
canaliculi, lacunar size, and lacunar density. The total number of canaliculi per osteocyte
decreased with age, specifically in the periosteal region. A greater number of canaliculi
were found in the periosteal region than the endosteal region of the young samples. The
canalicular diameter increased with age, specifically in the periosteal region from young
to old. In the old samples the canalicular diameter decreased from the periosteal region to
the endosteal region. The lacunar size decreased in the periosteal region from young to
old. It also decreased across the young sample with larger lacuna in the periosteal region.
The lacunar density increased with age. The finite element analysis revealed strain
amplification factors of 2.19 for the young, young endosteal, and young periosteal
models. The finite element analysis revealed strain amplification factors of 2.15, 1.89,
and 2.10 for the old model, the old endosteal, and the old periosteal models, respectively.
The lower strains predicted in the old bone compared to the young bone may be
responsible for the well-known resistance to loading that occurs with aging.
APPROVAL PAGE

The faculty listed below, appointed by the Dean of the School of Computing and Engineering have examined a thesis titled “Osteocyte Mechanotransduction: Changes with Age – A Parametric Finite Element Study,” presented by Teri L. Cline, candidate for the Master of Science degree, and certify that in their opinion it is worthy of acceptance.

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CHAPTER 1

INTRODUCTION AND PRELIMINARY STUDIES

According to the National Institute of Health Osteoporosis and Related Bone Diseases National Resource Center, more than 40 million people are currently affected by osteoporosis or are at a high risk due to low bone mass \(^{(1)}\). As humans age, the ability to form bone faster than it is lost decreases, with peak bone mass between the ages of 18 and 25 \(^{(2,3)}\). A comparison between images of normal bone and bone with osteoporosis reveals the severity of the effects of osteoporosis (Figure 1). While osteoporosis can affect anyone, it is found to be most common in older women \(^{(1)}\). Aging plays an important role in the remodeling of bone.

![Osteoporosis causes weak bones.](image)

Figure 1. A comparison between normal trabecular bone and trabecular bone affected by osteoporosis \(^{(4)}\).

The nature of bone is to adapt its structure in response to mechanical loading. Bone adaptation can occur as bone remodeling or bone resorption (bone loss from lack of
mechanical loading). Bone adaptation is controlled by three types of cells: (1) osteoclasts, that resorb bone, (2) osteoblasts, that lay down new bone, and (3) osteocytes, that send signals of bone remodeling or resorption to other bone cells (Figure 2).

Figure 2. Diagram of bone featuring osteoblasts, osteoclasts, and osteocytes (5).

Osteocytes make up over 90% of bone cells (6) and have been shown to be the mechanosensors in bone (7). Osteocytes have the ability to sense mechanical energy and send out biological signals. For example, engaging in physical activities like sports and exercise will strain bones at the macroscopic level. Osteocytes have the ability to sense the macroscopic strain at a microscopic level and transmit resulting biological signals through an extensive communicative network. Bone can adapt its structure based on this communication in response to mechanical loading by adding new bone in the presence of loading and remove bone in response to lack of mechanical loading (7). This process by which mechanical energy is converted into biological signals is called
mechanotransduction. Osteocytes act as the mediator in the mechanotransduction process within the bone microarchitecture\(^8\).

The microarchitecture of bone immediately surrounding the osteocyte is the focus of this study. Osteocytes are embedded in a mineralized matrix, housed in shell-like voids called lacunae. The cell processes of osteocytes are connected to each other through communication tunnels called canaliculi\(^7\). The region surrounding the lacuna is called the perilacunar region. The bone matrix surrounds the perilacunar region. Understanding the age-related changes in the microarchitecture of bone is essential in the study of the ability of the bone to sense macroscopic mechanical loading.

![Microscopic image of an osteocyte within its lacuna](image)

**Figure 3.** Microscopic image of an osteocyte within its lacuna\(^9\).
Previous studies have measured strain in vivo, during exercise\textsuperscript{(10,11)}. These studies were performed on the mid-shaft of the tibia at the macroscopic level. Additional in vitro studies have been conducted to study the effects of mechanical loading on bone in compression and tension, but the models vary enormously\textsuperscript{(12)}.

Measuring applied strains on the osteocytes ex vivo is also difficult with current measuring techniques. The avenues for studying the behavior of osteocytes under mechanical loading ex vivo have been compiled in recent articles\textsuperscript{(13,14)}. These avenues include imaging microscopic strain patterns in cortical bone subjected to a macroscopic loading equivalent to 2000 microstrain\textsuperscript{(14)} (Figure 4).

\textbf{Figure 4.} Cortical bone image showing microstructural strain patterns after the bone was subjected to 2000 microstrain (left) and a graph showing the global strains versus the strain concentration which parallels published values for amplified strain factors in cortical bone (right)\textsuperscript{(14)}.
A study presented current \textit{ex vivo} techniques, including scanning electron microscopy, which is utilized in this study \textsuperscript{(13)}. Imaging techniques are an important part in studying osteocytes. These images help researchers visualize the microarchitecture of bone. The \textit{in vivo} studies of bone assume that bone is a homogeneous or continuum material with constant material properties throughout its structure. Bone is not a continuum material and the inhomogeneity can be directed at the microarchitecture, namely the lacuna and the canaliculi. Therefore, understanding localized strains at the lacunar-canicular interface will provide more information of the deformation that acts on the osteocyte \textsuperscript{(15)}.

Previous studies \textsuperscript{(15, 16)} have investigated tissue strain amplification at the lacuna through finite element modeling. Both studies included ten canaliculi in the model and previously published values for the modulus of elasticity. The values for the modulus of elasticity varied between the perilacunar and non-perilacunar regions. The perilacunar region is the region surrounding the lacuna. This area is of interest because it may be hypo- or hyper mineralized compared to the rest of the bone matrix. This property of the perilacunar region may affect the strain sensed by the osteocyte \textsuperscript{(17)}. An example of one of the finite element studies is shown in Figure 5.
Figure 5. Finite element models comparing osteocyte microarchitecture with a perilacunar modulus of elasticity of 15 GPa (left) and a perilacunar modulus of elasticity of 35 GPa (right). Ten canaliculi were modeled in both. The fringe plot shows the variation in strain throughout the osteocyte microarchitecture. 

Utilizing the finite element method is essential to understanding microscopic strains because of the difficulty of measuring strain in vivo. Finite element modeling of the osteocyte microarchitecture includes using material properties, osteocyte microarchitecture, and appropriate boundary conditions to solve constitutive equations, using quasi-static numerical methods. The solutions to the constitutive equations, equations based on the material properties, help visualize the behavior of the model. The output studied in this case is the strain sensed at the lacunar-canalicular interface. Also, finite element modeling is an efficient means to evaluate data through parametric studies. The finite element model in the Bonivtch 2007 study was parametric in that the width of the perilacunar region, the diameter of the canaliculi, and the modulus of elasticity were all varied in the model without having to recreate geometry in every computer simulation run. Building from the previous investigations by quantitatively analyzing the
microarchitecture of murine bone will provide the information needed to build a more physiologically accurate computer model. The objective of this study was to analyze the age effects of the microarchitecture of murine bone and utilize the data found to construct more anatomically correct finite element models. The results from these studies will lead to a better understanding of the in vivo strains experienced at the lacuna-canalicular interface surrounding the osteocyte.
CHAPTER 2
METHODS

The methods for analyzing the microarchitecture of the murine bone included understanding the source of the samples, how the samples were prepared, imaged, and quantitatively analyzed. The finite element methods are also described.

Animal Subjects

The bone samples in the study came from mice from The Jackson Laboratory. The specific strain of mouse chosen was C57BL/6J. This particular strain of mouse is the most widely used inbred strain, an advantage because the mice show similar characteristics, including a low susceptibility to tumors and resistance to seizures. This strain is used in many different types of studies, including cardiovascular biology, developmental biology, diabetes and obesity, genetics, immunology, and neurobiology. An image of one of the mice is shown in Figure 6.

Figure 6. C57BL/6J Mouse from the Jackson Laboratory.
Ten murine bone samples were extracted from ten separate mice. The mice were all female. There were 5 old samples (2-years-old) and 5 young samples (5-months-old). All samples were blinded to the researcher.

Sample Preparation

Ten murine bone samples were prepared using the following protocol adapted from Kubek et al. 2010. The samples were fixed in 10% Neutral Buffered Formalin (NBF) for three days. The samples were stored in 70% ethanol (EtOH). The samples were then dehydrated using the procedure shown in Table 1.

Table 1. Murine bone dehydration procedure\(^{(18)}\).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Duration (hours)</th>
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<tbody>
<tr>
<td>70% EtOH</td>
<td>1</td>
</tr>
<tr>
<td>95% EtOH</td>
<td>1</td>
</tr>
<tr>
<td>95% EtOH</td>
<td>1</td>
</tr>
<tr>
<td>100% EtOH</td>
<td>2</td>
</tr>
<tr>
<td>100% EtOH</td>
<td>1</td>
</tr>
<tr>
<td>100% EtOH</td>
<td>1</td>
</tr>
<tr>
<td>100% EtOH</td>
<td>1</td>
</tr>
<tr>
<td>Xylene</td>
<td>1</td>
</tr>
<tr>
<td>Xylene</td>
<td>1</td>
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</tbody>
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Each specimen was then infiltrated using a mixture of 8.4 mL of Methylmethacrylate (Sigma-Aldrich M55909, St. Louis, MO), 1.4 mL of Dibutylphthalate (Sigma-Aldrich 524980, St. Louis, MO), 100 μL of Poly(ethylene glycol) (Hampton Research HR-2-603, Aliso Viejo, CA), and 70 mg of Benzoyl Peroxide (Sigma-Aldrich 179981, St. Louis, MO).
MO). After shaking for two days in sealed containers the samples were ready to be embedded. The infiltration solution was poured out of the containers and 20 mL of embedding solution consisting of 168 mL of Methylmethacrylate (Sigma-Aldrich M55909, St. Louis, MO), 28 mL of Dibutylphthalate (Sigma-Aldrich 524980, St. Louis, MO), 2mL of Poly(ethylene glycol) (Hampton Research HR-2-603, Aliso Viejo, CA), and 800 mg of Benzoyl Peroxide (Sigma-Aldrich 179981, St. Louis, MO) was added to the vials. As a catalyst, 66 μL of DMT (N,N-dimethyl-p-toluidine (MP Biomedicals 157844, Solon, OH) was added to each vial. The vials containing the bone samples were capped and stored at 4 °C for 5 days. Preparation of the resin-embedded samples began after using a band saw to cut the samples down to size in order to fit into the SEM. An image of a resin-embedded sample is shown in Figure 7.

![Image](image_url)

**Figure 7.** Murine bone resin-embedded sample.

The samples required sanding and acid etching to prepare for imaging. The samples were polished using sand paper and rinsed with DI water between each sanding as follows; 400 Grit (DUR WD-400), 600 Grit (DUR WD-600), 800 Grit (DUR-WD 800), and 1200 Grit (DUR-WD 1200). After an ultrasonic wash, the samples were then
polished on Gold Pad using high viscosity DIAMAT PC Diamond Suspension as follows; 0.25 micron Diamond Suspension (PC-0110-250) for 5 minutes, 0.1 micron Diamond Suspension (PC-0110-250) for 5 minutes, and 0.05 micron Diamond Suspension (PC-0105-250) for 5 minutes. After another ultrasonic wash, the samples were acid etched to remove a layer of bone matrix.

To acid-etch the samples, each was immersed in 35% phosphoric acid (Acros Organics 201145000, Pittsburgh, PA) for 10 seconds, then washed with DI water for 3 minutes, 3 times. The samples were each then rinsed with 5.25% Sodium Hypochlorite Reagent for 15 minutes. The samples were again washed with DI water for 3 minutes, 3 times. The samples were then put into a container with a loose cover and dried overnight. The samples were then gold sputter coated.

**Imaging Techniques**

After the samples were gold sputter-coated, they were imaged using scanning electron microscopy (SEM). Each of the images was taken in the mid-shaft of the femur. Using the SEM (Philips XL30, Electron Optics, FEI, Hillsboro, OR), a minimum of five images were captured in both the periosteal region and endosteal region of the bone (Figure 8) at 3000x magnification and 10 images were captured at 300x magnification.
An SEM image showing the difference between the endosteal and periosteal region can be found in Figure 9.

**Figure 8.** Diagram of long bone from the National Cancer Institute showing the endosteal region, closest to the bone marrow, and the periosteal region, closest to outer part of the bone (20).
Figure 9. SEM image of the endosteal and periosteal regions of bone. Photo courtesy of Yixia Xie, Department of Oral Biology and Craniofacial Sciences, University of Missouri - Kansas City.

The images taken at 3000x magnification were used to investigate the number of canaliculi, the diameter of the canaliculi, and the lacunar cross sectional area. The images taken at 300x magnification were used to investigate lacunar density. Examples images of both 3000x magnification and 300x magnification can be found in Figures 10 and 11.
Figure 10. Representative SEM image captured at 3000x magnification, used to measure the number of canaliculi, the diameter of canaliculi, and lacunar cross sectional area.

Figure 11. Representative SEM image captured at 300x magnification, used to measure lacunar density.
ImageJ software was used to analyze the images (ImageJ 1.45k, NIH, United States). The “Cell Counter” plugin was used to calculate the number of canaliculi and the lacunar density. Lacunar density was calculated as the number of lacunae per area. In order to calculate the lacunar cross sectional area, the area was modeled as an idealized ellipse in ImageJ. The minor and major axes were also recorded. An example image of the lacunar cross sectional area can be found in Figure 12.

Figure 12. SEM image of a sample from the old periosteal region, showing an idealized ellipsoid as the lacunar cross sectional area measurement.

Several rules were generated to ensure image analysis accuracy which included:

(1) count the canaliculi within 2 to 3 microns of the base, (2) measure 1 micron from the
base for the canalicular diameter, (3) do not calculate diameters on the branches of the
canaluli, and (4) no ‘stringy’ canaliculi chosen for the canalicular diameter
measurements. ‘Stringy’ can be defined in this reference as canaliculi that were very thin
or had a very thick bottom in comparison to the thickness at the tip. These canaliculi were
not chosen for the canalicular diameter measurements. At least five canalicular diameter
measurements were taken for each sample. More than five measurements were taken if
the image quality was good enough to take more than five. An example image of the
canaliculi count is shown in Figure 13. Each of the blue dots represents a count. ImageJ
automatically sums all the counts.

Figure 13. SEM image of a sample from the old periosteal region with the
count of the number of canaliculi represented by the blue dot and number near
each of the canaliculi.
Image Analysis Techniques

The statistical method of choice to determine significant differences was a Two-Sample t-test. There were 4 treatments tested; the number of canaliculi, the diameter of the canaliculi, the size of the lacuna, and the lacunar density. These were compared by the age of the murine bone sample and the location of the lacuna in the bone. The table below shows the tests for each of the analyses performed (Table 2).

Table 2. Tests performed for each treatment.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Treatments</th>
<th>Treatments</th>
<th>Treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number of Canaliculi</strong></td>
<td><strong>Canaliculi Diameter</strong></td>
<td><strong>Lacunar Size</strong></td>
<td><strong>Lacunar Density</strong></td>
</tr>
<tr>
<td>Old vs. Young</td>
<td>Old vs. Young</td>
<td>Old vs. Young</td>
<td>Old vs. Young</td>
</tr>
<tr>
<td>Old Endosteal Region vs. Young Endosteal Region</td>
<td>Old Endosteal Region vs. Young Endosteal Region</td>
<td>Old Endosteal Region vs. Young Endosteal Region</td>
<td></td>
</tr>
<tr>
<td>Old Periosteal Region vs. Young Periosteal Region</td>
<td>Old Periosteal Region vs. Young Periosteal Region</td>
<td>Old Periosteal Region vs. Young Periosteal Region</td>
<td></td>
</tr>
<tr>
<td>Old Endosteal Region vs. Old Periosteal Region</td>
<td>Old Endosteal Region vs. Old Periosteal Region</td>
<td>Old Endosteal Region vs. Old Periosteal Region</td>
<td></td>
</tr>
<tr>
<td>Young Endosteal Region vs. Young Periosteal Region</td>
<td>Young Endosteal Region vs. Young Periosteal Region</td>
<td>Young Endosteal Region vs. Young Periosteal Region</td>
<td></td>
</tr>
</tbody>
</table>

Finite Element Analysis Techniques

Six finite element models were created based on the geometry measurements determined from the image analysis. A summary of the geometry data can be found in Table 3.
Table 3. Summary of the data used to generate the finite element models. Each of the values represents the mean for each of the data sets. The number of canaliculi was rounded to the nearest whole number to generate the geometry.

<table>
<thead>
<tr>
<th>Summary of FEA Geometry Data</th>
<th>Old</th>
<th>Young</th>
<th>Old Endosteal</th>
<th>Young Endosteal</th>
<th>Old Periosteal</th>
<th>Young Periosteal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of canaliculi</td>
<td>45</td>
<td>52</td>
<td>43</td>
<td>47</td>
<td>46</td>
<td>56</td>
</tr>
<tr>
<td>Number of canaliculi - corrected (+2/5)</td>
<td>63</td>
<td>73</td>
<td>60</td>
<td>66</td>
<td>64</td>
<td>78</td>
</tr>
<tr>
<td>Canaliculi Diameter (μm)</td>
<td>0.2771</td>
<td>0.2641</td>
<td>0.2651</td>
<td>0.2603</td>
<td>0.2909</td>
<td>0.268</td>
</tr>
<tr>
<td>Area (μm²)</td>
<td>88.6</td>
<td>92.7</td>
<td>89</td>
<td>87.6</td>
<td>88.3</td>
<td>97.7</td>
</tr>
<tr>
<td>Major Axis (μm)</td>
<td>13.95</td>
<td>15.59</td>
<td>14.27</td>
<td>14.49</td>
<td>13.57</td>
<td>16.7</td>
</tr>
<tr>
<td>Minor Axis (μm)</td>
<td>8.22</td>
<td>7.66</td>
<td>8.09</td>
<td>7.77</td>
<td>8.35</td>
<td>7.56</td>
</tr>
<tr>
<td>Lacunar Density (lacunae/mm²)</td>
<td>1492</td>
<td>1345</td>
<td>1492</td>
<td>1345</td>
<td>1492</td>
<td>1345</td>
</tr>
<tr>
<td>Non-perilacunar dimensions (x-dir,μm)</td>
<td>28.91</td>
<td>31.53</td>
<td>29.16</td>
<td>30.83</td>
<td>28.63</td>
<td>32.22</td>
</tr>
<tr>
<td>Non-perilacunar dimensions (y-dir,μm)</td>
<td>23.18</td>
<td>23.6</td>
<td>22.98</td>
<td>24.11</td>
<td>23.41</td>
<td>23.08</td>
</tr>
<tr>
<td>Non-perilacunar dimensions (z-dir,μm)</td>
<td>23.18</td>
<td>23.6</td>
<td>22.98</td>
<td>24.11</td>
<td>23.41</td>
<td>23.08</td>
</tr>
<tr>
<td>Forced displacement (μm)</td>
<td>0.05872</td>
<td>0.06306</td>
<td>0.05832</td>
<td>0.06166</td>
<td>0.05726</td>
<td>0.06444</td>
</tr>
</tbody>
</table>
The number of canaliculi were corrected by a factor of 0.4 to account for hidden canaliculi unable to be accounted for through the two dimensional SEM images. This factor was determined in house using a three dimensional reconstruction of several osteocytes from a confocal image stack. The forced displacement in Table 3 is the amount of displacement required to induce 2000 microstrain on the model. The osteocyte lacuna was modeled as an ellipsoid revolved around the major axis. There are similar published representations of the osteocyte lacuna \(^{15}\). The thickness of the perilacunar region (the area surrounding the lacuna) was held constant throughout all simulations at 5 micrometers. The dimensions of the non-perilacunar region were calculated based on the lacunar density.

The density calculated for the old murine samples was used for the old periosteal model and the old endosteal model. Similarly, the density calculated for the young murine samples was used for the young periosteal model and the young endosteal model. The thickness of the non-perilacunar region was determined by the size of the width of the region. The same dimensions were used for both the width and the depth. Because the osteocyte lacuna was modeled as a revolved ellipsoid, this method was determined as viable. An image of example geometry (from old murine sample) can be found in Figure 14.
Figure 14. Geometry generated from the old murine image analysis results using half symmetry.

The geometry, quasi-static simulation, and post processing were all executed using Abaqus 6.12 (Simulia, Dassault Systemes, Providence, RI). The actual analysis was performed using one eighth symmetry on the model. The material properties used in the analysis can be found in Table 4 below.
Table 4. Summary of finite element material property data.

<table>
<thead>
<tr>
<th>Summary of FEA Material Data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Non-perilacunar Modulus of Elasticity (N/μm²)</td>
</tr>
<tr>
<td>Perilacunar Modulus of Elasticity (N/μm²)</td>
</tr>
<tr>
<td>Density (kg/μm³)</td>
</tr>
<tr>
<td>Poisson’s Ratio</td>
</tr>
</tbody>
</table>

The modulus of elasticity for the non-perilacunar region and the perilacunar region for both the old and young models was determined through a nano-indentation study of material properties (21). The modulus of elasticity for the old murine bone was also used for the old periosteal and old endosteal models. Likewise, the modulus of elasticity determined for the young murine sample was used for the young periosteal and young endosteal models. The Poisson’s ratio of 0.3 was used for each of the models which has been used in previous studies, as well (15). The material density was found in a published article (22) that investigated the bone density from several different mice, including the strain of mouse used in this study.

The boundary conditions for the analysis included a forced axial displacement perpendicular to the long axis in Figure 14. The displacement was calculated to model an
overall structural strain of 2000 microstrain, based on an investigation of \textit{in vivo} strain during human rigorous physical activity \cite{10}. The nodes on the two long sides were fixed using symmetry boundary conditions, so that the results could be mirrored to show overall results. The nodes on the side opposing the displacement boundary conditions were fixed in all degrees of freedom.

A mesh density study was conducted in order to determine the optimal number of nodes and elements to use for accurate convergence in the model. In a particular study about mesh density on a simplified femoral model \cite{23}, it was shown that linear tetrahedral elements allowed for results more closely related to theoretical ones when compared to hexahedral quadratic elements. The article also showed that linear tetrahedral elements are highly dependent on the density of the mesh. This study utilizes linear tetrahedral elements. The mesh was gradually made finer along fifteen steps. A graph of the mesh density study can be found in Figure 15. As one can see from the graph, linear tetrahedral elements are in fact dependent on the mesh density in determining accuracy in the results from the model.
The lacunar strain was measured as the maximum first principal strain in the model. Mesh convergence was determined accurate when the lacunar strain differed by less than 2% \(^{15}\). The optimal density was measured between 682560 nodes and 799650 nodes. All the models constructed had a mesh density on this same order.

Each of the simulations took approximately 20 minutes to run on 144 CPU’s each. The average lacunar strain was calculated for each model as well as strain amplification ratios. Fringe plots were also prepared to visualize the strain contours and concentrations.

The fringe plots used in the displacement analyses are in units of micrometers with the highest value for displacement in red and the lowest value for displacement in blue.

Figure 15. Mesh density analysis results.
Similarly, the fringe plots for the strain comparisons are in units of microstrain. The highest value for microstrain is red, while the lowest value for strain is in blue. These fringe plots all have the same numerical breakdown for ease of comparison.

This lacunar strain was used to find the strain amplification factor which was used to compare the various models. The strain amplification factor is calculated by dividing the strain from the finite element model by 2000 microstrain, the strain measured during rigorous physical activity\(^{(10)}\).
CHAPTER 3
RESULTS

Image Analysis Results

There were statistically significant findings (p < 0.05) from the SEM images. The number of canaliculi decreased from the young samples to the old samples by 14.1% (p < 0.001). The number of canaliculi also decreased by 18% in the old periosteal region when comparing it to the young periosteal region (p < 0.001). In the young sample specifically, the number of canaliculi also decreased by 15.5% from the young periosteal region to the young endosteal region (p = 0.001). There were also statistically significant changes in canaliculi diameter with age and location within the bone. The canaliculi diameter increased with age by 5% (p < 0.001), and the canaliculi diameter increased by 8.5% from the young periosteal region to the old periosteal region (p < 0.001). When investigating the old sample specifically, the canaliculi diameter decreased by 8.9% from the periosteal region to the endosteal region (p < 0.001). The cross sectional area of the lacunar also changed significantly in some of the comparisons. The lacunar cross sectional area decreased by 9.6% from the young periosteal region to the old periosteal region (p = 0.034). The lacunar cross sectional area also decreased by 10.3% in the young sample, specifically a decrease from the young periosteal region to the young endosteal region (p = 0.027). The lacunar density increased with age by 10.9% (p = 0.001). Graphs of all of the data can be found in Figure 16 through Figure 31 below. A graph that is marked with an asterisk signifies significant changes. The young and old samples include both the endosteal and periosteal regions.
The results of the number of canaliculi can be found in Figures 16 through 20.

**Figure 16.** Number of canaliculi comparing young and old samples.

**Figure 17.** Number of canaliculi comparing samples in the young endosteal region and old endosteal region.

**Figure 18.** Number of canaliculi comparing samples in the young periosteal region and old periosteal region.

**Figure 19.** Number of canaliculi comparing samples in the old periosteal region and the old endosteal region.

**Figure 20.** Number of canaliculi comparing samples in the young periosteal region and the young endosteal region.
The results of the canaliculi diameter can be found in Figures 21 through 25.

**Figure 21.** Canaliculi diameter comparing young and old samples.

**Figure 22.** Canaliculi diameter comparing samples in the young endosteal region and old endosteal region.

**Figure 23.** Canaliculi diameter comparing samples in the young periosteal region and old periosteal region.

**Figure 24.** Canaliculi diameter comparing samples in the old periosteal region and the old endosteal region.

**Figure 25.** Canaliculi diameter comparing samples in the young periosteal region and the young endosteal region.
The results of the analysis of the lacunar area can be found in Figures 26 through 30.

**Figure 26.** Lacunar area comparing young and old samples.

**Figure 27.** Lacunar area comparing samples in the young endosteal region and old endosteal region.

**Figure 28.** Lacunar area comparing samples in the young periosteal region and old periosteal region.

**Figure 29.** Lacunar area comparing samples in the old periosteal region and the old endosteal region.

**Figure 30.** Lacunar area comparing samples in the young periosteal region and the young endosteal region.
The results of the analysis of the lacunar density can be found in Figure 31.

![Lacunar Density: Young vs Old](image)

**Figure 31.** Lacunar density comparing young and old samples.

A summary of all of the results is shown in Table 5 below.
Table 5. Summary results of the image analysis.

<table>
<thead>
<tr>
<th></th>
<th>Number of canaliculi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young vs. Old</td>
<td>14.1% Decrease from Young to Old</td>
</tr>
<tr>
<td>YP vs. OP</td>
<td>18.0% Decrease from YP to OP</td>
</tr>
<tr>
<td>YP vs. YE</td>
<td>15.5% Decrease from YP to YE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Canaliculi Diameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young vs. Old</td>
<td>5.0% Increase from Young to Old</td>
</tr>
<tr>
<td>YP vs. OP</td>
<td>8.5% Increase from YP to OP</td>
</tr>
<tr>
<td>OP vs. OE</td>
<td>8.9% Decrease from OP to OE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Lacunar Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>YP vs. OP</td>
<td>9.6% Decrease from YP to OP</td>
</tr>
<tr>
<td>YP vs. YE</td>
<td>10.3% Decrease from YP to YE</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Lacunar Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young vs. Old</td>
<td>10.9% Increase from Young to Old</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Acronym Key</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE</td>
<td>Old Endosteal</td>
</tr>
<tr>
<td>OP</td>
<td>Old Periosteal</td>
</tr>
<tr>
<td>YE</td>
<td>Young Endosteal</td>
</tr>
<tr>
<td>YP</td>
<td>Young Periosteal</td>
</tr>
</tbody>
</table>

Finite Element Model Results

The results of the finite element analysis are the focus of this section. In order to provide confidence in the models, it was important to check the displacement plots against the prescribed calculated displacement values. The displacement in each of the models matches with the prescribed calculated displacements assigned in each of the models. Each of the displacement plots for the models can be found below. The units in the legend are in micrometers which are the base units for all models and analyses in this study. The values in red show the maximum displacement for each of the models.
Figure 32. Displacement plot of the old bone finite element model.

Figure 33. Displacement plot of the young bone finite element model.

Figure 34. Displacement plot of the old endosteal bone finite element model.
Figure 35. Displacement plot of the young endosteal bone finite element model.

Figure 36. Displacement plot of the old periosteal bone finite element model.

Figure 37. Displacement plot of the young periosteal bone finite element model.
Part of this study involved using experimental values from nano-indentation of the perilacunar and non-perilauncar regions of the bone \(^{(21)}\). This is different than a previous study \(^{(15)}\) which varied the values for the modulus of elasticity as a guesstimate rather than from measured values. The current investigation included comparing each of the models to the counterpart with a constant modulus of elasticity in both the perilacunar and non-perilacunar regions in the bone to isolate the results attributed to changes in microarchitecture alone. Images of each of the comparisons can be found in Figures 38 through 43 below. A summary table of the results of the average lacunar strain in each of the models can be found in Table 6. As shown in the figures below, the changes in the modulus of elasticity affect the strain patterns in the non-perilacunar region more than the perilacunar region.

![Figure 38. Average lacunar strain shown (in microstrain) in the old model with different values of the modulus of elasticity for the perilacunar and non-perilacunar regions (left) and in the old model with a constant modulus of elasticity (right).](image)
Figure 39. Average lacunar strain shown (in microstrain) in the young model with different values of the modulus of elasticity for the perilacunar and non-perilacunar regions (left) and in the young model with a constant modulus of elasticity (right).

Figure 40. Average lacunar strain shown (in microstrain) in the old endosteal model with different values of the modulus of elasticity for the perilacunar and non-perilacunar regions (left) and in the old endosteal model with a constant modulus of elasticity (right).
Figure 41. Average lacunar strain shown (in microstrain) in the young endosteal model with different values of the modulus of elasticity for the perilacunar and non-perilacunar regions (left) and in the young endosteal model with a constant modulus of elasticity (right).

Figure 42. Average lacunar strain shown (in microstrain) in the old periosteal model with different values of the modulus of elasticity for the perilacunar and non-perilacunar regions (left) and in the old periosteal model with a constant modulus of elasticity (right).
Figure 43. Average lacunar strain shown (in microstrain) in the young periosteal model with different values of the modulus of elasticity for the perilacunar and non-perilacunar regions (left) and in the young periosteal model with a constant modulus of elasticity (right).
Table 6. Comparison of osteocyte models based on the modulus of elasticity in the perilacunar and non-perilacunar regions.

<table>
<thead>
<tr>
<th>Model</th>
<th>Measured Modulus</th>
<th>Same Modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Strain amplification factor</td>
<td>Strain amplification factor</td>
</tr>
<tr>
<td>Old</td>
<td>2.15</td>
<td>2.14</td>
</tr>
<tr>
<td>Young</td>
<td>2.19</td>
<td>2.16</td>
</tr>
<tr>
<td>Old Endosteal</td>
<td>1.89</td>
<td>1.89</td>
</tr>
<tr>
<td>Young Endosteal</td>
<td>2.19</td>
<td>2.16</td>
</tr>
<tr>
<td>Old Periosteal</td>
<td>2.10</td>
<td>2.09</td>
</tr>
<tr>
<td>Young Periosteal</td>
<td>2.19</td>
<td>2.16</td>
</tr>
</tbody>
</table>

Table 6 shows the value of the strain amplification factor when comparing between the models. The lacunar strain decreases in all of the models when a constant modulus of elasticity is used across the model. The young model, the young endosteal model, and the young periosteal model have the same results in each case. The old model, the old endosteal model, and the old periosteal model all have varying results, but are all lower than the values of the young models.

The next part of the study involved comparing the average lacunar strain values between models. The measured values for the modulus of elasticity were used in this analysis. Therefore, the young models had a different modulus of elasticity compared to the old models. Also, the modulus of elasticity in the non-perilacunar region varied from the perilacunar region in all models.
The main focus of this study involved understanding the changes in average lacunar strain with age. The old model is compared with the young model in Figure 44. The old endosteal model is compared with the young endosteal model in Figure 45. The old periosteal model is compared with the young periosteal model in Figure 46. Another important comparison is across the bone regions. Therefore, the old endosteal model is compared with the old periosteal model in Figure 47. Likewise, the young endosteal model is compared with the young periosteal model in Figure 48. A summary table of the results can be found in Table 6.

Figure 44. Average lacunar strain shown (in microstrain) in the old model (left) and in the young model (right).
Figure 45. Average lacunar strain shown (in microstrain) in the old endosteal model (left) and in the young endosteal model (right).

Figure 46. Average lacunar strain shown (in microstrain) in the old periosteal model (left) and the young periosteal model (right).
Figure 47. Average lacunar strain shown (in microstrain) in the old endosteal model (left) and in the old periosteal model (right).

Figure 48. Average lacunar strain shown (in microstrain) in the young endosteal model (left) and in the young periosteal model (right).
Table 6 contains the complete summary of the strain amplification factors. When comparing the old murine model to the young murine model, the strain amplification decreased with age from 2.19 in the young model to 2.15 in the old model. The strain amplification in the young endosteal model was greater when comparing to the old endosteal model at 2.19 and 1.89, respectively. Similar results were found in the young periosteal model compared to the old periosteal model with strain amplification values of 2.19 and 2.10, respectively. The young endosteal model and the young periosteal model showed the same results of a strain amplification factor of 2.19. The old endosteal and old periosteal models differed in the results with strain simplification factors of 1.89 and 2.10, respectively.
CHAPTER 4
DISCUSSION

Image Analysis Discussion

In a previous study on the microarchitecture of bone\textsuperscript{(24)}, it was found that in aged rats (80-weeks-old), there are fewer lacunae and canaliculi when compared to their young counterparts. The data from the present study matched the data describing the number of canaliculi. In both studies the number of canaliculi decreased. However, there was a difference in the trend concerning the number of lacunae. It was found in the present study that the number of lacunae increased with age by 10.9%.

It is important to mention that one main difference between the previous study and this one is that in the previous study, the samples were taken from the mandible (or the jaw) of the rat where loading conditions are different than those in the femur, where the samples from this study were taken. Species variation may account for the differences in results. Also, the images analyzed in this study were captured with scanning electron microscopy and were therefore two-dimensional images. Because only the lacunae were seen, there was no concrete evidence to show that an osteocyte inhabited the lacuna. A histological study on the murine bone will be conducted in future studies in order to find out the percentage of lacunae that actually house an osteocyte.

Finite Element Model Discussion

The strain amplification factors for the young murine samples, including the young endosteal and the young periosteal samples (all at 2.19) was greater than that of
the old endosteal and old periosteal samples (1.89 and 2.10, respectively). It is interesting to note that the old endosteal model had a much lower strain amplification factor than any other model. It is well known that bone resorption occurs primarily on the endosteal surface of the bone, while bone remodeling occurs on the periosteal surface. The results from this study follow that knowledge and show, especially in the old model, that age changes, with respect to mechanical loading, have a great effect on the ability of bone to remodel. When looking at moving averages of the elements in the finite element study that were used to calculate the strain amplification factor (APPENDIX A), it is easy to see the trend in the old endosteal sample.

This study supports the hypothesis that young bone is able to lay down new bone better than older bone because of greater strain at the osteocyte lacuna-canaliculi interface. This study is an advancement in the current knowledge of osteocyte behavior. While previous studies\(^{(15,16)}\) investigated lacunar strain, these studies did not include actual tested perilacunar and non-perilacunar material properties. Also, these same previous studies included only 10 canaliculi, while the current study focused on a more anatomically correct number of canaliculi in both the endosteal and periosteal regions.

After further investigation, it was found that changing the placement of the canaliculi also changed the average lacunar strain values. The fidelity of the current model can be increased by further investigating the effect of changing the placement of the canaliculi. For future studies, performing a finite element analysis where all the canaliculi are equidistant from one another will prove to be beneficial in gaining a better understanding as to how the average lacunar strain is affected by this placement.
The current model is idealized and the placement of the canaliculi was at the discretion of the researcher. A set of foundational instructions or requirements for building and analyzing an osteocyte network would also be beneficial for future studies. The material properties used in this study were from murine samples that were fixed and resin-embedded\(^{(21)}\). For future studies, fresh samples will be tested and that data will help provide a better representation of the behavior of the microarchitecture under loading conditions.

This study also utilized linear tetrahedral elements based on a study of a simplified femur that also used linear tetrahedral elements\(^{(23)}\). Since that study was published, new technologies in the area of finite element modeling have become more advanced. There are new element formulations, like the “improved surface stress formulation” tetrahedral element offered by Abaqus that may supplement the current methods and aid in building a more efficient parametric osteocyte finite element model.

Although the presented models utilize idealized geometry, they are arguably the most physiological accurate in regards to lacunar size and density, and canalicular number and diameter to date. Future models could include actual non-idealized geometry, however this type of modeling does not lend itself to the type of parametric study that was conducted here.
CHAPTER 5
CONCLUSION

This study serves as one of the building blocks in advancing knowledge of the magnitude of strain applied to the osteocyte with aging. Through physical imaging of murine femur bones, it was shown that there are significant changes in the microarchitecture of murine bone with regards to age. There is also a difference in the lacunar strain with regards to age and bone location as shown in the finite element models. Also shown, the old murine sample had lower average values of strain at the lacuna canaliculi interface, supporting the hypothesis that the response to load is decreased in older bone due to decreased strain on the osteocyte in older bone. Another finding support to the observation that bone is resorbed more in the endosteal region compared to the periosteal region. The knowledge gained from this study will be used to advance research into the prevention and cure of osteoporosis that occurs with aging.
Figure A1. Magnitude of maximum lacunar strain of the elements used to calculate the strain amplification factor in the models with the same modulus of elasticity for the entire model.
Figure A2. Magnitude of maximum lacunar strains of the elements used to calculate the strain amplification factor in the models with measured values for the modulus of elasticity for the perilacunar and non-perilacunar regions.
Appendix B

**Two-Sample T-Test and CI: Count, Age**

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>50</td>
<td>44.50</td>
<td>8.41</td>
<td>1.2</td>
</tr>
<tr>
<td>Y</td>
<td>50</td>
<td>51.8</td>
<td>10.0</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Difference = \( \mu (O) - \mu (Y) \)
Estimate for difference: -7.32
95% CI for difference: (-10.99, -3.65)
T-Test of difference = 0 (vs not =): T-Value = -3.96 P-Value = 0.000 DF = 95

**Figure B1.** Canaliculi count - young versus old.

**Two-sample T for Count**

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE</td>
<td>25</td>
<td>42.96</td>
<td>9.14</td>
<td>1.8</td>
</tr>
<tr>
<td>YE</td>
<td>25</td>
<td>47.48</td>
<td>9.82</td>
<td>2.0</td>
</tr>
</tbody>
</table>

Difference = \( \mu (OE) - \mu (YE) \)
Estimate for difference: -4.52
95% CI for difference: (-9.92, 0.88)
T-Test of difference = 0 (vs not =): T-Value = -1.68 P-Value = 0.099 DF = 47

**Figure B2.** Canaliculi count – young endosteal versus old endosteal.
Appendix B (continued)

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td>25</td>
<td>46.04</td>
<td>7.47</td>
<td>1.5</td>
</tr>
<tr>
<td>YP</td>
<td>25</td>
<td>56.16</td>
<td>8.25</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Difference = μ (OP) − μ (YP)
Estimate for difference: -10.12
95% CI for difference: (-14.51, -5.63)
T-Test of difference = 0 (vs not =): T-Value = -4.54  P-Value = 0.000  DF = 47

**Figure B3.** Canaliculi count – young periosteal versus old periosteal.

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE</td>
<td>25</td>
<td>42.96</td>
<td>9.14</td>
<td>1.8</td>
</tr>
<tr>
<td>OP</td>
<td>25</td>
<td>46.04</td>
<td>7.47</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Difference = μ (OE) − μ (OP)
Estimate for difference: -3.08
95% CI for difference: (-7.83, 1.67)
T-Test of difference = 0 (vs not =): T-Value = -1.30  P-Value = 0.199  DF = 46

**Figure B4.** Canaliculi count – old endosteal versus old periosteal.
Appendix B (continued)

**Two-Sample T-Test and CI: Count, Location**

Two-sample T for Count

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>YE</td>
<td>25</td>
<td>47.48</td>
<td>9.52</td>
<td>2.0</td>
</tr>
<tr>
<td>YP</td>
<td>25</td>
<td>56.16</td>
<td>8.28</td>
<td>1.7</td>
</tr>
</tbody>
</table>

Difference = mu (YE) - mu (YP)
Estimate for difference: -8.68
95% CI for difference: (-13.85, -3.51)
T-Test of difference = 0 (vs not =): T-Value = -3.38, P-Value = 0.001, DF = 46

**Figure B5.** Canaliculi count – young endosteal versus young periosteal.

**Two-Sample T-Test and CI: Width, Age**

Two-sample T for Width

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>334</td>
<td>0.2771</td>
<td>0.0447</td>
<td>0.0024</td>
</tr>
<tr>
<td>Y</td>
<td>296</td>
<td>0.2641</td>
<td>0.0458</td>
<td>0.0027</td>
</tr>
</tbody>
</table>

Difference = mu (O) - mu (Y)
Estimate for difference: 0.01298
95% CI for difference: (0.00588, 0.02008)
T-Test of difference = 0 (vs not =): T-Value = 3.59, P-Value = 0.000, DF = 614

**Figure B6.** Canaliculi diameter – old versus young.
Appendix B (continued)

**Two-Sample T-Test and CI: Width, Location**

Two-sample T for Width

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE</td>
<td>178</td>
<td>0.2651</td>
<td>0.0430</td>
<td>0.0032</td>
</tr>
<tr>
<td>YE</td>
<td>148</td>
<td>0.2603</td>
<td>0.0456</td>
<td>0.0037</td>
</tr>
</tbody>
</table>

Difference = mu (OE) - mu (YE)

Estimate for difference: 0.00476

95% CI for difference: (-0.00497, 0.01449)

T-Test of difference = 0 (vs not =): T-Value = 0.96 P-Value = 0.336 DF = 305

**Figure B7.** Canaliculi diameter – old endosteal versus young endosteal.

**Two-Sample T-Test and CI: Width, Location**

Two-sample T for Width

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OP</td>
<td>156</td>
<td>0.2909</td>
<td>0.0426</td>
<td>0.0034</td>
</tr>
<tr>
<td>YP</td>
<td>148</td>
<td>0.2680</td>
<td>0.0459</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

Difference = mu (OP) - mu (YP)

Estimate for difference: 0.02291

95% CI for difference: (0.01290, 0.03291)

T-Test of difference = 0 (vs not =): T-Value = 4.51 P-Value = 0.000 DF = 297

**Figure B8.** Canaliculi diameter – old periosteal versus young periosteal.
Appendix B (continued)

**Figure B9.** Canaliculi diameter – old endosteal versus old periosteal.

**Two-Sample T-Test and CI: Width, Location**

Two-sample T for Width

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE</td>
<td>178</td>
<td>0.2651</td>
<td>0.0430</td>
<td>0.0032</td>
</tr>
<tr>
<td>OP</td>
<td>156</td>
<td>0.2909</td>
<td>0.0426</td>
<td>0.0034</td>
</tr>
</tbody>
</table>

Difference = mu (OE) - mu (OP)
Estimate for difference: -0.02583
95% CI for difference: (-0.03506, -0.01660)
T-Test of difference = 0 (vs not =): T-Value = -5.50  P-Value = 0.000  DF = 327

**Figure B10.** Canaliculi diameter – young endosteal versus young periosteal.

**Two-Sample T-Test and CI: Width, Location**

Two-sample T for Width

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>YE</td>
<td>148</td>
<td>0.2603</td>
<td>0.0456</td>
<td>0.0037</td>
</tr>
<tr>
<td>YP</td>
<td>148</td>
<td>0.2680</td>
<td>0.0459</td>
<td>0.0038</td>
</tr>
</tbody>
</table>

Difference = mu (YE) - mu (YP)
Estimate for difference: -0.00768
95% CI for difference: (-0.01814, 0.00278)
T-Test of difference = 0 (vs not =): T-Value = -1.45  P-Value = 0.149  DF = 293
Appendix B (continued)

**Figure B11.** Lacunar cross sectional area – old versus young.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>50</td>
<td>88.6</td>
<td>13.7</td>
<td>1.9</td>
</tr>
<tr>
<td>Y</td>
<td>50</td>
<td>92.7</td>
<td>16.3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

Difference = \( \mu (O) - \mu (Y) \)
Estimate for difference: -4.04
95% CI for difference: (-10.01, 1.94)
T-Test of difference = 0 (vs not =): T-Value = -1.34 P-Value = 0.183 DF = 95

**Figure B12.** Lacunar cross sectional area – old endosteal versus young endosteal.

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>OE</td>
<td>25</td>
<td>99.0</td>
<td>13.8</td>
<td>2.8</td>
</tr>
<tr>
<td>YE</td>
<td>25</td>
<td>97.6</td>
<td>14.9</td>
<td>3.0</td>
</tr>
</tbody>
</table>

Difference = \( \mu (OE) - \mu (YE) \)
Estimate for difference: 1.33
95% CI for difference: (-6.83, 9.49)
T-Test of difference = 0 (vs not =): T-Value = 0.33 P-Value = 0.744 DF = 47
Figure B13. Lacunar cross sectional area – old periosteal versus young periosteal.

Figure B14. Lacunar cross sectional area – old endosteal versus old periosteal.
Appendix B (continued)

**Figure B15.** Lacunar cross sectional area – young endosteal versus young periosteal.

<table>
<thead>
<tr>
<th>Location</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>YE</td>
<td>25</td>
<td>87.6</td>
<td>14.9</td>
<td>3.0</td>
</tr>
<tr>
<td>YP</td>
<td>25</td>
<td>97.7</td>
<td>16.3</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Difference = μ(YE) - μ(YP)
Estimate for difference: -10.11
95% CI for difference: (-19.00, -1.23)
T-Test of difference = 0 (vs not =): T-Value = -2.29 P-Value = 0.027 DF = 47

**Figure B16.** Lacunar density – old versus young.

<table>
<thead>
<tr>
<th>Age</th>
<th>N</th>
<th>Mean</th>
<th>StDev</th>
<th>SE Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>O</td>
<td>49</td>
<td>0.001492</td>
<td>0.000178</td>
<td>0.000025</td>
</tr>
<tr>
<td>Y</td>
<td>50</td>
<td>0.001345</td>
<td>0.000240</td>
<td>0.000034</td>
</tr>
</tbody>
</table>

Difference = μ(O) - μ(Y)
Estimate for difference: 0.000148
95% CI for difference: (0.000063, 0.000232)
T-Test of difference = 0 (vs not =): T-Value = 3.48 P-Value = 0.001 DF = 90
REFERENCE LIST


17. Lane NE, Yao W, Balooch M, Nalla RK, Balooch G, Habelitz S, Kinney JH, Bonewald, LF (2006) Glucocorticoid-treated mice have localized changes in trabecular bone material properties and osteocyte lacunar size that are not observed in placebo-treated or estrogen-deficient mice. J.Bone Miner.Res. 21, 466-476.


VITA

Teri Lynne Cline was born in Independence, Missouri on the 17th of July 1983 to Kelly Ann Cline and Charles Edgar Cline, Jr. She has an older brother, Charles. She attended Lee’s Summit High School in Lee’s Summit, MO where she lettered in Music. She graduated high school in May of 2001. She went on to complete leadership development missionary training from Teen Mania Ministries in December of 2002, having worked on missions projects in Tyler, TX and Rome, Italy. She completed another missionary trip to Chiang Mai, Thailand in 2005. She completed her Bachelor’s Degree in Mechanical Engineering from the University of Missouri – Kansas City in May of 2011. While a student there, she co-founded the local university chapter of Engineers Without Borders, supporting a project to provide a clean water system to Kilometer 6 in the Dominican Republic. She was also President of the Society of Women Engineers during her junior and senior years. She received several academic scholarships during her undergraduate career. She interned at the U.S. Geological Survey in Lee’s Summit, MO during her junior and senior years, as well. During the summer of 2011, she joined the Advanced Engineering Simulations and Analysis team at Honeywell in Kansas City, MO as a Finite Element Analysis Engineer. She will obtain her Master’s Degree in Mechanical Engineering from the University of Missouri – Kansas City on the 17th of May 2013. Upon completion of her degree requirements, Ms. Cline plans to continue her career at Honeywell as a Finite Element Analysis Engineer. During her free time, Ms. Cline likes to play tennis, travel, enjoy time with her friends and family, play violin and guitar, and enjoy the outdoors.