MATERIAL DIFFERENCES IN EQUINE CORTICAL AND TRABECULAR BONE

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ABSTRACT

Material Differences in Equine Cortical and Trabecular Bone

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A greater understanding of bone materials would be beneficial in creating more accurate computer models and in the making of biomedical products involving bone. This study set out to determine whether cortical and trabecular bone are two separate materials, or whether they are the same material with a variance in porosity. To answer this question, samples were taken from different sections of the equine metacarpus, underwent densitometry analysis and were statistically analyzed. The majority of results suggest that the material is the same between varying densities of bone and thus the same between cortical and trabecular bone. These particular results are consistent with current standard practices. However, in several instances specifically regarding high porosity trabecular bone, a variance in density was found likely indicating a combination of differences in both material and architecture. Further studies should be done with specific focus on material variances to high porosity trabecular bone to improve the accuracy of computer models and general knowledge.

Keywords: Material Differences, Bone Density, Equine, Equivalent Thickness of Aluminum (ETA)

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TABLE OF CONTENTS

LIST OF TABLES

Table Page

LIST OF FIGURES

Figure Page

CHAPTER 1

Introduction

Relevance

Bones are rigid organs that contribute to the endoskeleton of all vertebrates and are vital to the structure and function of the body. The have been analyzed and studied for thousands of years and our knowledge of their structure, function, and composition is still growing. With a greater understanding of these aspects of bone, many things could be accomplished.

Broken bones are extremely common with over 6.8 million cases in the United States each year [13]. For U.S. military men and women, a major cause of amputation of limbs is from complex fractures [14]. Although the basis of how bones repair fracture is widely known, further knowledge of exact material properties could greatly benefit the healing and remodeling process specifically with regard to improving the accuracy of computer models.

Using a computer to model bone is becoming increasingly common and can be an extremely effective tool. Development of advanced computer based models has permitted researchers to explore a vast area of musculoskeletal science from the complex stimuli at the cellular level to the mechanical behavior of heterogeneous skeletal structures [15]. Additionally, computer modeling has greatly helped the progress of modern bone implant development [15]. However,

it is not known if trabecular bone, also known as cancellous or spongy bone, should be treated as a different material than cortical bone, also known as compact bone, or if it is the same material with a lower density. A deeper understanding of the material characteristics of bone would greatly improve the accuracy and efficiency of computer models benefiting the production of bone implants and modeling of the skeletal system in general.

Bone Structure and Function

The skeletal system is a functional unit found in many animals and consists of bone, cartilage, ligaments and tendons. Each part plays its own vital purpose in the support and function of the body. Bone provides a framework to support the body, protection of vital organs, reservoirs for calcium homeostasis, blood cell formation, and attachment sites for muscles [3]. It is an osseous tissue meaning it is calcified tissue that provides bone with its hard structure, allowing it to support the demands of the body [8]. Cartilage acts like a cushion separating diarthrodial joints providing bone on bone interactions with an almost frictionless surface for joints. Tendons and ligaments aid in the movement of the body and prevent unwanted movement of the joints. Tendons connect muscle to bone while ligaments directly connect bone to bone.

The types of bones in the body of a vertebrate consist of long bones, short bones, flat bones, and irregular bones. Long bones, such as the femur, phalanges and tibia, are generally longer than they are wide, while short bones, such as the talus and capitate, as the name implies, are much shorter. Flat

bones, like the occipital, parietal and nasal, are somewhat flat in structure whose principal requirement is either extensive protection or the provision of broad surfaces for the attachment of muscle. Irregular bones, such as the vertebrae, sacrum, and coccyx, serve various purposes throughout the body and do not fall under the category of long, short or flat bones.

As seen in Figure 1 below, the vertebrate skeleton is mainly composed of two types of tissue: compact (cortical) bone that forms the outer layer of bones, and spongy (trabecular) bone found inside bones.

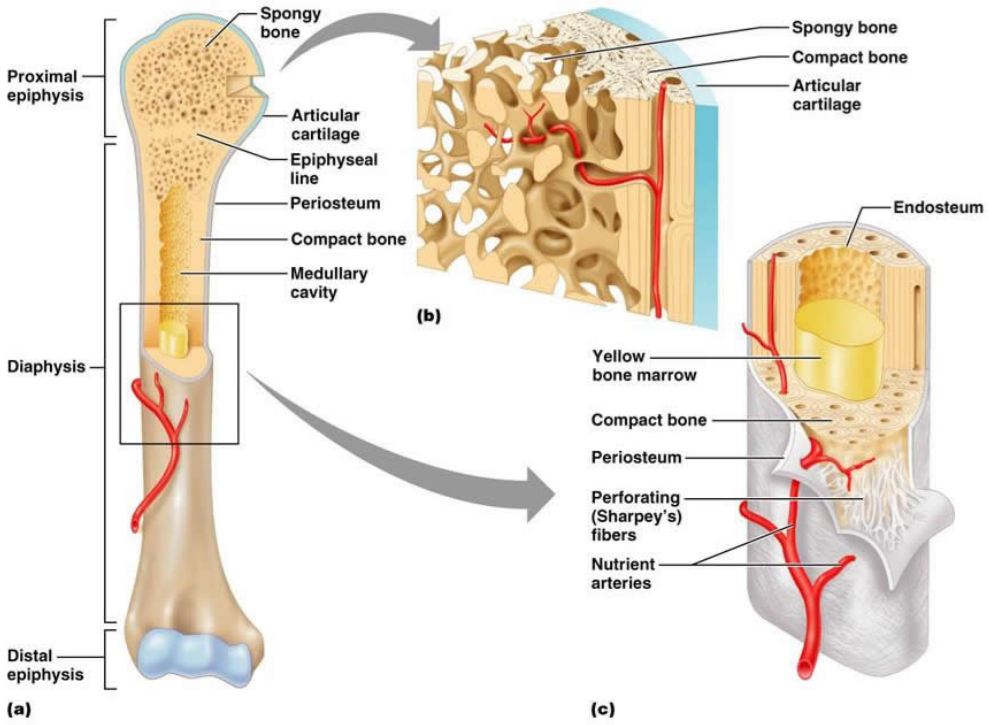


Figure 1: Components of an adult human long bone [9].

Compact bone is generally found throughout the diaphysis of the bone comprising of only about 5%-10% of the bone where trabecular bone comprises

about 75%-95% of a bone and is usually found in the bone epiphyses and houses the bone morrow [10]. The inner surface of the bone is lined with endosteum while the outer surface is covered by periostium. The epiphyses are located on either side of the diaphysis and are separated by the epiphyseal line. The epiphyseal line, commonly referred to as the growth plate, is the location of prenatal, postnatal, and adolescent growth but remains as a “scar” throughout life, delineating where the growth plate used to be.

Bone is usually formed in layers or lamellae containing collagen fibers, proteoglycans, and other substances [1]. In compact bone, lamellae form concentric cylinders called osteons that generally run parallel to the bone axis. In the center of the osteon is a passageway, called the Haversian canal, for nerves and blood vessels.

An osteon is formed from bone remodeling, in which bone resorption proceeds radially outward from a blood vessel as seen in Figure 3 below. The cells responsible for bone resorption are called osteoclasts. Bone deposition is preformed by osteoblasts proceeding radially inward and ending at the Haversian canal. Throughout the remodeling process a small percentage of osteoblasts become trapped within the bone matrix forming osteocytes that help with communication throughout the bone. Osteoblasts also remain on the surface of the bone and are called bone-lining cells [10].

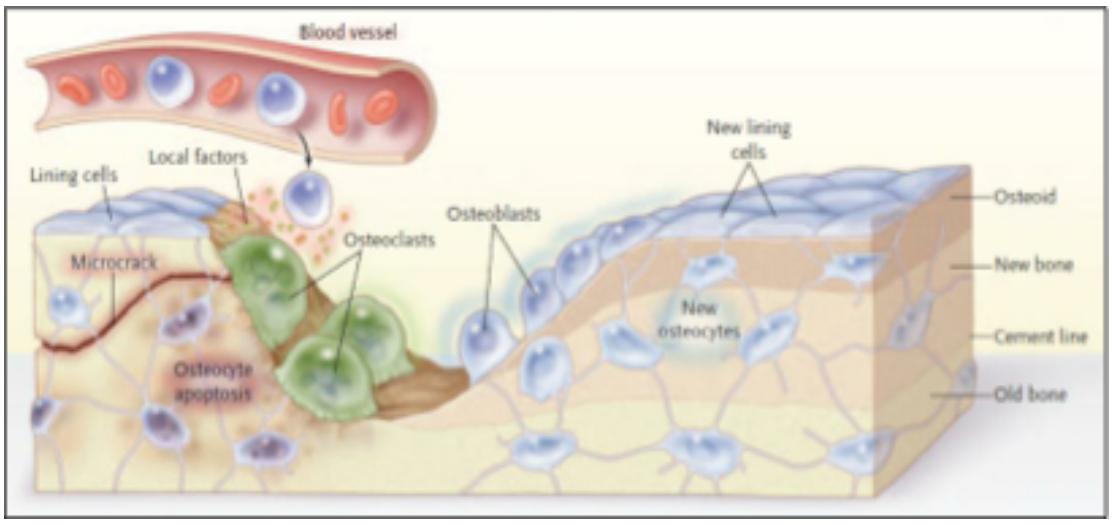


Figure 2: The human adult bone remodeling process [11].

When analyzing bone at the tissue level, it is necessary to distinguish the physical properties of the different regions in the bone. For instance, the anterior side of a human tibia may be denser than the posterior possibly as a result of cyclical loading that is more distributed along the front side of the bone as a result of walking and running. In general, axial sections of bone are categorized into the following regions: anterior (facing the front), posterior (facing the end), medial (facing the midline), and lateral (facing the sides of the body) [2].

Analysis of Bone

The density of bone can be determined in several different ways. A bone mineral density (BMD) test can be done using a dual-energy x-ray absorptiometry (DEXA) scan to measure how many grams of calcium and other bone minerals are packed into a specific segment of bone [6]. Although this test

is somewhat inaccurate and imprecise, it can be preformed on a live patient without an invasive test or surgery.

A more accurate method to determine the density of bone is to find the ash density. The ash density is the ratio between ash weight and volume of a sample of bone tissue and can be calculated by removing a sample of bone, measuring its volume, reducing the sample to ash in a furnace, and weighing the remaining ashes. Although effective, this method is usually only used post-mortem, as it requires a sample of bone to be fully removed. Ash density was calculated for each specimen used in the current study, however is not included in this discussion.

Another more accurate method, used in the current study, is to measure the density of bone using densitometry. Densitometry is the measuring of optical density of a substance by shining light on it and measuring its transmission. The exact methodology used in this study is described in greater detail in the methods section. Since osteoporosis is a lack of bone density, these methods are an efficient tool in understanding, analyzing and treating osteoporosis.

A key question in progressing out understanding of osteoporosis and the materials that make up bone in general, is whether cortical bone is just trabecular bone with fewer holes in it, or whether cortical and trabecular bone are two different materials entirely. The standard operating procedure in most computer modeling of bone is the assumption that bone is a constant material and that the

material properties should be changed by punching or filling holes in it. The variation in the material itself is often taken as just representing new osteons that have not fully mineralized. Recently, literature studies with the use of bisphosphonates (such as that by Christopher Hernandez [7]) show an increase in local mineralization as the bone turnover decreases. This paper will address this question of variance in bone material and its consequences.

Animal Models

Animal models are useful for studying similar tissues in the human body, including bone tissue. Rats are a common animal model used in studies due to their short life cycle, relatively lower cost, and small size. However, their relative lack of Haversian canal remodeling makes them poor candidates for modeling human bone remodeling [2]. Rabbits, on the other hand, undergo Haversian canal remodeling and have been used to model osteoporosis [2]. Their relatively small size and fast growth period result in less maintenance cost and less downtime, making them a more attractive model. However, their small size is inadequate for testing implant dynamics on a human scale [2]. Sheep are adequately large in size and may provide a more accurate model for osteoporosis in humans. More importantly, ovariectomized sheep have been shown in multiple studies as a feasible model for bone mineral density loss [2]. This suggests that they have similar hormonal effects to human females, and may be used to model postmenopausal osteoporosis.

This study utilized equine bone to more adequately understand the material properties of that bone, specifically whether or not there are material differences in cortical and trabecular bone. Equine bone was chosen due to a large existing data set with a wide range of both animal age and material density. The intent is that this data would provide an accurate model for use with human bone. In many ways, such as the behavior of fatigue, racehorse bone is different from human bone [10]. However, the general principles of bone materials are the same.

Study Objectives

The purpose of this study was to analyze equine bone to determine if cortical bone and trabecular bone are one material with different densities or two separate materials altogether. Samples were taken from the metacarpus bone of ten different equine specimens and placed on microradiographs for analysis. Densitometry measurements were taken from each sample where the ratio of bone volume over total volume (BV/TV), the number of bone pixels per picture, and the mean, median, mode, standard deviation, minimum, and maximum equivalent thicknesses of aluminum (ETA) were recorded. The ETA was used to represent each animal specimen's bone density in density measurements. Since aluminum has a similar atomic number to hydroxyapatite (the most abundant material in bone), it is an effective way to measure bone density [12]. The current

standard operating procedure in most computer modeling of bone is the assumption that bone is a constant material and by changing the density of the material we can change its material properties, however there have been few studies regarding the validity of this. It is expected that the results of the study will either validate this assumption or shed new light on the material properties of bone.

CHAPTER 2

Methods and Materials

Animal Preparation

Ten horses were used throughout the study, ranging in age from 5 months to 20 years. The left metacarpus (second, third and fourth metacarpal bones) was harvested from these ten horses within 12 hours postmortem. Each horse died at the Veterinary Medical Teaching Hospital at the University of California Davis from causes not involving the musculoskeletal system. The collection consisted of seven Thoroughbreds, two Arabians, and one Quarterhorse. Five horses were female, three were intact males, and two were castrated males. The age of each horse was determined from tooth wear or medical records with a range of 5 months to 20 years with a mean age of 6.7 years. Average skeletal maturity of the equine metacarpus occurs between 15 – 18 months for the distal epiphysis and before birth for the proximal epiphysis [16]. This implies that there were one to three horses (the 5 month old foal and the two yearlings) that had not reached skeletal maturity in the metacarpus bone used for this study. The bones were wrapped on saline-soaked paper towels and kept in plastic bags at -20°C when not being processed.

Equine Specimen Preparation

The equine 3rd metacarpal bone, also called the cannon bone, was divided into five proximodistal levels (numbered 1-5, from the distal end to the proximal end). Each proximmodistal level was then divided into 6 cortical sectors (dorsomedial, dorsal, dorsolateral, palmaromedial, palmar, and palmarolateral). In addition, the proximal levels 3, 4, and 5 also had, depending on the size of the horse, sectors from the 2nd metacarpal (medial splint bone), 4th metacarpal (lateral splint bone), and central, trabecular 3rd metacarpal (cannon bone). The distal level 1 also had specimens taken from the central sector. The Metacarpal bones used in the study are depicted below in Figure 3. The division of proximodistal levels and points representing sectors within each level are depicted in Figure 4.

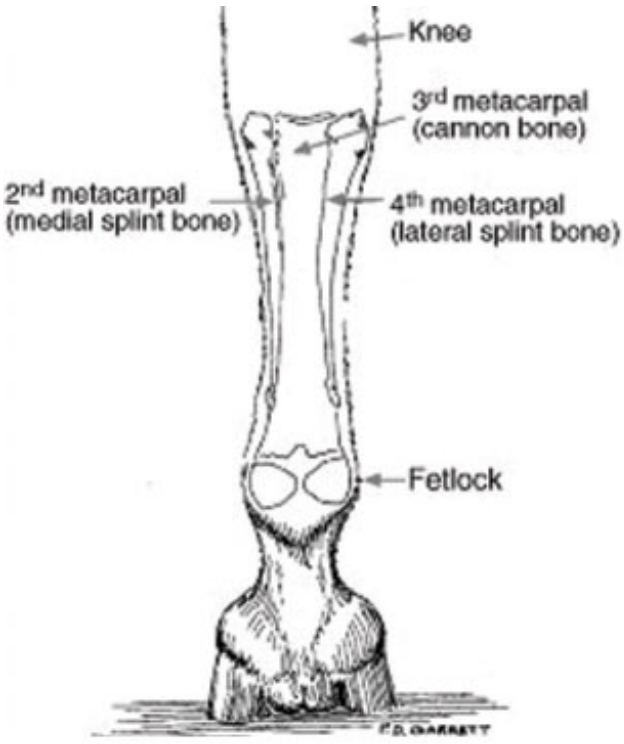


Figure 3: Equine metacarpal bone structure.

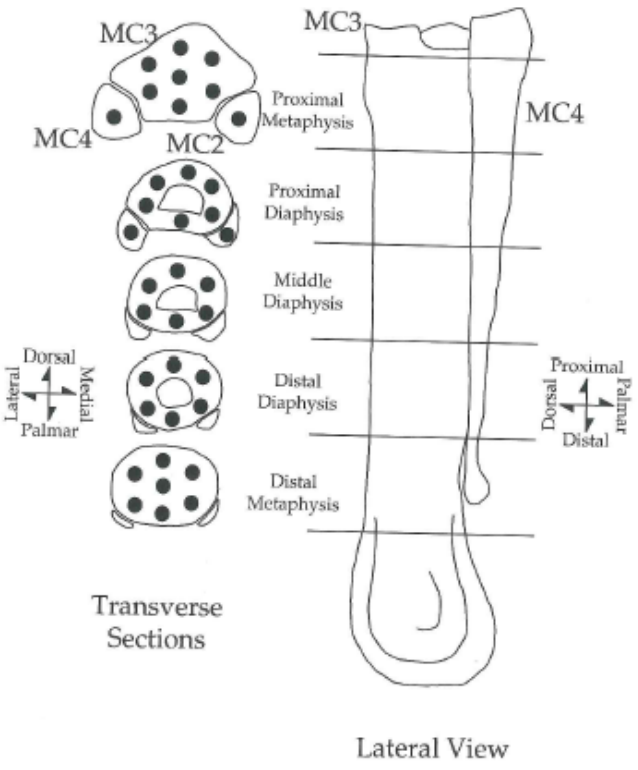


Figure 4: The division of proximodistal levels and the division of sectors within each level used for the current study.

Specimens were milled under cold deionized water irrigation on a lathe in order to obtain the largest possible right cylindrical specimen, oriented such that the length of the cylinder was parallel to the long axis of the metacarpus, from each sample. Mean final dimensions were length 15.73 (SD 3.18, range 7.16-20.46)mm, diameter 6.36 (SD 1.55, range 3.10-10.60)mm.

After mechanical testing (see Les et al., J Orthop Res 12:822-833, 1994 [5]), the largest fragment from each specimen was subjected to drying and

ashing. The next largest fragment was embedded in polymethylmethacrylate, cut and ground to a 100-micron (+/-5 micron) thickness, and microradiographed by Faxitron, Hewlett-Packard in McMinnville Oregon, USA. The microlithography plate used was a Pol-edged HRP-la with an exposure time of 40 minutes at 20 kVp and 3mA. The stepwedge was made using Reynolds Wrap aluminum foil.

Imaging

To image the equine bone samples, an Olympus BX-41 Microscope and an attached Retiga EXi Q color camera (QImaging, Surrey, BC, Canada) were used. Images were taken at California Polytechnic University San Luis Obispo. Each anatomical sector on the microradiograph was imaged at 100x using the attached camera and its program, QCapture Pro (QImaging). The images were taken in an 8-bit black and white format to allow for more accurate histogram measurements. During the imaging process, the appropriate camera and microscope settings including light intensity, camera exposure, color scheme, focus, and magnification were selected for the entire microradiograph and remained constant between each image. When viewed through the microscope, the sample was ensured to be bright enough to be visible, however not so intense the bone characteristics were not visible. It was important to ensure each bone specimen was visible both through the microscope as well as through the imaging camera and software.

When imaging the specimens, each anatomical sector was broken into 4 quadrants: northeast, northwest, southeast, and southwest. The results from each quadrant were averaged in order to capture the variation between images from a single specimen. Each quadrant was imaged individually at 100x total magnification. An example of the quadrant breakdown is seen in Figure 5.

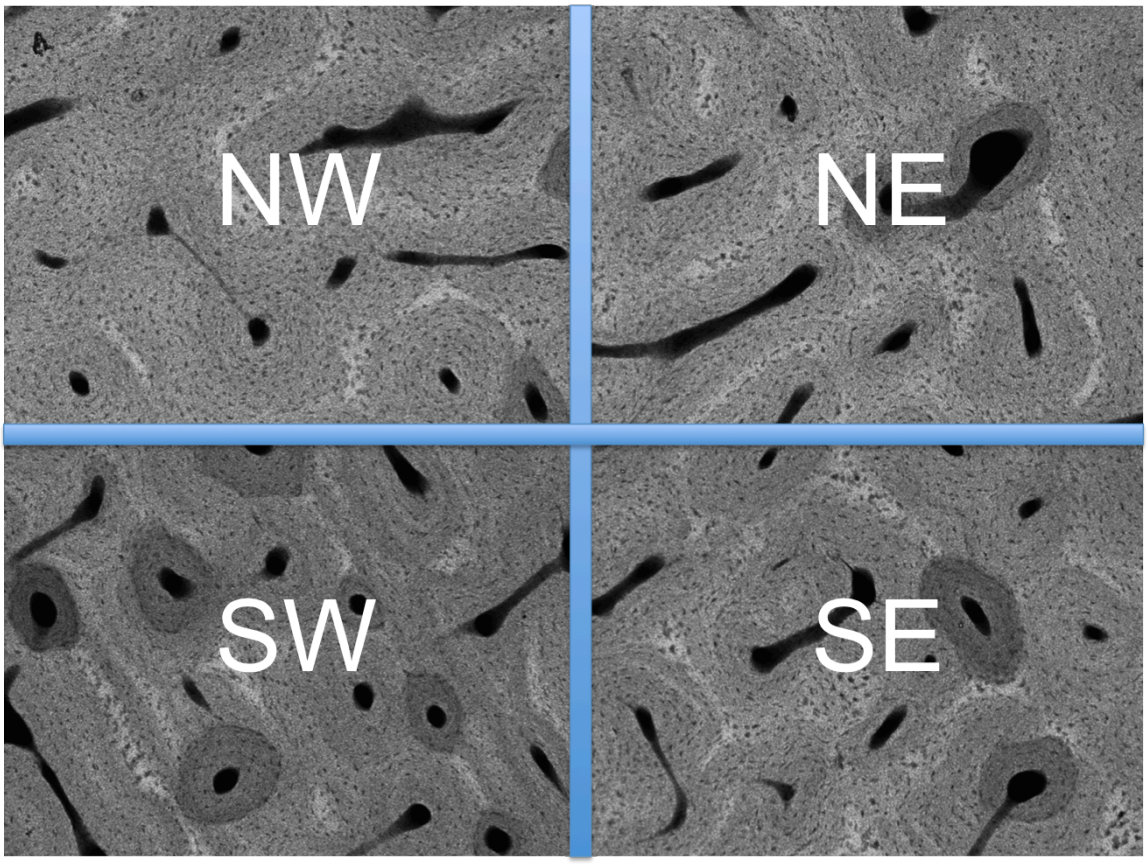


Figure 5: Image at 40x magnification showing the four quadrants that were individually imaged throughout the study.

Using the same intensity as the bone specimens, the aluminum stepwedge was imaged at a 100x total magnification with the camera and saved. The aluminum stepwedge is composed of 9 steps of different aluminum layers,

including a blank microradiograph with a zero thickness image, beginning with 0.0mm of aluminum increasing by 0.02mm with each step, for a total of 8 steps, Figure 6. Each step was only imaged a single time.

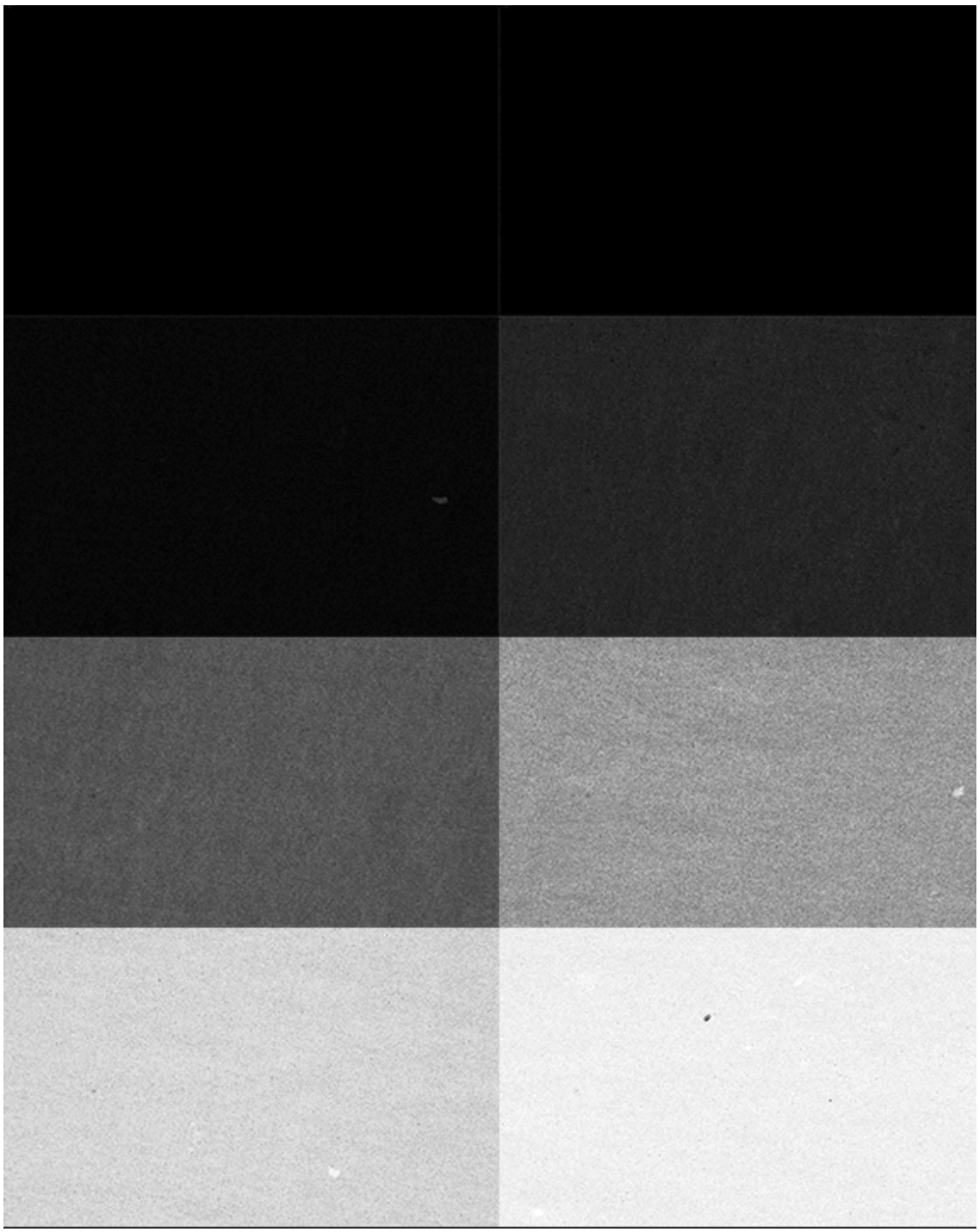


Figure 6: An example of the aluminum stepwedge, showing the increase in thickness. From left to right, top to bottom, the stepwedge demonstrated an increase from 0.0mm to 0.02mm, 0.06mm, 0.08mm, 0.1mm, 0.12mm and 0.14mm.

It was important to keep the brightness and intensity of the microscope the same throughout each individual bone specimen on the microradiograph as each specimen corresponded to its particular step wedge. If a bone specimen was not optimally visible at certain brightness when compared to another image on the same microradiograph, the brightness or intensity was changed and the stepwedge was imaged for each subsequent brightness or intensity.

Densitometry Analysis

To quantify bone density, the intensity of each bone specimen was compared to the intensity of its corresponding aluminum stepwedge. Each image, including both sectors and stepwedges were loaded into ImageJ (Wayne Rashband (NIH)) and analyzed with the histogram tool. Using this software tool, it was possible to produce a numerical pixel value corresponding to the intensity of light captured from the microscope. Aluminum is often used because it is repeatable in its makeup and X-ray attenuation properties. It is a consistent standard to which a bone section of known thickness can be compared; therefore it is possible to calculate a density for each specific bone specimen [4]. The numerical values for each specimen can be compared to the numerical values of the aluminum stepwedge, thereby producing a repeatable density value. Typically thicker and/or denser materials appear lighter, while thinner and/or less dense materials appear darker.

Each step in the stepwedge correlates with a thickness increase of 0.02mm. Using the histogram feature in ImageJ (Wayne Rashband (NIH)), it was possible to create a table of data, which includes the number of pixels at every pixel density from 0 to 255. The information gathered from the histogram was then exported into its corresponding Stepwedge Template Excel file. The mean pixel intensity (along with the median, mode and standard deviation pixel intensity) was determined for each level in the step wedge to serve as the key. An example of this can be seen in Table 1 below.

Table 1: *Mean Pixel Intensity to corresponding aluminum step wedge thickness.*

Level	Al Thickness	Mean Pixel Intensity	Median Pixel Intensity	Mode Pixel Intensity	SD Pixel Intensity
Camera Off	0	0	0	0	0
0	0	0	0	0	0
1	0.02	2.018036445	2	2	0.34926245
2	0.04	9.576594966	7	9	1.654339533
3	0.06	25.16005471	19	25	3.326552333
4	0.08	57.75438702	43	58	6.498784088
5	0.1	94.1046274	63	92	12.79052265
6	0.12	162.910932	108	164	18.34403374
7	0.14	219.7213631	185	221	9.244433961

The corresponding figure below (Figure 7) is a graph of stepwedge aluminum thickness verses the mean pixel intensity from the same example

stepwedge template. This particular graph portrays a non-gaussian (right skewed) distribution and, although different specimens showed slightly varied curves and distributions, this example portrays what was most commonly found. The reason for this shape may be due to the upper limit of .14mm thickness of aluminum. In addition, the attenuation of light is a function of the square of the distance of aluminum the photons have to travel to reach the photosensitive material that is then read by a digital imaging system.

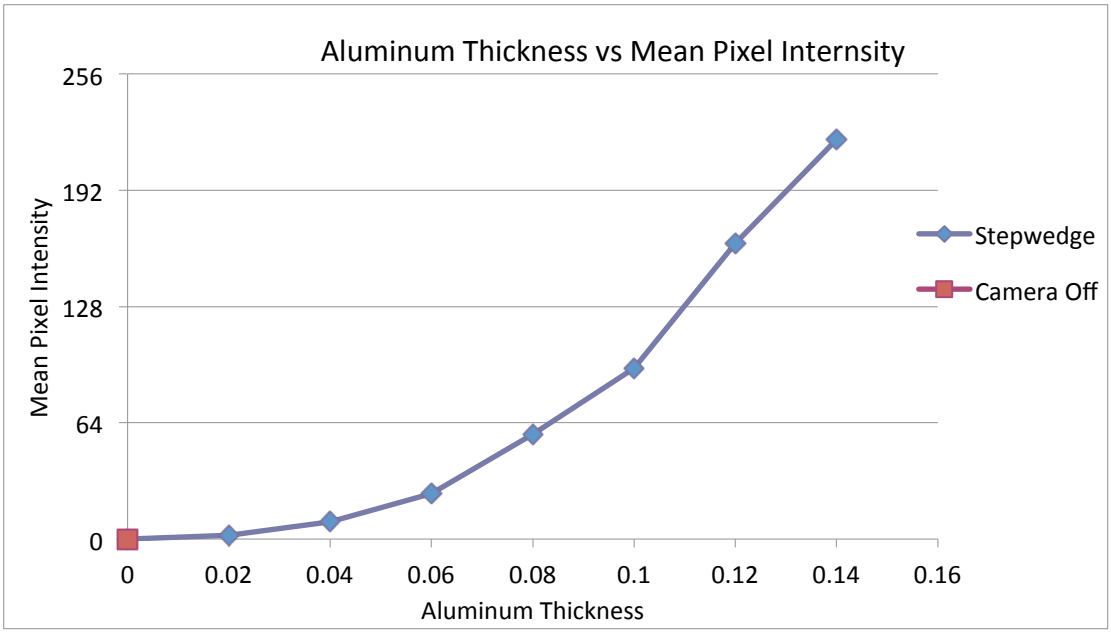


Figure 7: A example graph of aluminum thickness verses mean pixel intensity.

By graphing the aluminum thickness and the mean pixel intensity it is also possible to produce a 4-parameter sigmoid curve (Equation 1) in Sigma Plot 11.0 (Systat Software, Inc.) [4].

$$y = y_0 + \frac{a}{1+e^{-(\frac{x-x_0}{b})}}$$ Equation 1

In Equation 1, "y" represents the pixel intensity that would fall between 0 and 255 and "x" represents the thickness, in mm, of the aluminum stepwedge (in this system, between 0 and 0.14mm). "x_0" represents the x-value at which the curve (uncorrected for "y_0") reaches 50% of “a” allowing us to translate our curve from left to right until the curve fits assuming that below a certain thickness of aluminum, we are unable to tell completely black from almost completely black. “y_0" corrects the curve vertically until it fits, adjusting for electrical or background noise in the imaging system. “a” is the y asymptote and should generally be close to 255 in our system. Therefore, the general shape of the curve should be defined by “b”. The distribution of the pixel counts was used to determine the ETA of each specimen using the parameters of the stepwedge, using the Stepwedge Template and the Quantitative Densitometry Template seen in Appendix B. Based on the distribution of the stepwedge pixels, it is necessary to use the complex curve to achieve an accurate fit while also making the fewest assumptions.

With the parameters determined by the sigmoid curve, it was possible to determine the ETA of each specimen. Again, the histogram feature in ImageJ (Wayne Rashband (NIH)) was used to determine the density in each quadrant of a given specimen. From the histogram, the pixel count at each pixel density was

converted to ETA using the parameters calculated from the corresponding stepwedge.

Also included in this calculation was the porosity of each sector to represent the proportion of pixels in the image representing non-bone pixels [28]. The porosity was determined with the use of a “Merz Grid” seen in Figure 8 by counting all intersecting points that were not considered bone and subtracting that from 36, the number of possible points.

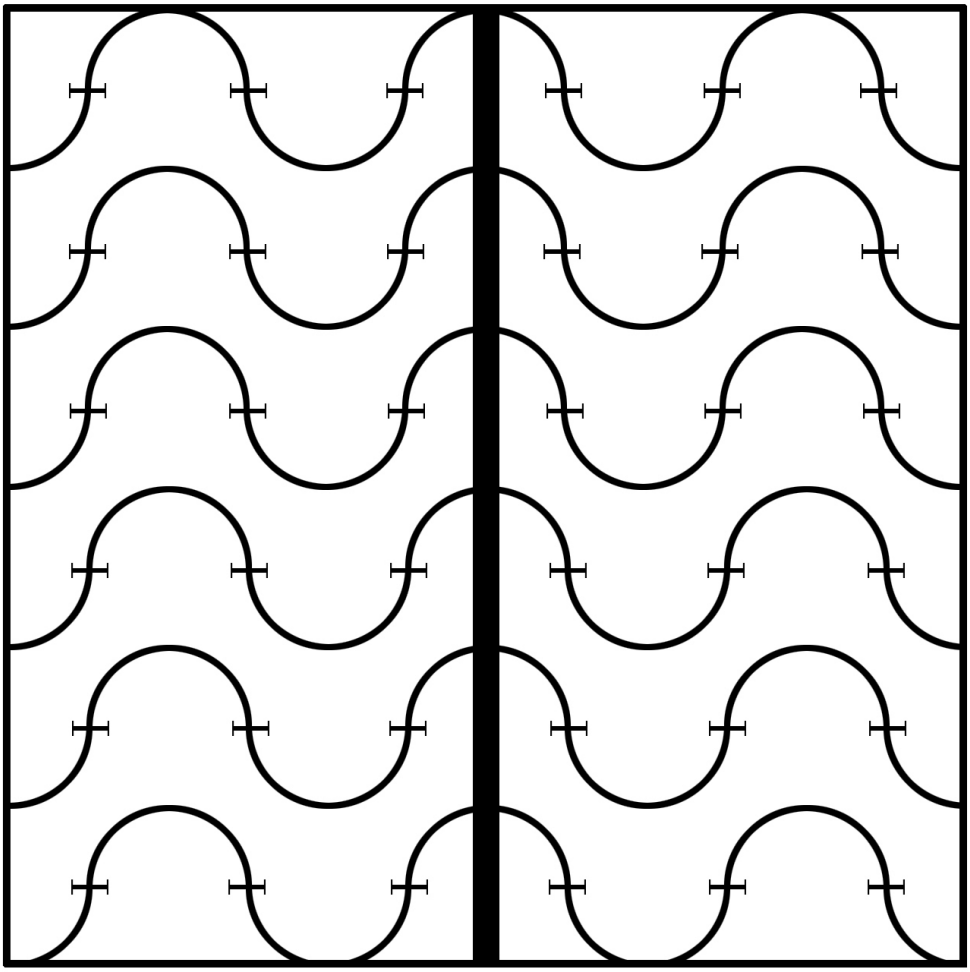


Figure 8: Merz Grid used to calculate BV/TV values.

The Merz Grid was used in Adobe Photoshop loaded into a stack and placed on top of the bone images and resized appropriately. This enabled us to quickly and accurately calculate the porosity of several bone images, one after another. With the use of the Quantitative Densitometry Template the overall bone volume over total volume (BV/TV) was then calculated from the porosity values. For each specimen the bone histogram values from all four quadrants were averaged.

Once the BV/TV values, averaged quadrant bone histogram values, calibration parameters from SigmaPlot, and other identification information such as the plate and specimen number were entered into the Quantitative Densitometry Template, the key output variables were saved into a Final Values template that can be seen in Appendix D. The key parameters consisted of the plate number, specimen, horse, age (in years), gender (male, female or gelding), breed, proximodistal level, radial sector, BV/TV, bone pixels, mean ETA, median ETA, Mode ETA, SD ETA, min ETA and max ETA.

All densitometry analysis was performed at California Polytechnic University San Luis Obispo.

Statistics

To analyze the collected data a mixed-model ANOVA was used following the guidelines of the SPSS Mixed Model Instructions provided by Dr. Clifford Les (Appendix E). A mixed model was chosen because there is a correlation between data from the same horse in several occasions. Unlike a repeated-measures analysis, a mixed model is also more forgiving when dealing with missing data. Repeated-measures analysis will typically drop the entire animal out of the analysis if a single value is missing.

Initially, basic statistics were preformed comparing both level and sector to the BV/TV and the mean ETA to get an idea of how the data generally behaved. Next, three separate analyses were preformed.

Analysis 1 used 2-way Repeated Measures ANOVAs on levels 1, and 5, sectors 1-6, 9 for each parameter. This was done to answer the questions (a) is the ETA (or its mean, median, maximum, BV/TV ect.) different overall between the proximal and distal ends of the bone, (b) Is the ETA different between different sectors (lumping proximal and distal levels), and (c) is the ETA different between different spots on the same level (strictly speaking, that's the interaction term).

Analysis 2 used a mixed-model ANOVA on sector while only looking at level 5. Level 5 was chosen because it encompasses every sector. Level 1 does not contain splint bones and would thus not contain sectors 7 or 8. This would

hopefully tell us the differences, if they exist, between material characteristics of bone at different locations on the same level.

Additionally, because there was a pattern of missing data, the sectors were grouped into three larger sets dubbed supersectors. The dorsal supersector consisted of sectors 1, 2, and 3 and portrayed the relatively tensile side of bone. The palmar supersector consisted of sectors 4, 5, and 6 and portrayed the compressive side of the bone. The central supersector consisted only of sector 9. For each parameter the average, standard deviation (SD), and coefficient of variation (CV) was calculated.

The CV is found dividing the mean from the standard deviation, as seen in Equation 2 below where σ, is the standard deviation and μ is the mean.

$$CV = \frac{\sigma}{\mu} \qquad \text{Equation 2}$$

The CV value represents the dispersion of data points in a data series around the mean. A CV value close to 0.0 means that the data is closely centered around the mean, higher CV values imply a widely-dispersed data set. Table 2 below displays the CV values for each parameter of every horse. “N/A” means that there was only one data point for that particular supersector and horse and thus not enough to give a trustworthy standard deviation and trustworthy CV.

Table 2. *Coefficient of Variation (CV) values for each Supersector of each horse.*

Horse	Supersector	BV/TV	Bone Pixels	Mean ETA	Median ETA	Mode ETA	SDETA	min ETA	max ETA
25	Dorsal	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Palmar	0.725	0.732	0.052	0.057	0.064	0.355	0.372	0.071
	Central	0.097	0.100	0.126	0.097	0.106	0.727	0.701	0.057
23	Dorsal	0.504	0.410	0.190	0.121	0.451	0.721	0.284	0.177
	Palmar	0.392	0.392	0.035	0.051	0.060	0.435	0.180	0.159
	Central	0.735	0.736	0.067	0.022	0.011	0.265	0.017	0.006
22	Dorsal	0.232	0.230	0.007	0.001	0.002	0.095	0.039	0.086
	Palmar	0.177	0.178	0.085	0.078	0.073	0.315	0.476	0.069
	Central	0.184	0.184	0.019	0.004	0.014	0.365	0.276	0.136
21	Dorsal	0.131	0.131	0.245	0.229	0.225	0.232	0.235	0.157
	Palmar	0.303	0.305	0.133	0.118	0.118	0.378	0.339	0.100
	Central	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
18	Dorsal	0.012	0.012	0.100	0.097	0.102	0.579	0.647	0.180
	Palmar	0.303	0.326	0.135	0.123	0.121	0.433	0.174	0.120
	Central	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
17	Dorsal	0.293	0.292	0.075	0.068	0.071	0.420	0.288	0.057
	Palmar	0.264	0.264	0.066	0.064	0.081	0.372	0.326	0.120
	Central	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
16	Dorsal	0.204	0.204	0.085	0.077	0.076	0.584	0.465	0.080
	Palmar	0.447	0.447	0.063	0.045	0.055	0.396	0.202	0.020
	Central	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15	Dorsal	0.153	0.153	0.125	0.099	0.080	0.322	0.417	0.110

	Palmar	0.044	0.045	0.138	0.135	0.131	0.256	0.314	0.104
	Central	0.780	0.773	0.634	0.620	0.642	0.319	0.417	0.472
14	Dorsal	0.251	0.248	0.142	0.125	0.121	0.392	0.319	0.205
	Palmar	0.182	0.182	0.067	0.062	0.070	0.140	0.201	0.100
	Central	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
11	Dorsal	0.373	0.372	0.071	0.080	0.116	0.571	0.446	0.074
	Palmar	0.376	0.372	0.078	0.063	0.496	0.290	0.461	0.076
	Central	0.616	0.620	0.121	0.083	0.079	0.364	0.646	0.134

Two-way repeated measure ANOVAs were then preformed on level and supersector using SigmaPlot and then rerun excluding the central supersector in order to pick up differences between compact bone supersectors. Fishers LSD (Least Square Difference) test was used for *post-hoc* analysis.

Next, X-Y scattergrams and linear regressions comparing BV/TV to each additional parameter (Bone Pixels, Mean ETA, Meadian ETA, Mode ETA, SD ETA, Min ETA, and Max ETA) were created for each specimen. This addresses the trabecular-vs-compact bone question as a continuous variable rather than as categorical. X-Y scattergrams and linear regressions comparing Mean, Median, and Mode ETA were also created.

Finally, plots comparing ETA values converted from pixel intensity and the numbers of pixels were created from a random sample of specimens. These

graphs were visually analyzed for a Gaussian curve, skew, and to ensure that there was no “max-out” phenomenon. A “max-out” phenomenon would occur if the range of thicknesses on the aluminum stepwedge that were too small.

CHAPTER 3

Results

Densitometry

This study was designed to ascertain whether cortical and trabecular bone are two different materials or if they are the same materials that only differ in porosity. In order to quantify bone material density, the intensity profile of each bone specimen was compared to the intensity profile of its corresponding aluminum step wedge. An example of this output can be seen in Figure 9 below.

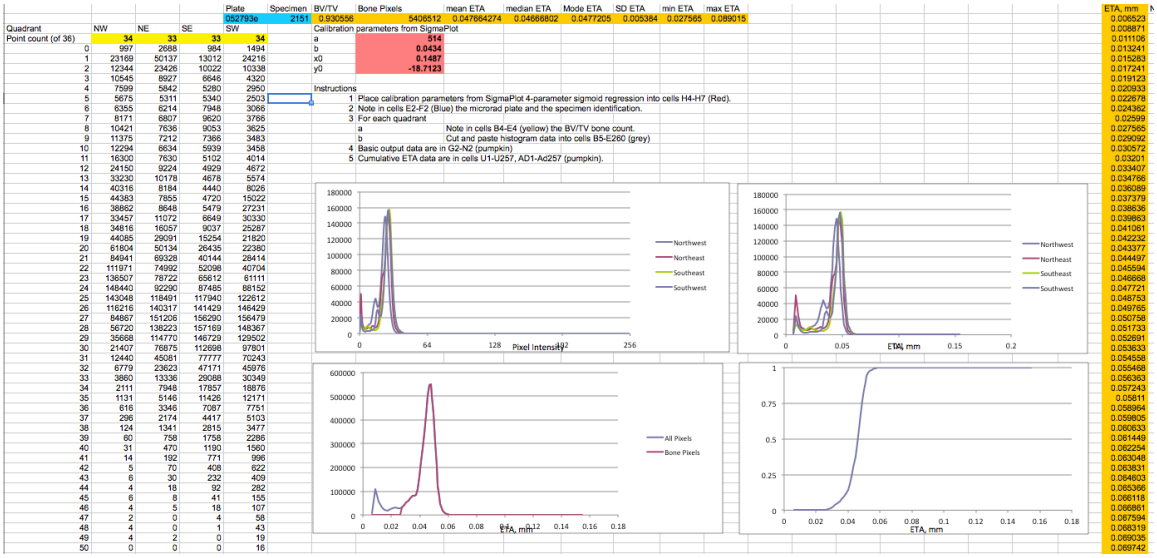


Figure 9: Pixel intensity for each quadrant of bone imaged (Left) with merz grid results (Yellow). Stepwedge calibration parameters from SigmaPlot (Red). Graphical display of pixel intensity and ETA (Bottom Center). Output used in analysis (Top Orange) for each corresponding specimen (Blue).

From the excel spreadsheet shown in Figure 9 above, output values of BV/TV, Bone Pixels, Mean ETA, Median ETA, Median ETA, Mode ETA, SD ETA, Minimum ETA, and Maximum ETA were created. A list of the final results for each specimen can be found in Appendix G.

Pre-Supersector Analysis 1

The first analysis used 2-way Repeated Measures ANOVAs on levels 1, and 5, sectors 1-6,9 for each parameter. There were nine parameters; BV/TV, Bone Pixels, Mean ETA, Median ETA, Median ETA, Mode ETA, SD ETA, Minimum ETA, and Maximum ETA. A table of significant differences is displayed below by its represented p-value ($p < 0.05$ significant). The effect of level, and sector was analyzed for each parameter. In Table 3 below the significant differences are highlighted in yellow while those with a low but not significantly significant p-value ($0.05 < p < 0.1$) are highlighted in grey.

Table 3. *2-way RM ANOVAs significant difference results for Analysis 1.*

	Analysis 1 P-Values			
Parameter	**Level**	**Sector**	**Level x Sector**	**Post Hoc Required**
BV/TV	0.388	0.041	0.38	Yes
Mean ETA	0.707	0.24	0.421	No
Meadian ETA	0.767	0.314	0.42	No
Mode ETA	0.799	0.198	0.355	No
SD ETA	0.842	0.073	0.74	No
Min ETA	0.525	0.058	0.089	No
Max ETA	0.304	0.034	0.805	Yes

To fully evaluate differences, it is necessary to use a *post-hoc* Fisher LSD analysis. The Fisher LSD test results for each tested parameter that required one are shown in Appendix H. Figures 10 through 16 below depict a graphical representation for the comparison of BV/TV and Mean ETA in Level 1 and Level 5. To see a full list of Analysis 1 results please see Appendix H.

From this analysis it is important to note that BV/TV (Figure 10) has shown a significant difference between sectors. *Post-hoc* analysis showed that sector 2 was significantly different from sectors 3, 5, 6, and 9 and that sector 4 was significantly different from sector 9.

Because sectors 1, 2, and 3 represent the relatively tensile side of bone it is expected that they show some significant differences from sectors 4, 5, and 6 (the more compressive side of the bone) and from sector 9 (the central sector). It is also important to note that there were no significant differences between the proximal and distal ends of bone (Level) or between different spots on the same

level (the interaction term, Level x Sector). In all subsequent figures, error bars represent the standard error.

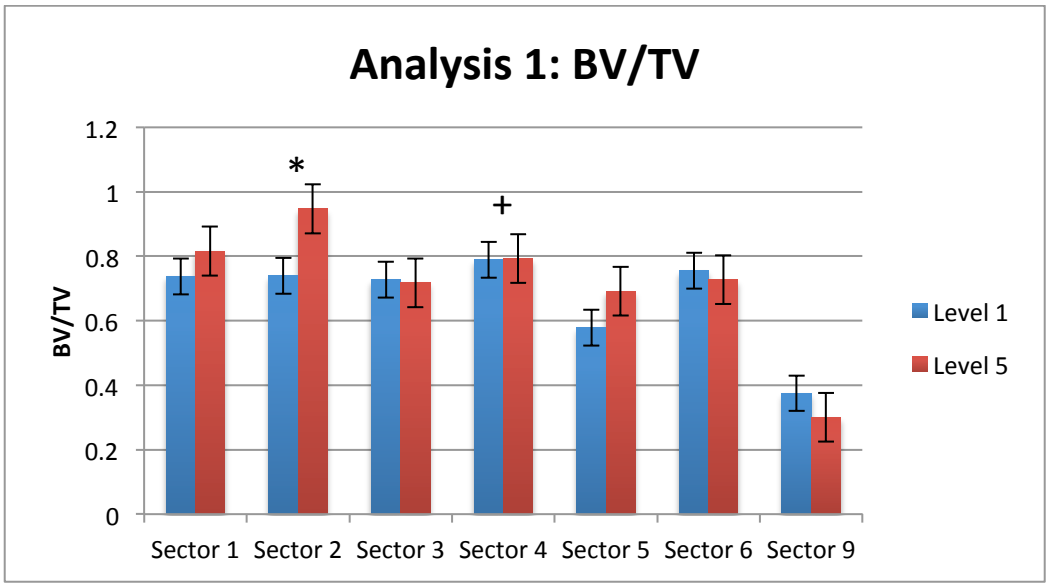


Figure 10: Graphical depiction of the comparison of BV/TV across Level 1 and Level 5 for Analysis 1.

* Represents statistical significance from sector 2 to sectors 3, 5, 6, and 9 on Levels 1 and 5.

+ Represents statistical significance from sector 4 to sector 9 on Levels 1 and 5.

To display these statistical differences in a visual manner that is more easily understood, the table below (Table 4) was created. Here, sectors that are significantly different from one another do not share a corresponding symbol (*, +, or #). For example, Sector 2 is significantly different from Sectors, 3,5,6, and 9 because they do not share a common symbol and, similarly, Sector 4 is significantly different from Sector 9

Table 4. *Analysis 1 significant differences in Sectors for BV/TV.*

Analysis 1: BV/TV			
Sector			
2	*		
4	*	+	
1	*	+	#
3		+	#
5		+	#
6		+	#
9			#

There were no significant differences noticed in Mean ETA. This expresses that, when comparing levels 1 and 5, the ETA is not different overall between proximal and distal ends of the bone, between different sectors, or between different sectors on the same level (the interaction term).

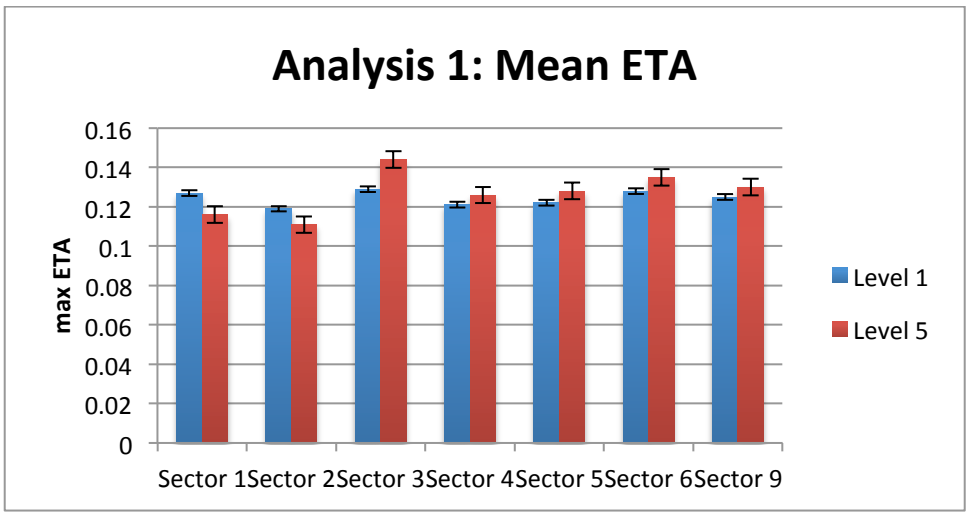


Figure 11: Graphical depiction of the comparison of Mean ETA across Level 1 and Level 5 for Analysis 1.

When looking at maximum ETA a significant difference was found in sector. *Post-hoc* analysis showed that sector 3 was significantly different from sectors 1, 2, 4, and 5 and that sector 9 was significantly different from sectors 1 and 4.

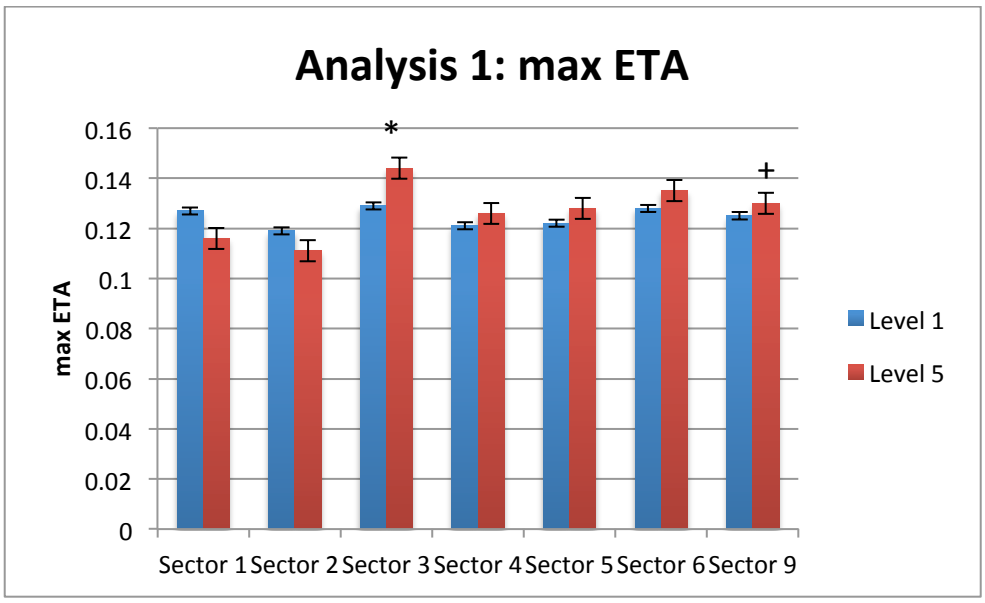


Figure 12: Graphical depiction of the comparison of maximum ETA across Level 1 and Level 5 for Analysis 1.

* Represents statistical significance from sector 3 to sectors 1, 2, 4, and 5 on Levels 1 and 5.

+ Represents statistical significance from sector 9 to sectors 1 and 4 on Levels 1 and 5.

Table 5: *Analysis 1 significant differences in Sectors Maximum ETA.*

Analysis 1: max ETA			
Sector			
3	*	+	
9	*	+	
6	*	+	#
2		+	#
4		+	#
1			#
5			#

The following graphs (Figures 13 through 16) display the comparisons of Median, Mode, SD, and Minimum ETA and show no significant differences. It is important to note that although there was no statistical significance between Sectors for Min ETA it was close with a p-value of 0.058.

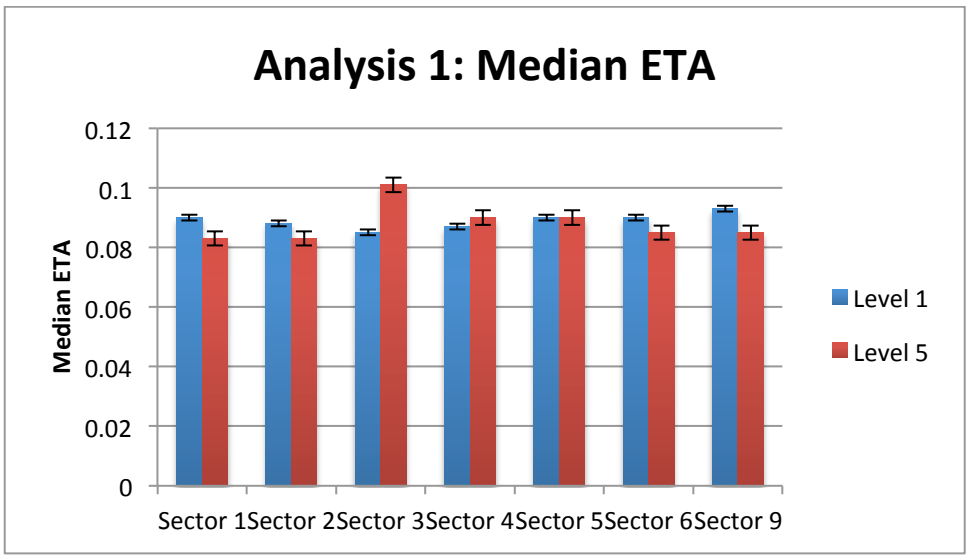


Figure 13: Graphical depiction of the comparison of Median ETA across Level 1 and Level 5 for Analysis 1.

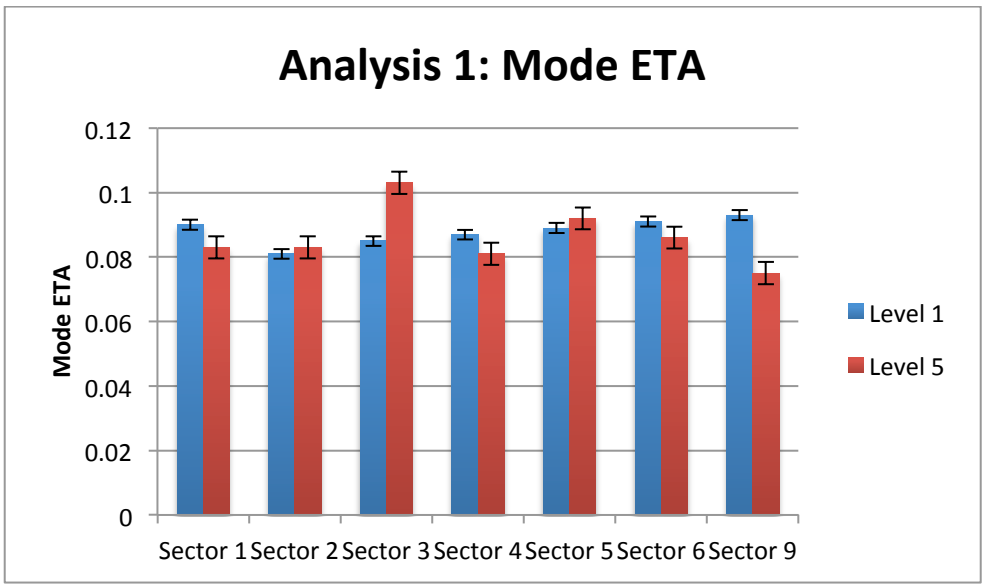


Figure 14: Graphical depiction of the comparison of Mode ETA across Level 1 and Level 5 for Analysis 1.

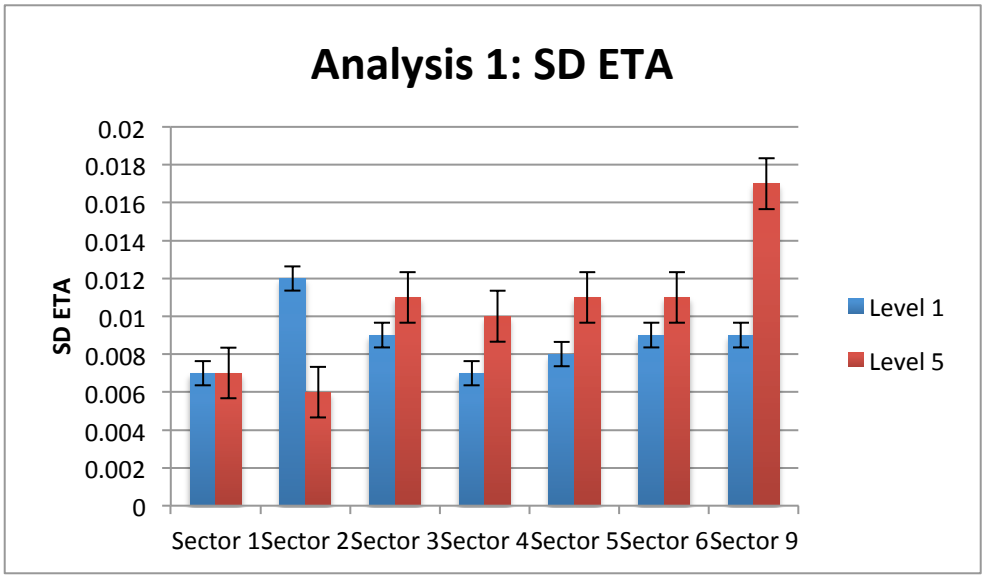


Figure 15: Graphical depiction of the comparison of SD ETA across Level 1 and Level 5 for Analysis 1.

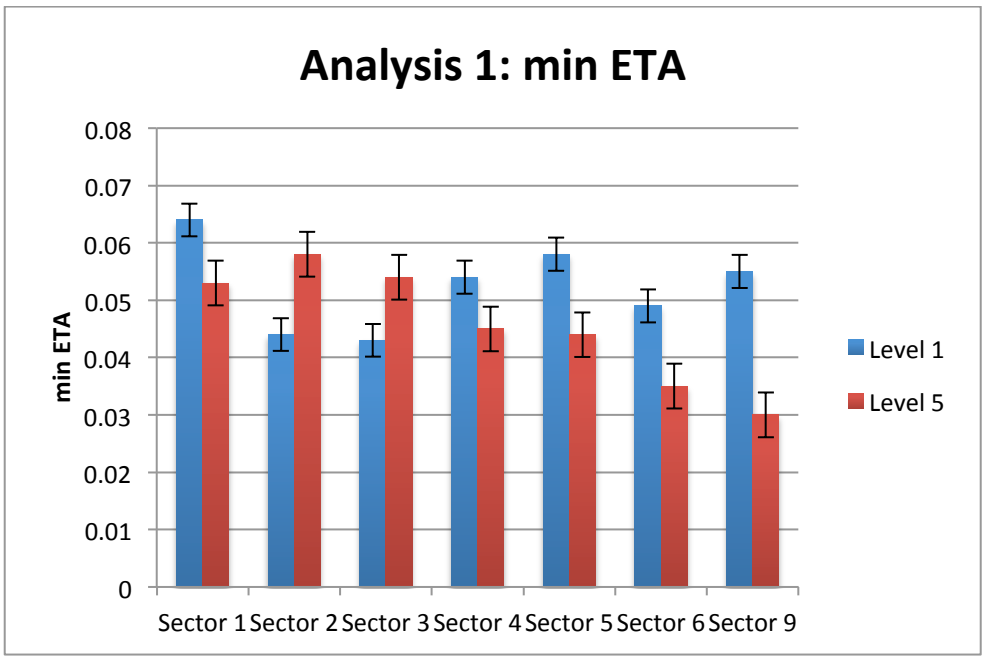


Figure 16: Graphical depiction of the comparison of minimum ETA across Level 1 and Level 5 for Analysis 1.

Pre-Supersector Analysis 2

For the second analysis a 1-way ANOVA was performed on level 5, all sectors. A table of significant differences is displayed below by its represented p-value ($p < 0.05$ significant). Analysis 2 compared the sectors on level 5 for each of the seven parameters. In Table 6 below the significant differences are again highlighted in yellow and those close are highlighted in gray.

Table 6. *1-way ANOVA significant difference results for Analysis 2.*

	Analysis 2 P-Values	
Parameter	**Sector**	**Post Hoc Required**
BV/TV	0.005	Yes
Mean ETA	0.059	No
Median ETA	0.118	No
Mode ETA	0.358	No
SD ETA	0.031	Yes
Min ETA	0.132	No
Max ETA	0.163	No

Again, Fisher LSD tests were performed *post-hoc* to further distinguish differences. The Fisher LSD test results for each tested parameter that required one are shown in Appendix I. A graphical depiction of the BV/TV, Mean ETA, and SD ETA sector comparisons are also shown in Figures 17 through 23 below. To see a full list of Analysis 2 results please see Appendix I.

From this analysis we see that, as to be expected, there was a significant difference between sectors for BV/TV. *Post-hoc* analysis revealed that sector 9 was significantly different than all other sectors (1-8). Because sector 9 is located centrally, this is to be expected. There was also a significant difference observed between sectors 2 and 8.

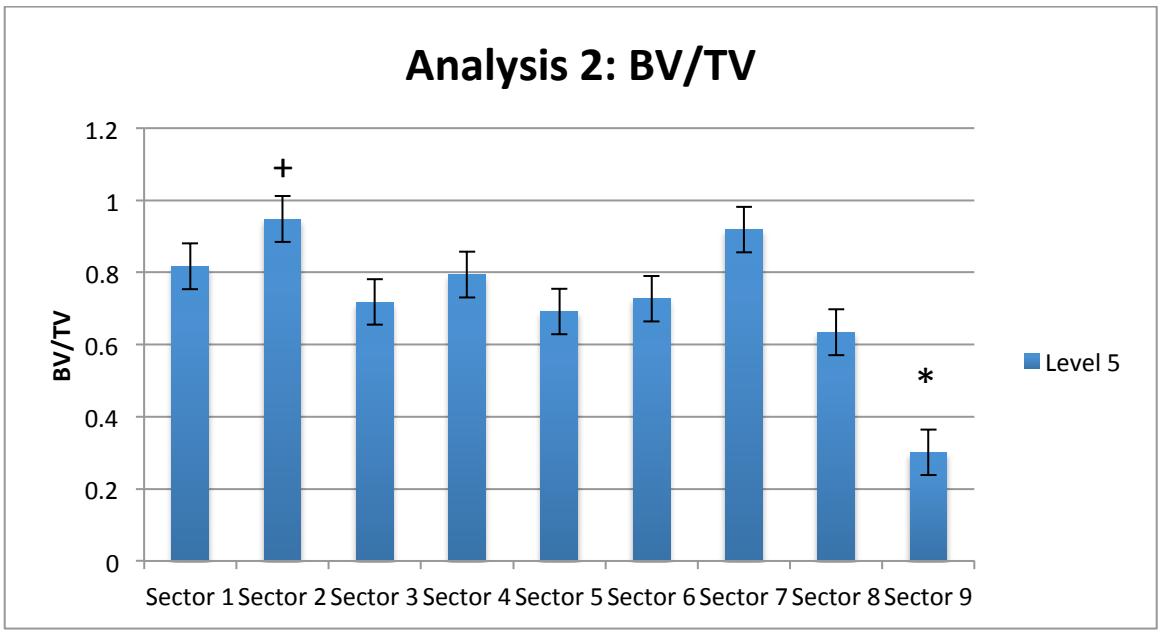


Figure 17: Graphical depiction of the comparison of BV/TV across Level 5 for Analysis 2.

* Represents statistical significance from sector 9 to sectors 1 through 8.

+ Represents statistical significance from sector 2 to sector 8.

Again, these statistical differences are displayed in a visual manner in Table 7 below. Sectors that are significantly different from one another do not share a corresponding symbol (*, +, or #).

Table 7. *Analysis 2 significant differences in Sectors for BV/TV.*

Analysis 2: BV/TV			
Sector			
2	*		
7	*	+	
1	*	+	
4	*	+	
6	*	+	
3	*	+	
5	*	+	
8		+	
9			#

It is also important to note that, although there were no significant differences in Mean ETA between sectors, it was close with a p-value of p=0.059. Here, a significant difference would indicate that there are differences between material characteristics of bone at different locations (Sectors) on the same level suggesting that cortical and trabecular bone may consist of different materials.

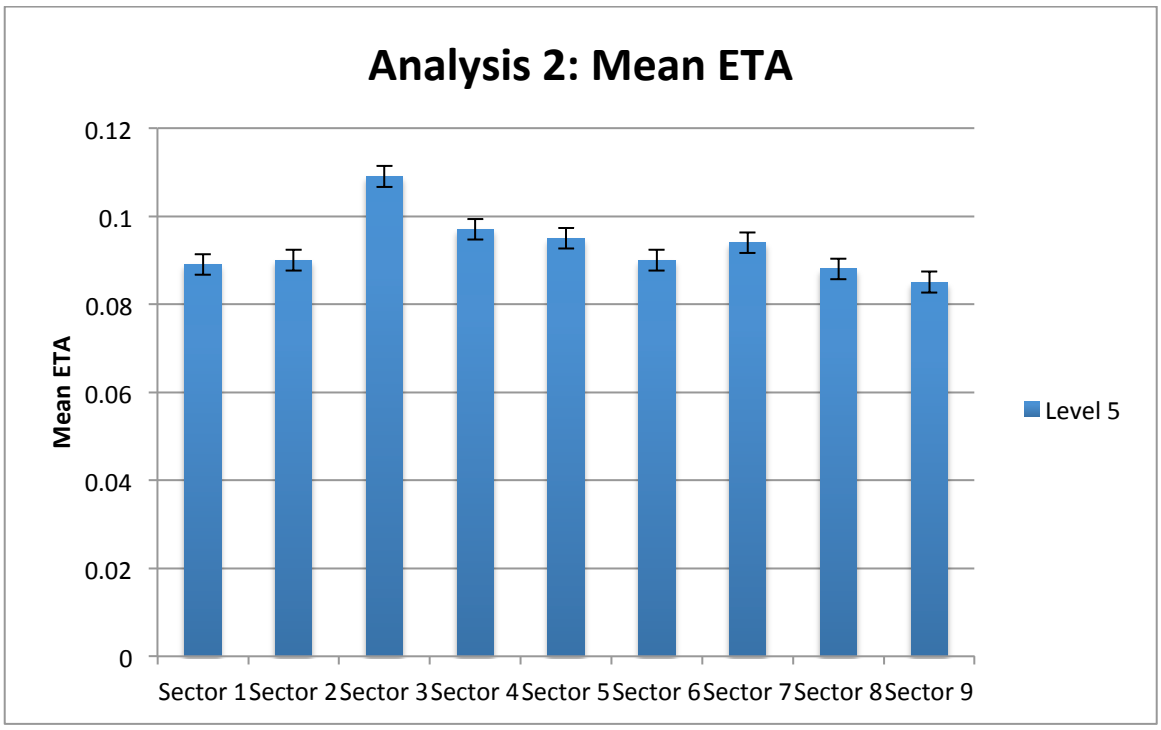


Figure 18: Graphical depiction of the comparison of Mean ETA across Level 5 for Analysis 2.

There was also a significant difference found in SD ETA between sectors. *Post-hoc* analysis showed that the fully trabecular bone (Sector 9) was significantly different from Sectors 1, 2, 3, 4, 7, and 8 and that Sector 2 was significantly different from Sector 6. This difference in SD ETA expresses differences in the variability of ETA within a sector. With a significantly larger SD (as was the case for Sector 9, the central trabecular sector) there is a much larger variability of ETA within the sector. This variability of ETA once again suggests that there are material differences between the fully trabecular bone and the more compact bone.

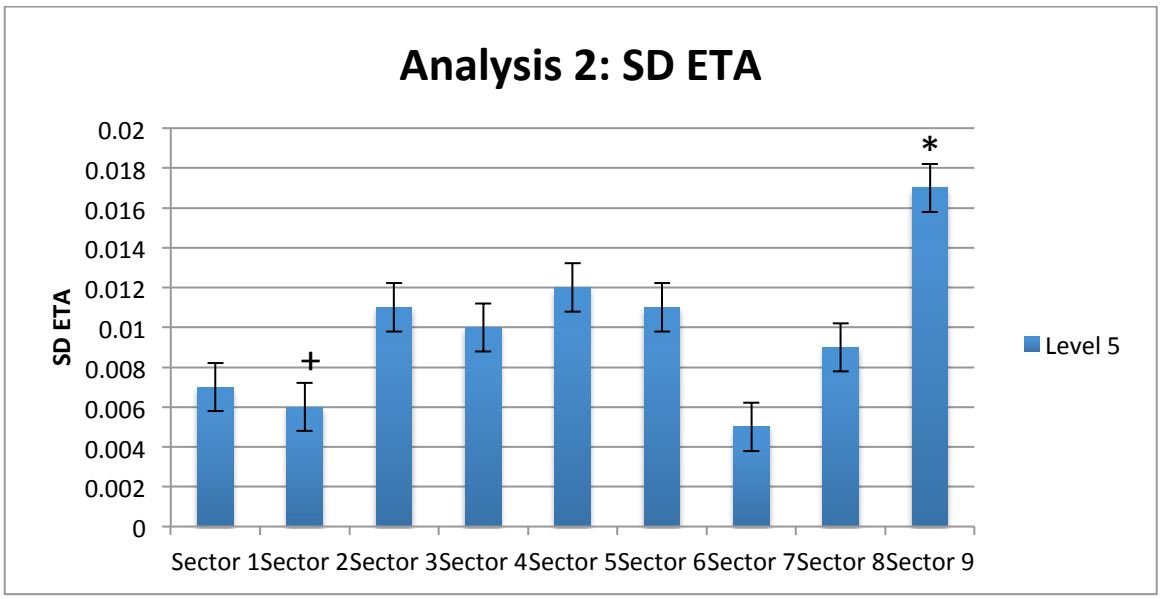


Figure 19: Graphical depiction of the comparison of SD ETA across Level 5 for Analysis 2.

* Represents statistical significance from sector 9 to sectors 1, 2, 3, 4, 7, and 8.

+ Represents statistical significance from sector 2 to sector 6

Table 8. *Analysis 2 significant differences in Sectors for SD ETA.*

Analysis 2: SD ETA			
Sector			
9	*		
5	*		
6	*	+	
3		+	#
4		+	#
8		+	#
1		+	#
2			#
7			#

The following graphs (Figures 20 through 23) display the comparisons of Median, Mode, Minimum and Maximum ETA and show no significant differences.

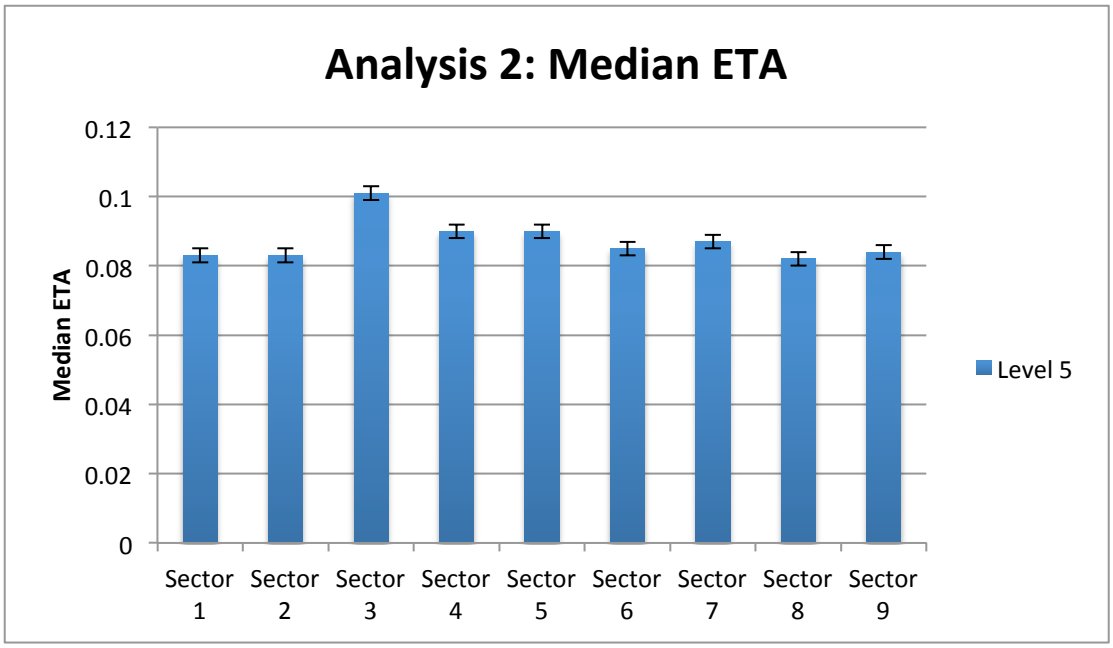


Figure 20: Graphical depiction of the comparison of Median ETA across Level 5 for Analysis 2.

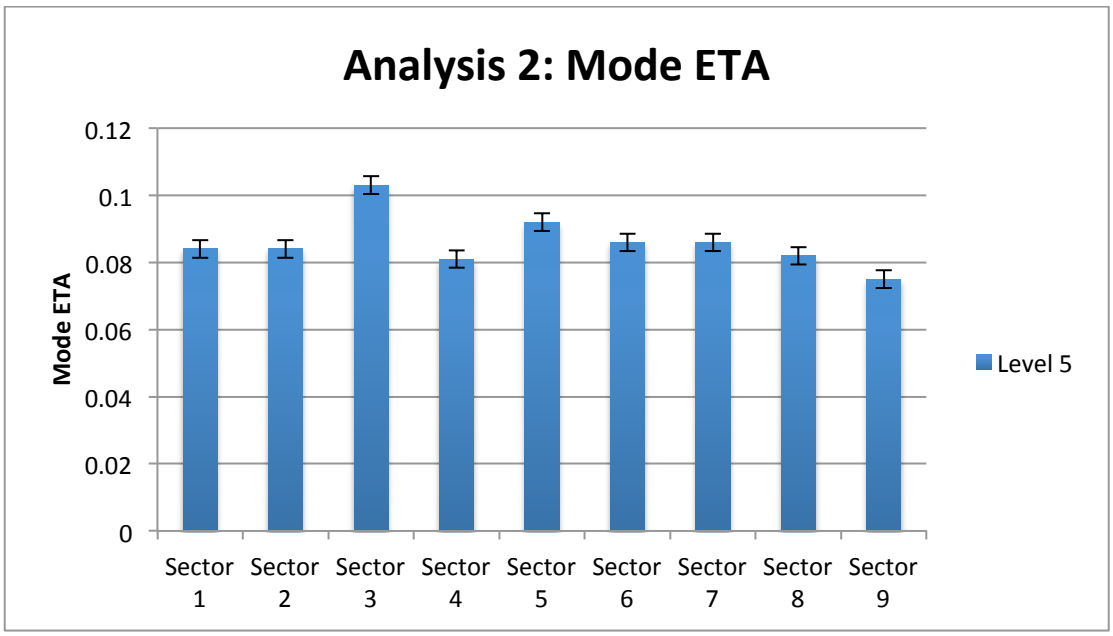


Figure 21: Graphical depiction of the comparison of Mode ETA across Level 5 for Analysis 2.

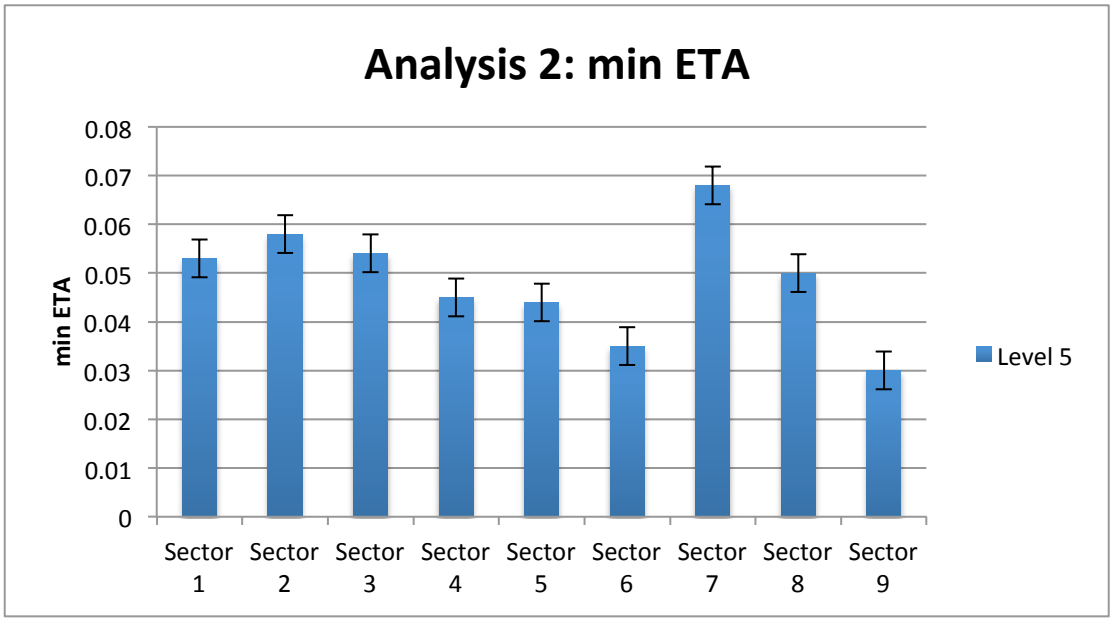


Figure 22: Graphical depiction of the comparison of SD ETA across Level 5 for Analysis 2.

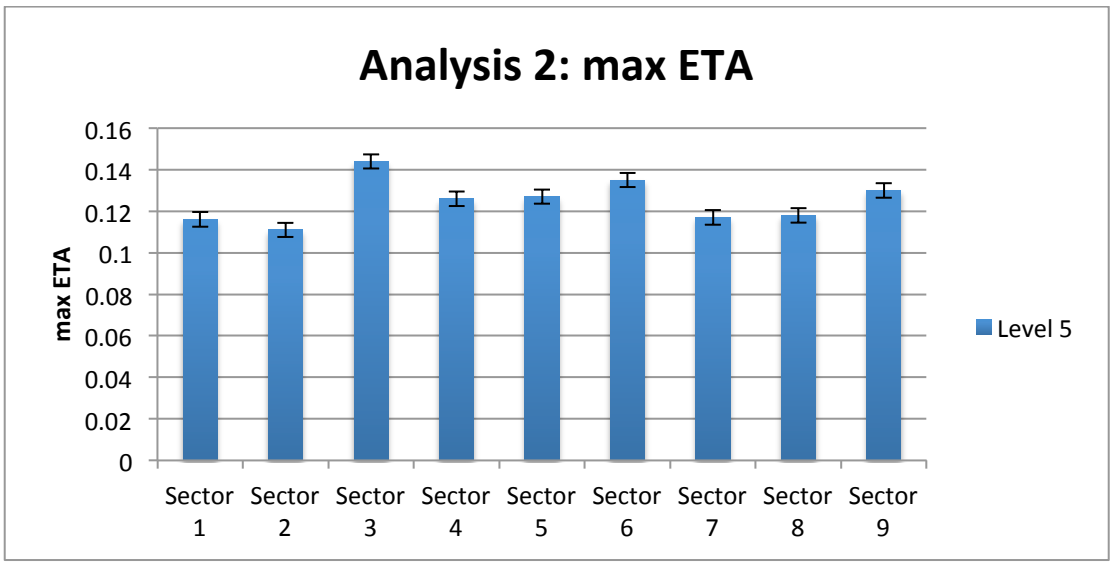


Figure 23: Graphical depiction of the comparison of SD ETA across Level 5 for Analysis 2.

Supersector Analysis

As a result of a pattern of missing data, a number of horses were dropped from the analyses above (see Appendix F for a full specimen inventory). This created problems with the accuracy and reliability of the pre-Supersector analyses. Because of this, all sectors were condensed into three categories (Dorsal, Central, Palmar) called supersectors. The dorsal supersector consisted of sectors 1, 2, and 3 and portrayed the relatively tensile side of bone. The palmar supersector consisted of sectors 4, 5, and 6 and portrayed the compressive side of the bone. The central supersector consisted only sector 9.

Two-way repeated measure ANOVAs were then preformed on level and Supersector for all three Supersectors (Dorsal, Central, and Palmer) and then preformed again excluding the central Supersector. P-values from these analyses, to show significant difference, are displayed in below in Table 9 and Table 10. Significantly different values are highlighted in yellow.

When comparing the Dorsal, Central, and Palmar Supersectors we notice that there are significant differences in several areas. For BV/TV, significant differences were found in Supersectors, and when comparing both Dorsal and Palmar Supersectors to the Central Supersector. For Bone Pixels, significant differences were found when comparing both Dorsal and Palmar Supersectors to the Central Supersector. There was also a significant difference found in Levels when looking at Min ETA. The significant differences found between horse are not relevant for the purpose this study.

These results agree with our pre-existing knowledge that bone is less dense in the central area. It is also important to note that there were no significant differences when comparing the Dorsal Supersector to the Palmar Supersector. It is because of this that an additional two-way RM ANOVA was preformed excluding the central Supersector.

Table 9. *Two-way RM ANOVVAs p-values on all Supersector regions.*

Dorsal, Central and Palmar Supersector P-values							
	Horse	Level	Supersector	Level x Supersector	Dorsal vs. Central	Dorsal vs. Palmar	Palmar vs. Central
BV/TV	0.001	0.981	0.001	0.898	0.001	0.642	0.001
Bone Pixels	0.001	0.96	0.001	0.889	0.001	0.621	0.001
Mean ETA	0.602	0.253	0.2	0.122	na	na	na
Median ETA	0.476	0.304	0.517	0.16	na	na	na
Mode ETA	0.507	0.633	0.277	0.48	na	na	na
SD ETA	0.141	0.15	0.053	0.266	na	na	na
Min ETA	0.575	0.024	0.254	0.199	na	na	na
Max ETA	0.651	0.319	0.509	0.863	na	na	na

When comparing the Dorsal and Palmar Supersectors there were no significant differences found.

Table 10. *Two-way RM ANOVVAs p-values on Dorsal and Palmar Supersector regions.*

Dorsal and Palmar Supersector P-values				
	Horse	Level	Supersector	Level x Supersector
BV/TV	0.98	0.818	0.312	0.878
Bone Pixels	0.864	0.789	0.296	0.909
Mean ETA	0.887	0.601	0.991	0.11
Median ETA	0.808	0.572	0.872	0.218
Mode ETA	0.97	0.543	0.76	0.162
SD ETA	0.458	0.373	0.355	0.225
Min ETA	0.712	0.363	0.426	0.366
Max ETA	0.433	0.219	0.924	0.998

Linear Regressions

Linear regressions were calculated from X-Y scattergrams comparing BV/TV to each additional parameter (Mean ETA, Median ETA, Mode ETA, SD ETA, Min ETA, and Max ETA) for each horse in order to address the trabecular-vs-compact bone question as a continuous variable rather than as categorical. Figures 24 through 29 display these scattergrams and regressions where the equation of the line tells us the slope, the R^2 value indicates how well the data points fit the line, and the p value indicates whether the slope is significantly different from 0.

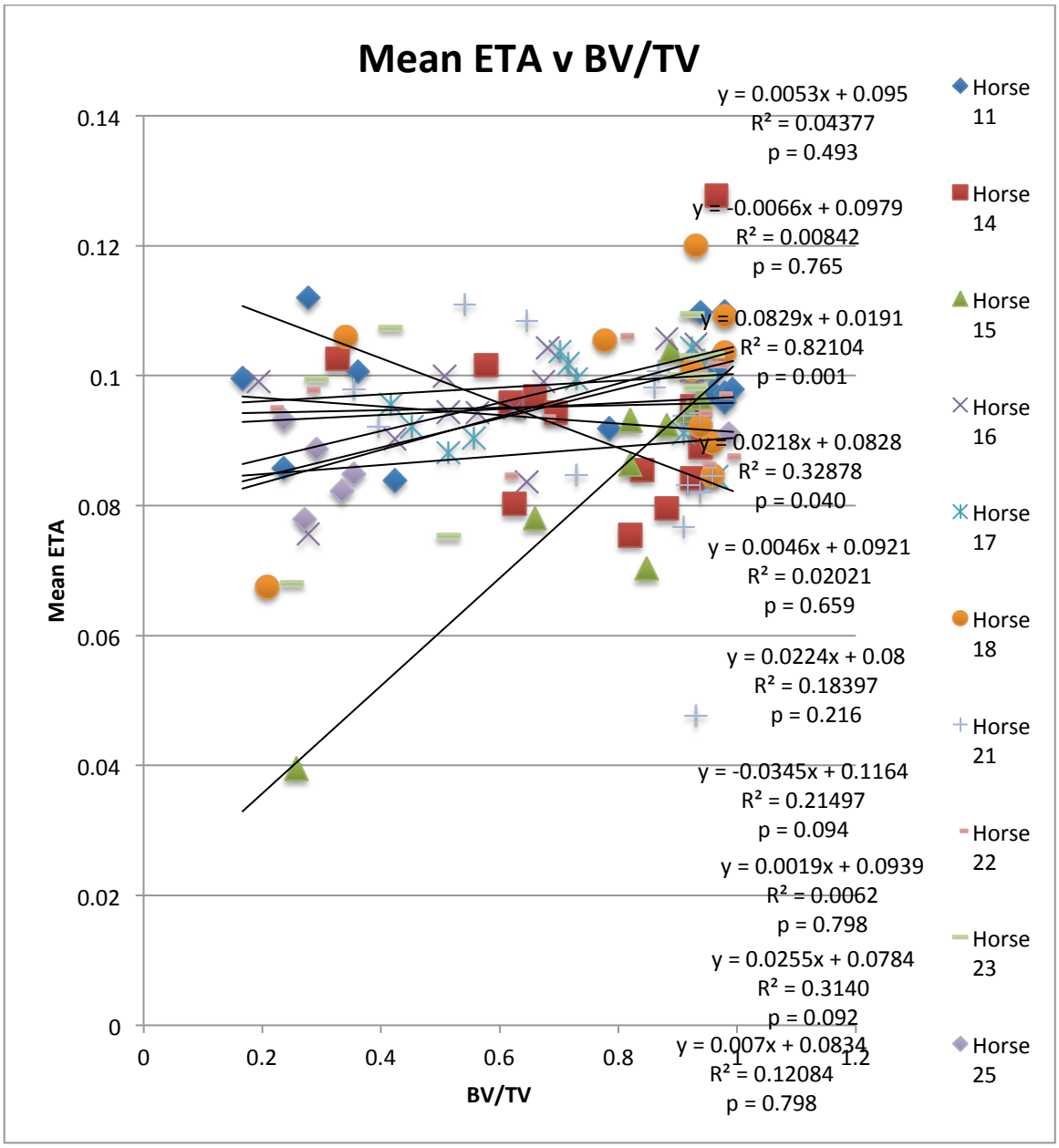


Figure 24: Scattergram with linear regressions comparing Mean ETA to BV/TV for each equine specimen.

From Figure 24 above we see that in most cases there is not a significant relationship, meaning that Mean ETA does not change with BV/TV.

Horse 15 (a one year old Thoroughbred filly) had a fairly high relationship (R^2 = 0.821) with a p value that shows significant difference from 0.0. (p = 0.001) indicating that the lower the porosity, the higher the mineralization. To ensure that the regression results were not driven by the single low data point (BV/TV=0.26, Mean ETA=0.039), the regression was redone with the removal of that point. Although the R^2 value decreased to 0.3192, the slope maintained its statistical significance from 0.0 (p = 0.038).

There is a negative relationship for both horse 14 and 21 indicating that the lower the porosity the lower the mineralization. However, because there is not a strong correlation between BV/TV and Mean ETA in general, this data supports that cortical and trabecular bone are the same material regardless of BV/TV.

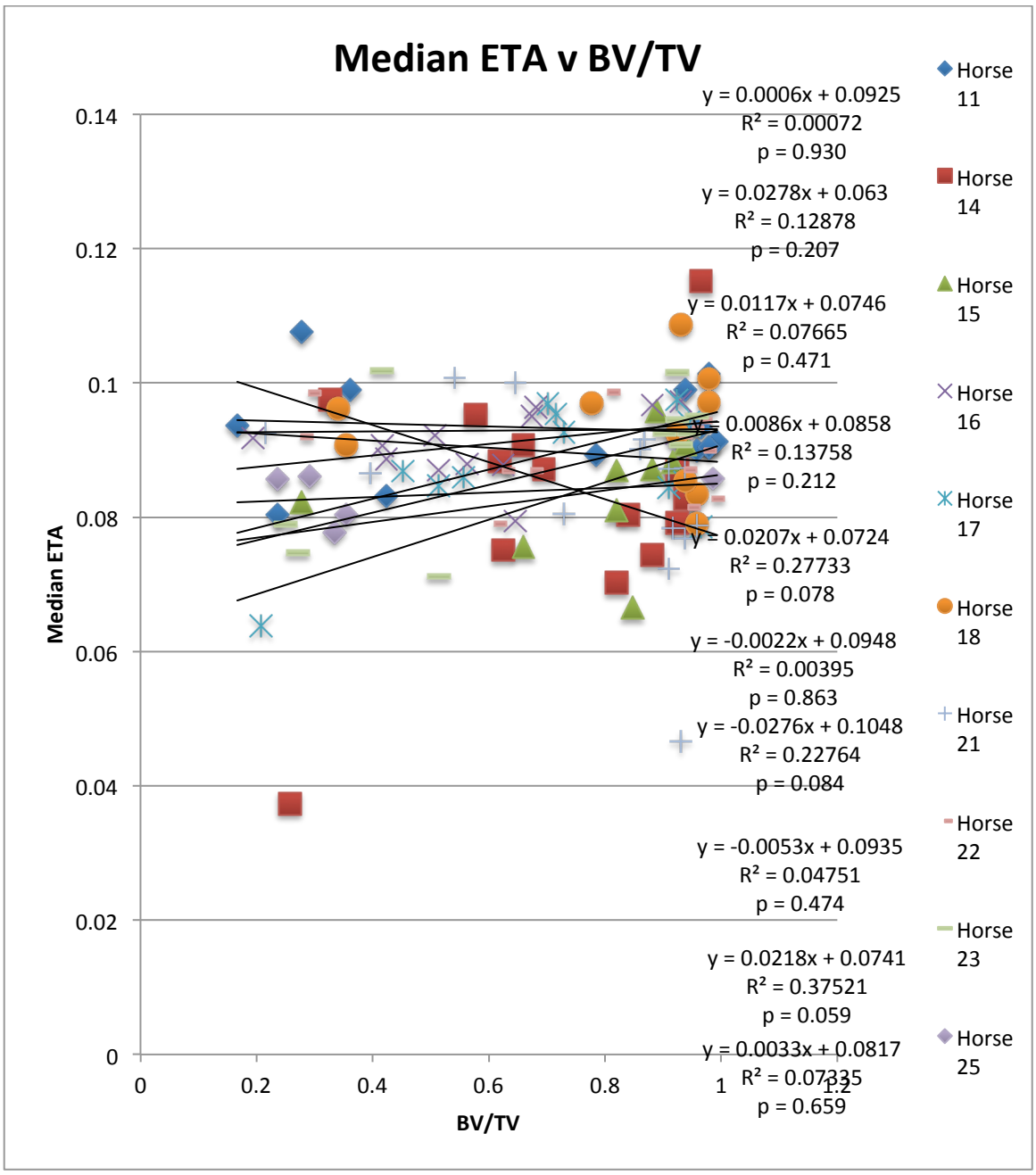


Figure 25: Scattergram with linear regressions comparing Median ETA to BV/TV for each equine specimen.

From Figure 25 above we see that there are no cases where there is a strong relationship between Median ETA and BV/TV. This is another, stronger, argument that bone is the same material regardless of BV/TV.

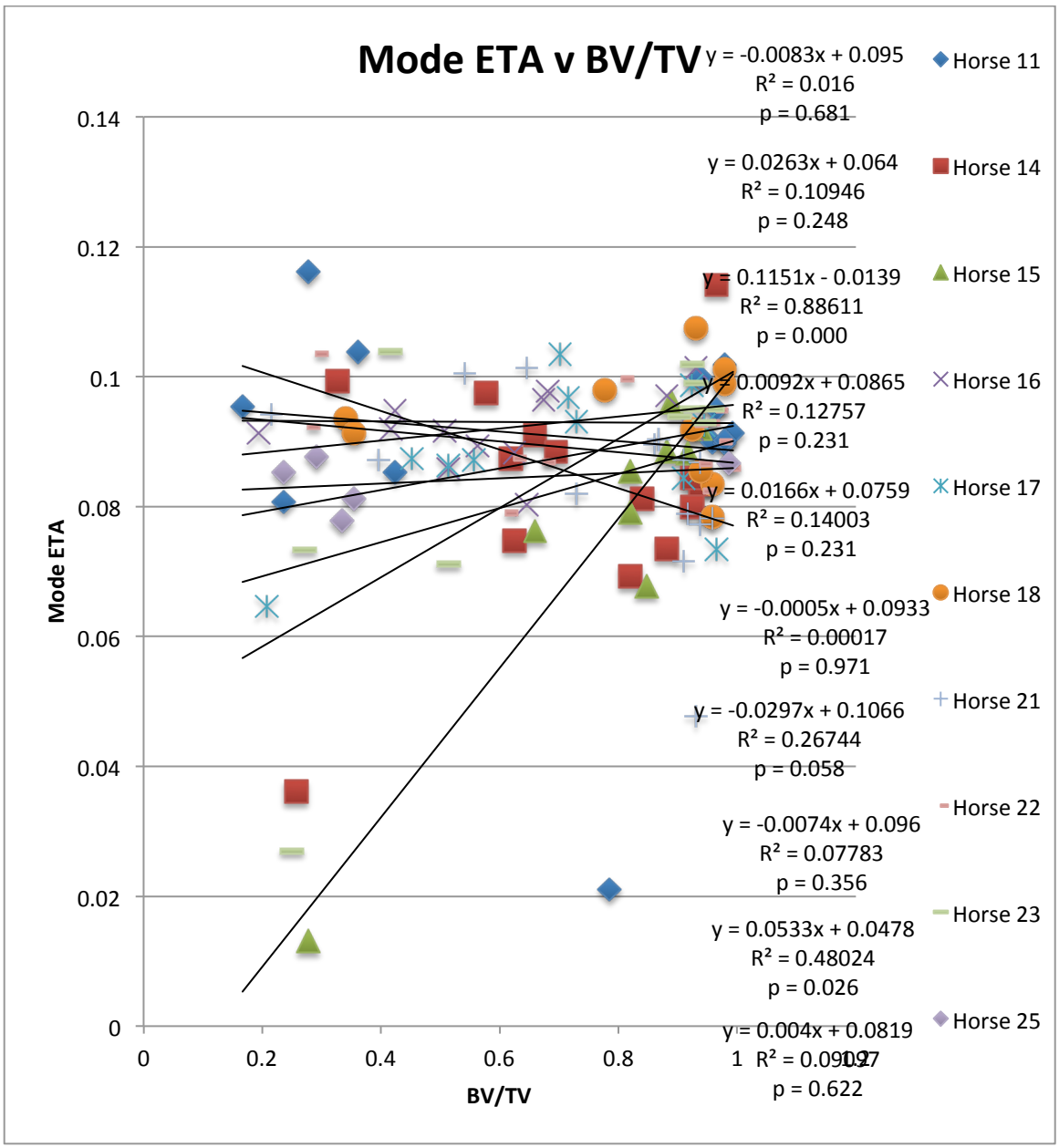


Figure 26: Scattergram with linear regressions comparing Mode ETA to BV/TV for each equine specimen.

Figure 26 above shows that we have a similar situation as Figure 24 (Mean ETA) where the only horse that shows a strong relationship is horse 15.

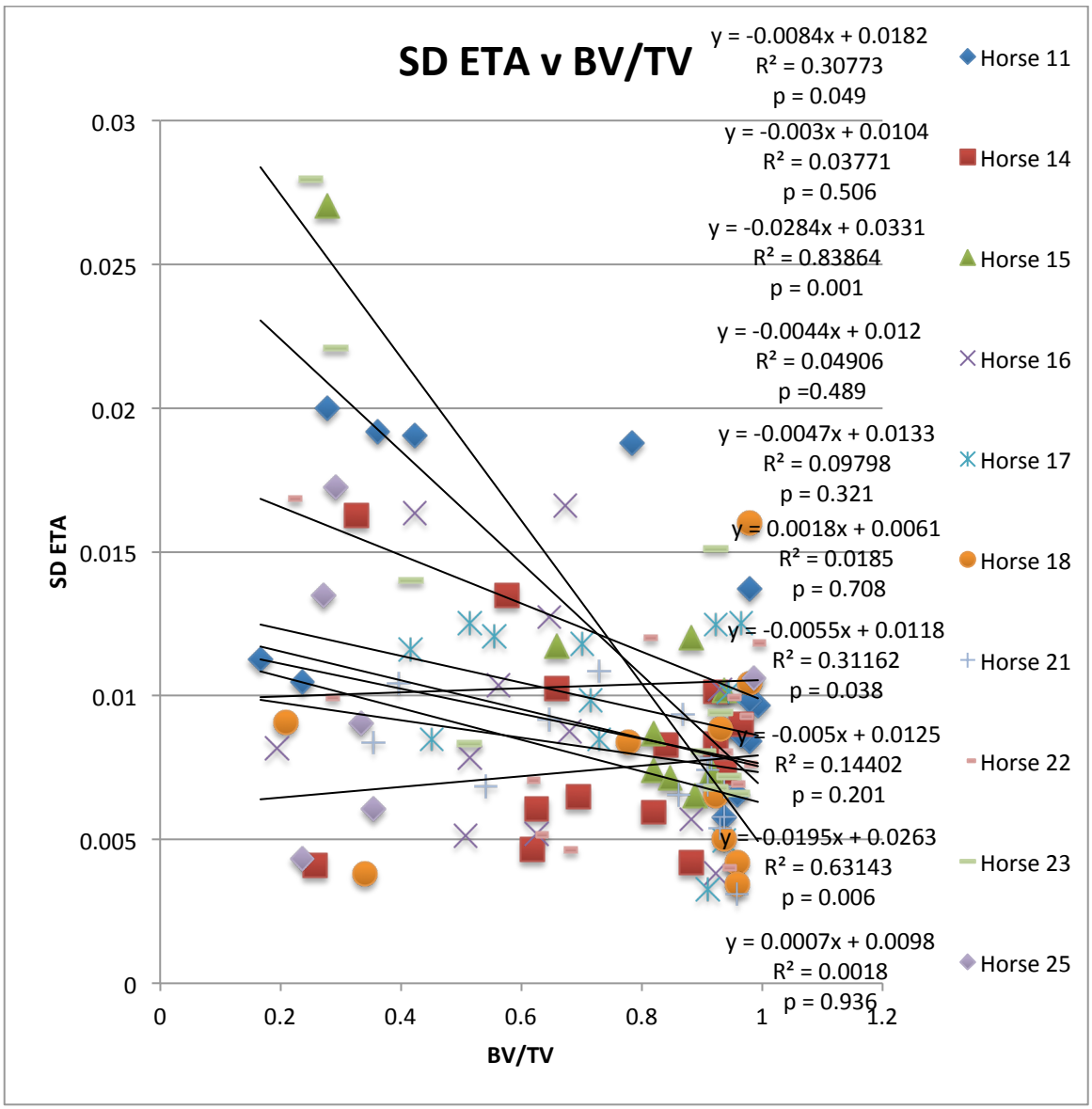


Figure 27: Scattergram with linear regressions comparing SD ETA to BV/TV for each equine specimen.

From Figure 27 above we see that there is a large range of standard deviations in high porosity bone (BV/TV 0.2-0.4) while the low porosity bone is relatively closely grouped indicating a wider range of materials in higher-porosity bone. Horses 11, 15, 21, and 23 are statistically significant indicating that the significance is not an outlier or a fluke. This regression shows that the less

porous the specimen, the less variability in the ETA there is. If the material was constant the porosity and ETA should be independent from one another. Therefore, this is an argument that the different ranges of BV/TV values (i.e. cortical vs trabecular) are different materials.

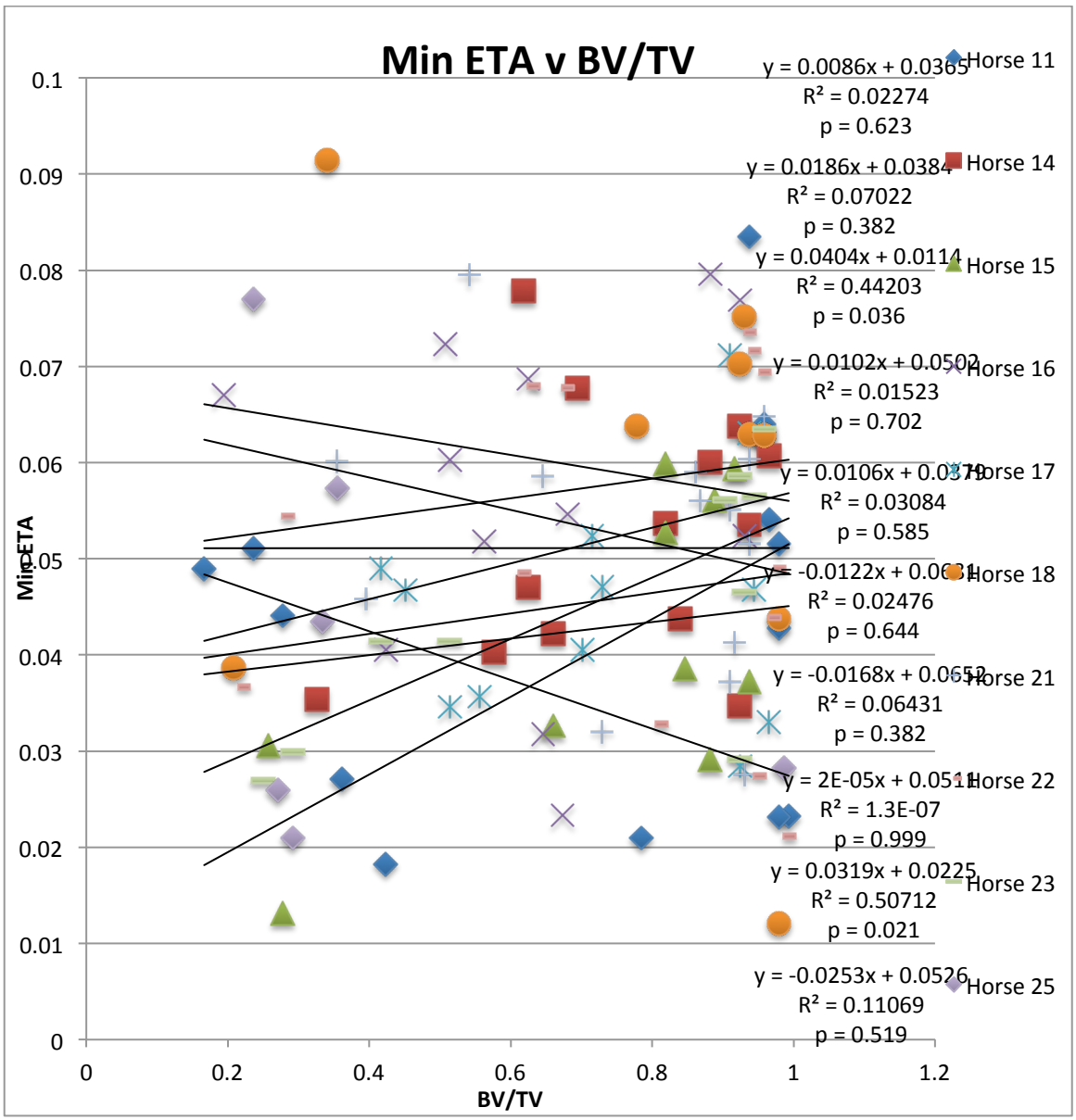


Figure 28: Scattergram with linear regressions comparing Min ETA to BV/TV for each equine specimen.

From Figure 28 we see that once again, aside from horse 15 and 23 which both have a positive relationship, the regressions are fairly flat. This is another same-material argument.

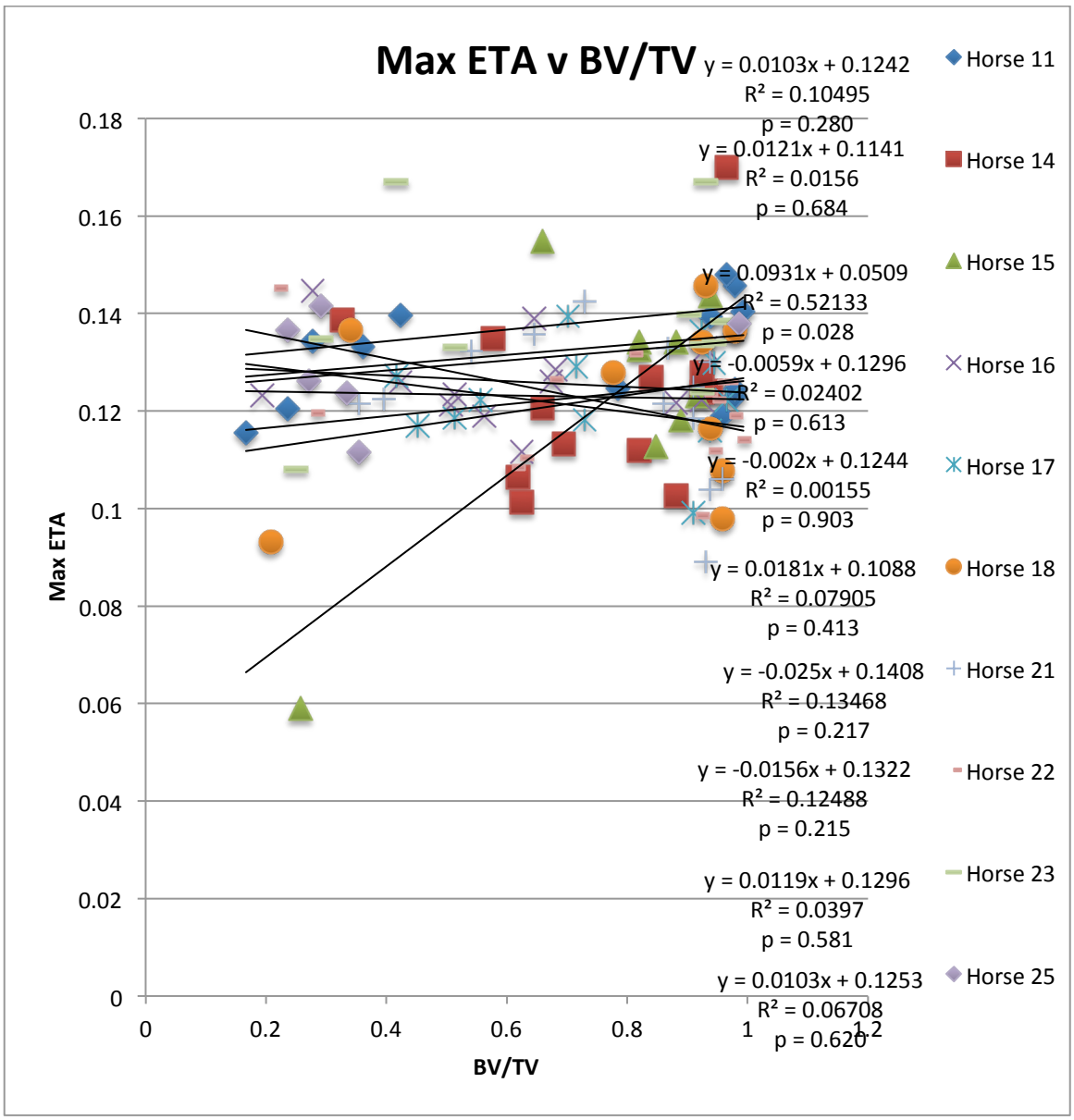


Figure 29: Scattergram with linear regressions comparing Max ETA to BV/TV for each equine specimen.

Figure 29 tells us that in most cases (besides horse 15) there is no significant relationship between Max ETA and BV/TV. Once again this is a same-material argument.

CHAPTER 4

Conclusion

Samples were taken from different sections of the equine metacarpus bone in order to better understand the material properties of bone. The equine metacarpus is an effective specimen because it exhibits a full spectrum of bone density from highly dense cortical bone to highly porous trabecular bone. The goal of this study was to determine if cortical and trabecular bone consist of the same material with only a variance in density or if they are different materials altogether.

The current standard operating procedure is the assumption that bone is a constant material and by changing the density of the material we can change its material properties, however there have been few studies regarding the validity of this.

The study used 114 equine samples from 10 horses of various age, sex, and breed. The samples came from 9 different radial sectors and 3 different proximodistal levels. Each sample underwent densitometry analysis to obtain the following parameters: Bone Volume / Total Volume (BV/TV), Bone Pixels, Mean Equivalent Thickness of Aluminum (ETA), Median ETA, Mode ETA, Standard Deviation ETA, Minimum ETA, and Maximum ETA.

The collected data from each specimen then underwent a series of statistical analyses. This included a mixed-model ANOVA on level while only looking at sector 9 the central sector (Analysis 1), a mixed-model ANOVA on sector while only looking at level 5 (Analysis 2), and two-way repeated measure ANOVAs were then preformed on level and supersector (a groping of sectors) and then rerun excluding the central supersector (Supersector Analysis). Finally, linear regressions were calculated from X-Y scattergrams comparing BV/TV to each additional parameter.

The results of the densitometry and statistical analysis showed a significant difference in Sector when looking at BV/TV for Analysis 1 and 2 and the Supersector Analyses. These results are not surprising yet they are a good quality control check telling us that we have a reasonable range of independent variables to use in considering our main objective. Similarly, as expected, significant differences were found in Bone Pixels throughout each analysis.

When considering Mean ETA no significant differences were found in any of the three analyses. It is important to note that, although it was not significantly different, Analysis 2 was close to statistical significance when looking at sector with a p-value of 0.059. A significant difference here would indicate that there are differences between material characteristics of bone at different locations on the same level.

There were no significant differences in Median or Mode ETA in Analysis 1 and 2 and the Supersector Analyses.

When considering SD ETA, Analysis 2 showed a significant difference in sectors. *Post-hoc* analysis revealed that sector 9 was significantly different than all other sectors (1-8) and that sector 2 was significantly different that sector 8. This expresses differences in the variability of ETA within a sector. With a significantly larger SD (such as was the case for Sector 9) there is a much larger variability of ETA within the sector.

For Min ETA, the Supersector Analysis comparing Dorsal, Central and Palmar Supersectors found a significant difference in level. This suggests that there may be material differences in the high porosity areas of bone.

When looking Max ETA there was a significant difference in Sector in Analysis 1. Sector 3 was significantly different from Sectors 1, 2, 4, and 5 on Levels 1 and 5 and Sector 9 was significantly different from Sectors 1 and both on Levels 1 and 5. This suggests that there may be material differences in some low porosity areas of bone.

With only two exceptions, the results from these analyses suggest that the material is the same between sectors and thus between varying densities of bone. That is, with the BV/TV varying between different sectors of bone, mean ETA (as well as Median, Mode, Min, and Max ETA) remains somewhat constant. However, in Analysis 2, when looking at SD ETA as a function of sector, we see

that while the mean values aren't different between cortical and trabecular bone, the variation in ETA is considerably higher in the trabecular bone. This is an argument that we have different materials between varying densities of bone while suggesting how those materials differ from one sector to another by the range of ETA values. Additionally, in the Supersector Analysis comparing Dorsal, Central, and Palmar Supersectors, the significant difference in levels tells us that there may be material differences in the high porosity areas of bone. This idea is further developed with the results from the linear regressions.

Overall, the linear regressions preformed showed a trend of data that suggests that trabecular and cortical bone consist of the same material with varying porosities. This can be expressed in the lack of correlation between BV/TV and Mean ETA, Median ETA, Mode ETA, minimum and maximum ETA. As previously stated, the majority of specimens in these regressions showed no correlation.

However, the regression comparing BV/TV and SD ETA showed heteroscedasticity, a large range of standard deviations in high porosity bone (BV/TV 0.2-0.4) while the low porosity bone is relatively closely grouped indicating a wider range of materials in higher-porosity bone. (Note that this was also shown in Analysis 2 where Sector 9, the high-porosity central sector, showed statistical differences from all other sectors.) Horses 11, 15, 21, and 23 were statistically significant indicating that the significance is not an outlier or a fluke. This variation of material in high-porosity bone could potentially be a matter

of differential remodeling activity but may simply be a matter of maxing out ETA on the cortical specimens. A “max-out” phenomenon would occur if the range of thicknesses on the aluminum stepwedge that were too small and may have limited the values in the ETA data. In order to clarify the meaning of these results, further testing should be done. To ensure that the ETA was not maxed out, a stepwedge with a larger range and more intervals should be used. To test whether these results were a result of differential remodeling activity, the effect of age on ETA variation (SD ETA) should be taken into account. Theoretically, a younger horse would have less remodeling resulting in a more consistent material whereas an older horse, having undergone greater amounts of remodeling, may have a larger variation in high-porosity bone material due to differential modeling.

A future study could also investigate the role that variation in SD plays with mechanical properties. To see what effects more varied materials have on mechanical properties (such as stiffness and strength) one could compare each mechanical property to the amount of material variation to see if there is any correlation. There is also a full set of mechanical data for the equine bones used in this study and a future study could investigate what material properties (modulus, stress, strain, energy, etc.) would be affected by a variance of materials.

Horse 15 (a one year old Thoroughbred filly) showed a stronger correlation in each category with the exception of Median ETA. This strong

correlation indicates that the lower the porosity of this particular specimen's bone, the higher the mineralization. The reason for this anomaly is not certain, however a number of factors including age, breed, diet, and lifestyle may have contributed.

There were several limitations to this study that should be taken into account. The equine specimens came from a wide range of ages including a 5-month-old foal and a twenty-year-old Thoroughbred. Bone constantly remodels throughout life yet material properties and characteristics may vary drastically depending on age. Therefore, this variation in age between specimens may have caused inconsistencies between samples taken from the same portion of bone. Additionally, although more samples were initially harvested, there were only 114 samples used from 10 different equine specimens. This shortage of bone specimens created a need to combine sectors of bone from different areas into larger general groups in order to maintain statistical value. A larger sample of bone specimens would have allowed more statistical accuracy and the ability to distinguish a greater number of sectors with the bone.

Ultimately, this study set out to answer the following question; "Are cortical and trabecular bone two separate materials, or are they the same material with a variance in density?" To answer this question, samples were taken from different sections of the equine metacarpus bone, underwent densitometry analysis and were statistically analyzed as described above. The majority of results from these analyses suggest that the material is the same between varying densities of bone

and thus the same between cortical and trabecular bone. However, there were several instances where varying materials were found specifically with regard to high porosity trabecular bone.

References

1. Bone. [Online] SUNY Downstate Medical Center, March 2008.http://ect.downstate.edu/courseware/histomanual/bone.html.

2. Calcagno, Joseph. *Seasonal and Anatomical Variation in Compact Bone Remodeling in Adult Sheep.* California Polytechnic State University. San Luis Obispo, 2011. MS Thesis.

3. Marieb, E.N. and K. Hoehn, Human anatomy & physiology. 2007: Pearson Benjamin Cummings.

4. Bugbee, Cailyn. EFFECT OF SHORT-TERM ESTROGEN DEPLETION ON COMPACT BONE MICRODENSITOMETRY IN THE EWE. California Polytechnic State University. San Luis Obispo, November 2012. MS Thesis

5. Les et al., J Orthop Res 12:822-833, 1994

6. Lim LS, Hoeksema LJ, Sherin K; ACPM Prevention Practice Committee. Screening for osteoporosis in the adult U.S. population: ACPM position statement on preventive practice. *Am J Prev Med*. 2009;36:366-375.

7. Christopher J. Hernandez, MS; Gary S. Beaupré, PhD; Dennis R. Carter, PhD, “A model of mechanobiologic and metabolic influences on bone adaptation” Journal of Rehibilitation reasearch & developmentVol. 37 No. 2, March/April 2000 Pages 235 – 244

8. Saladin, K.S., *Anotomy & Physiology: The Unity of Form and Function.* 5th ed, ed. M.H.H. Education 2010, Ney York: McGraw Hill Higher Education.

9. *Skeletal Cartilages*. Chapter 6: Bones and Skeleal Tissue [cited 2012 Sept. 3, 2012].

10. R. Bruce Martin, D.B.B., Neil A. Sharkey, *Skeletal Tissue Mechanics*2004, New York: Springer.

11. Seeman, E.a.P.D.D., *Bone quality--the material and structural basis of bone strength and fragility.* N Engl J Med, 2006. **354**(21): p. 2250-2261.

12. Wong, E., *Effects of Ovariectomy, Seasonal Changes, and Anatomical Position on the Compact Bone Remodeling as seen in the Adult Ovine Model*, in *Biomedical Engineering*2012, Cal Poly: San Luis Obispo. p. 93.

13. Broken Bone Injuries: Statistics, *Schwebel Goetz & Sieben.* http://www.schwebel.com/practice/broken-bone-injuries/statistics/

14. Peppers F., *UGA discovery uses 'fracture putty' to repair broken bones in days.* February 3, 2012. UGA Today

15. Karnezis I. A., Fragkiadakis E.G., *Computer modeling in bone research.* Surg Technol Int. 2000; 9: 299-307.

16. Bennet D. Ph.D., *Timing and Rate of Skeletal Maturation in Horses,* Equine Studies, Ranger Piece, 2008

Appendices

A. ImageJ Histogram Feature

B. Stepwedge Template Excel File

C. Quantitative Densitometry Worksheets

D. Final output Values

E. SPSS Mixed Model Instructions

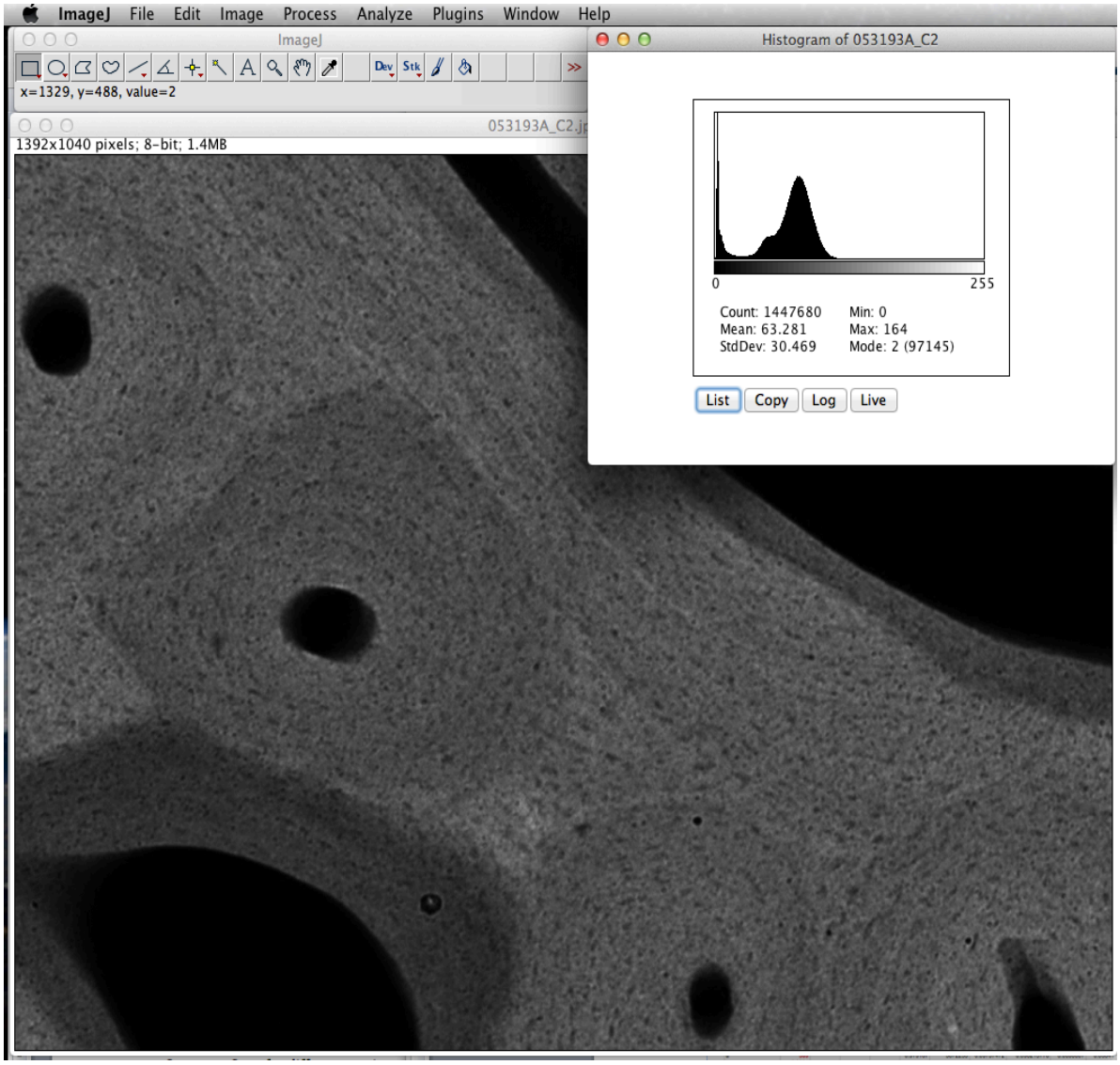
F. Specimen Inventory

G. Densitometry Output For All Specimens

H. Analysis 1 Full Results

I: Analysis 2 Full Results

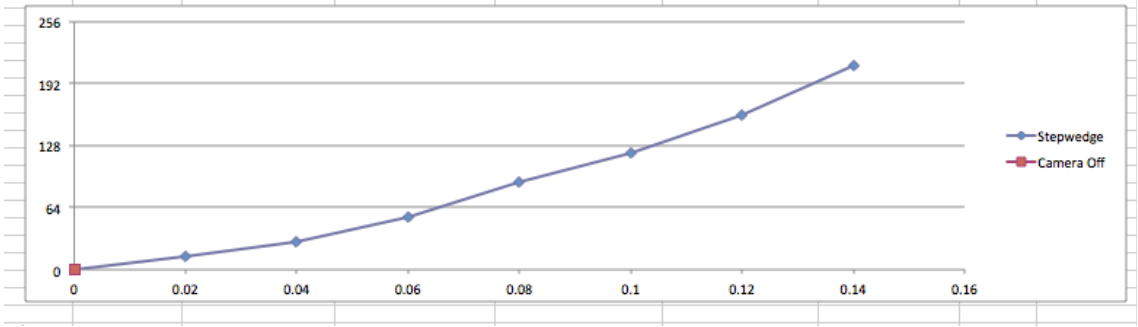
Appendix A: ImageJ Histogram Feature



Appendix B: Stepwedge Template Excel File

	A	B	C	D	E	F	G	H	I
1	Camera Off	Level 0	Level 1	Level 2	Level 3	Level 4	Level 5	Level 6	Level 7
2	1447680	1447680	0	1	0	0	0	0	0
3	0	0	3	2	2	0	0	0	0
4	0	0	10	3	1	0	0	0	0
5	0	0	39	6	6	0	0	0	0
6	0	0	106	6	1	1	0	0	0
7	0	0	363	9	3	0	0	0	0
8	0	0	938	8	7	3	0	0	0
9	0	0	2625	6	6	0	0	0	0
10	0	0	6995	13	5	0	0	3	0
11	0	0	18922	15	11	3	0	3	0
12	0	0	50655	7	10	6	0	1	0
13	0	0	125327	12	9	1	0	2	0
14	0	0	248408	19	21	8	0	8	0
15	0	0	350731	35	17	4	0	7	0
16	0	0	320618	76	28	11	0	2	0
17	0	0	196015	123	35	8	0	7	0
18	0	0	86350	213	32	12	0	13	0
19	0	0	29203	370	29	8	1	5	0
20	0	0	8062	718	46	14	1	15	0
21	0	0	1844	1152	50	8	0	13	0
22	0	0	344	2130	63	11	1	14	1
23	0	0	89	4308	64	13	1	15	0
24	0	0	25	8563	94	24	1	11	0
25	0	0	6	17732	131	10	0	9	1
26	0	0	1	35533	159	19	2	10	0
27	0	0	1	66340	191	19	0	8	1
28	0	0	0	110901	203	11	0	8	1
29	0	0	0	164165	260	11	2	10	1
30	0	0	0	210387	329	17	4	8	0
31	0	0	0	227828	394	15	4	13	1
32	0	0	0	209219	472	24	5	8	1
33	0	0	0	162202	555	13	8	5	0
34	0	0	0	106941	682	18	9	1	1
35	0	0	0	61277	738	14	7	6	4
36	0	0	0	31526	919	21	6	9	1

K	L	M	N	O	P	Q	R	S
l	Al Thickness	Mean Pixel Intensity	Median Pixel Intensity	Mode Pixel Intensity	SD Pixel Intensity		Camera Off	
era Off	0	0	0	0	0			
0	0	0	0	0	0		0	
1	0.02	13.24515915	10	13	1.746638715			
2	0.04	28.90613326	23	29	2.691083112			
3	0.06	53.92608864	41	54	5.267206029			
4	0.08	90.70767642	69	91	7.060791067			
5	0.1	120.3903238	90	121	9.562624119			
6	0.12	159.8239991	111	160	12.61669568			
7	0.14	210.1491987	145	215	14.04252507			



Appendix C: Quantitative Densitometry Worksheets

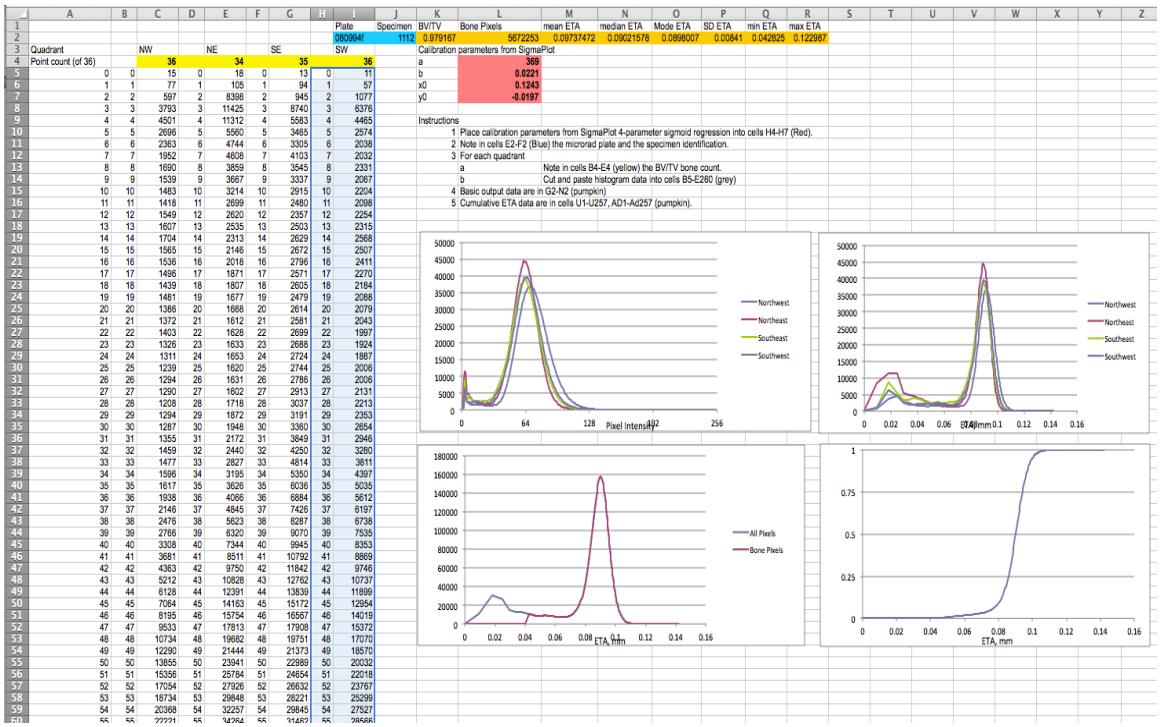


Plate	Specimen	BV/TV	Bone Pixels	mean ETA	median ETA	Mode ETA	SD ETA	min ETA	max ETA
080994f	1112	0.979167	5672253	0.09737472	0.09021578	0.0898007	0.00841	0.042825	0.122987

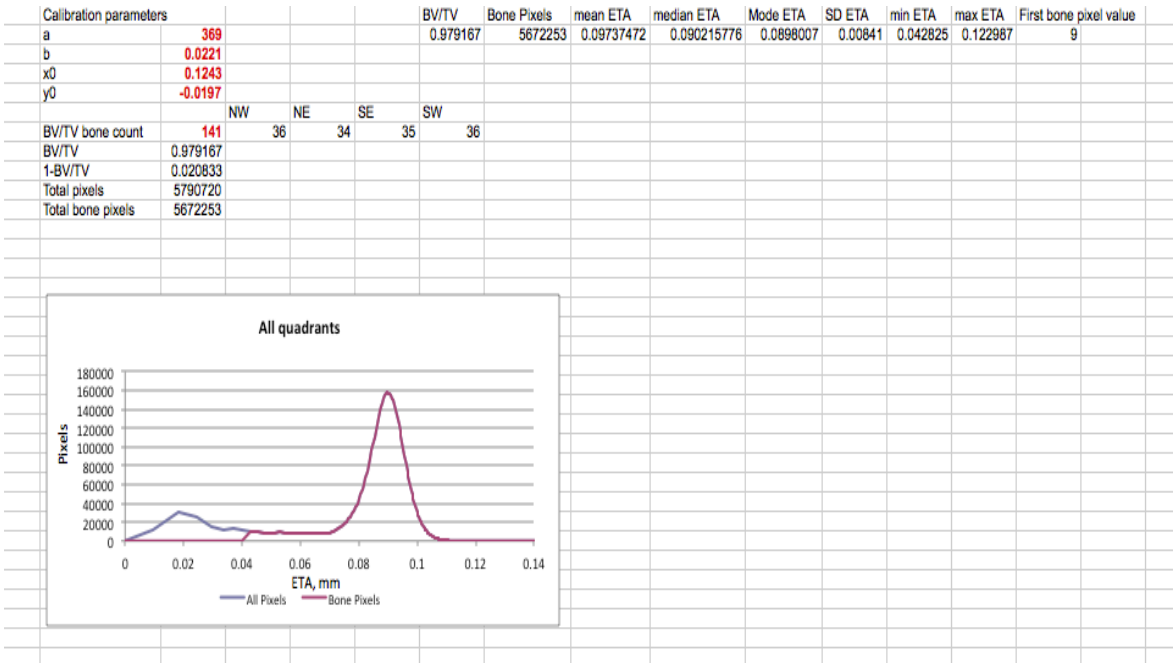
Calibration parameters from SigmaPlot

a	369
b	0.0221
x0	0.1243
y0	-0.0197

Instructions

1. Place calibration parameters from SigmaPlot 4-parameter sigmoid regression into cells H4-H7 (Red).
2. Note in cells E2-F2 (Blue) the microrad plate and the specimen identification.
3. For each quadrant
   - a. Note in cells B4-E4 (yellow) the BV/TV bone count.
   - b. Cut and paste histogram data into cells B5-E260 (grey)
4. Basic output data are in G2-N2 (pumpkin)
5. Cumulative ETA data are in cells U1-U257, AD1-Ad257 (pumpkin).

Quadrant		NW		NE		SE		SW	
Point count (of 36)		36		34		35		36	
0	0	15	0	18	0	13	0	11	
1	1	77	1	105	1	94	1	57	
2	2	597	2	8398	2	945	2	1077	
3	3	3793	3	11425	3	8740	3	6376	
4	4	4501	4	11312	4	5583	4	4465	
5	5	2696	5	5560	5	3465	5	2574	
6	6	2363	6	4744	6	3305	6	2036	
7	7	1952	7	4608	7	4103	7	2032	
8	8	1690	8	3859	8	3545	8	2331	
9	9	1539	9	3667	9	3337	9	2067	
10	10	1483	10	3214	10	2915	10	2204	
11	11	1418	11	2699	11	2480	11	2098	
12	12	1549	12	2620	12	2357	12	2254	
13	13	1607	13	2535	13	2503	13	2315	
14	14	1704	14	2313	14	2629	14	2568	
15	15	1565	15	2146	15	2672	15	2507	
16	16	1536	16	2018	16	2796	16	2411	
17	17	1496	17	1871	17	2571	17	2270	
18	18	1439	18	1807	18	2605	18	2184	
19	19	1481	19	1677	19	2479	19	2068	
20	20	1386	20	1668	20	2614	20	2079	
21	21	1372	21	1612	21	2581	21	2043	
22	22	1403	22	1628	22	2699	22	1997	
23	23	1326	23	1633	23	2688	23	1924	
24	24	1311	24	1653	24	2724	24	1867	
25	25	1239	25	1620	25	2744	25	2006	
26	26	1294	26	1631	26	2786	26	2006	
27	27	1290	27	1602	27	2913	27	2131	
28	28	1208	28	1718	28	3037	28	2213	
29	29	1294	29	1872	29	3191	29	2353	
30	30	1287	30	1946	30	3360	30	2654	
31	31	1355	31	2172	31	3849	31	2946	
32	32	1459	32	2440	32	4250	32	3280	
33	33	1477	33	2827	33	4814	33	3811	
34	34	1596	34	3195	34	5350	34	4397	
35	35	1617	35	3626	35	6036	35	5035	
36	36	1938	36	4066	36	6884	36	5612	
37	37	2146	37	4845	37	7426	37	6197	
38	38	2476	38	5623	38	8287	38	6738	
39	39	2766	39	6320	39	9070	39	7535	
40	40	3308	40	7344	40	9945	40	8353	
41	41	3681	41	8511	41	10792	41	8869	
42	42	4363	42	9750	42	11842	42	9746	
43	43	5212	43	10828	43	12762	43	10737	
44	44	6128	44	12391	44	13839	44	11899	
45	45	7064	45	14163	45	15172	45	12954	
46	46	8195	46	15754	46	16567	46	14019	
47	47	9533	47	17813	47	17908	47	15372	
48	48	10734	48	19682	48	19751	48	17070	
49	49	12290	49	21444	49	21373	49	18570	
50	50	13855	50	23941	50	22989	50	20232	
51	51	15356	51	25784	51	24654	51	22018	
52	52	17054	52	27926	52	26632	52	23767	
53	53	18734	53	29848	53	28221	53	25299	
54	54	20368	54	32257	54	29845	54	27527	



Calibration parameters		BV/TV	Bone Pixels	mean ETA	median ETA	Mode ETA	SD ETA	min ETA	max ETA	First bone pixel value
a	369	0.979167	5672253	0.09737472	0.090215776	0.0898007	0.00841	0.042825	0.122987	9
b	0.0221									
x0	0.1243									
y0	-0.0197									

		NW	NE	SE	SW
BV/TV bone count	141	36	34	35	36
BV/TV	0.979167				
1-BV/TV	0.020833				
Total pixels	5790720				
Total bone pixels	5672253				

Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ	AK	AL
	ETA, mm	NW	NE	SE	SW	All quadrants	Bone Pixels	cumulative bone pixels, proportion				
	0	15	18	13	11	57	0	0				
	0	77	105	94	57	333	0	0				
	0.009328	597	8398	945	1077	11017	0	0				
	0.018277	3793	11425	8740	6376	30334	0	0				
	0.024659	4501	11312	5583	4465	25861	0	0				
	0.02963	2696	5560	3465	2574	14295	0	0				
	0.033705	2363	4744	3305	2038	12450	0	0				
	0.037163	1952	4608	4103	2032	12695	0	0				
	0.040167	1690	3859	3545	2331	11425	0	0				
	0.042825	1539	3667	3337	2067	10610	10610	0.001870509				
	0.04521	1483	3214	2915	2204	9816	9816	0.003601038				
	0.047374	1418	2699	2480	2098	8695	8695	0.005133939				
	0.049356	1549	2620	2357	2254	8780	8780	0.006681825				
	0.051184	1607	2535	2503	2315	8960	8960	0.008261444				
	0.052882	1704	2313	2629	2568	9214	9214	0.009885843				
	0.054467	1565	2146	2672	2507	8890	8890	0.011453121				
	0.055954	1536	2018	2796	2411	8761	8761	0.012997657				
	0.057355	1496	1871	2571	2270	8208	8208	0.014444701				
	0.058679	1439	1807	2605	2184	8035	8035	0.015861246				
	0.059936	1481	1677	2479	2088	7725	7725	0.017223139				
	0.061132	1386	1668	2614	2079	7747	7747	0.01858891				
	0.062272	1372	1612	2581	2043	7608	7608	0.019930176				
	0.063363	1403	1628	2699	1997	7727	7727	0.021292421				
est	0.064408	1326	1633	2688	1924	7571	7571	0.022627164				
ist	0.065412	1311	1653	2724	1887	7575	7575	0.023962612				
ist	0.066378	1239	1620	2744	2006	7609	7609	0.025304055				
est	0.067308	1294	1631	2786	2006	7717	7717	0.026664537				
	0.068206	1290	1602	2913	2131	7936	7936	0.028063628				
	0.069074	1208	1718	3037	2213	8176	8176	0.029505031				
	0.069914	1294	1872	3191	2353	8710	8710	0.031040576				
	0.070728	1287	1948	3360	2654	9249	9249	0.032671145				
	0.071517	1355	2172	3849	2946	10322	10322	0.03449088				
	0.072284	1459	2440	4250	3280	11429	11429	0.036505776				
	0.073029	1477	2827	4814	3811	12929	12929	0.038785118				
	0.073754	1596	3195	5350	4397	14538	14538	0.04134812				
	0.074461	1617	3626	6036	5035	16314	16314	0.044224226				
	0.075149	1938	4066	6884	5612	18500	18500	0.047485717				
	0.075821	2146	4845	7426	6197	20614	20614	0.051119899				
	0.076477	2476	5623	8287	6738	23124	23124	0.055196586				
	0.077117	2766	6320	9070	7535	25691	25691	0.059725827				
	0.077744	3308	7344	9945	8353	28950	28950	0.064829619				
	0.078356	3681	8511	10792	8869	31853	31853	0.070445201				
	0.078956	4363	9750	11842	9746	35701	35701	0.076739172				
	0.079544	5212	10828	12762	10737	39539	39539	0.083709771				
	0.080119	6128	12391	13839	11899	44257	44257	0.091512138				
	0.080684	7064	14163	15172	12954	49353	49353	0.100212914				
	0.081238	8195	15754	16567	14019	54535	54535	0.109827259				
	0.081781	9533	17813	17908	15372	60626	60626	0.120515428				
	0.082315	10734	19682	19751	17070	67237	67237	0.132369096				
	0.08284	12290	21444	21373	18570	73677	73677	0.145358114				
0.16	0.083355	13855	23941	22989	20032	80817	80817	0.159605892				
	0.083862	15356	25784	24654	22018	87812	87812	0.175068866				
	0.084361	17054	27926	26632	23767	95379	95379	0.191901877				
	0.084851	18734	29848	28221	25299	102102	102102	0.209902132				
	0.085334	20368	32257	29845	27527	109997	109997	0.22929425				
	0.08581	22221	34264	31462	28566	116513	116513	0.249935118				
	0.086278	24124	36426	32947	30778	124275	124275	0.271744402				
	0.08674	25477	37840	34232	32735	130284	130284	0.294713053				
	0.087195	27386	39616	35501	34201	136704	136704	0.31881353				

Appendix D: Final output Values

082494e	1612	16	15	G	TB	1	2	Dorsal	0.674	3907335	0.099	0.095	0.096	0.017	0.023	0.126
181494f	1613	16	15	G	TB	1	3	Dorsal	0.646	3743115	0.084	0.079	0.080	0.013	0.032	0.139
081494e	1654	16	15	G	TB	5	4	Palmar	0.681	3941845	0.104	0.096	0.098	0.009	0.055	0.129
081294a	1655	16	15	G	TB	5	5	Palmar	0.424	2464727	0.090	0.089	0.095	0.016	0.041	0.126
081194g	1614	16	15	G	TB	1	4	Palmar	0.514	2977030	0.094	0.087	0.086	0.008	0.060	0.123
081994c	1616	16	15	G	TB	1	6	Palmar	0.194	1126352	0.099	0.092	0.091	0.008	0.067	0.123
080894a	1657	16	15	G	TB	5	7		0.924	5355342	0.101	0.092	0.092	0.004	0.077	0.122
080994h	1658	16	15	G	TB	5	8		0.563	3258269	0.094	0.088	0.089	0.010	0.052	0.119
081494a	1647	16	15	G	TB	4	7		0.931	5391493	0.105	0.097	0.101	0.010	0.052	0.133
080994j	1759	17	3	F	AR	5	9	Central	0.417	2414371	0.095	0.091	0.092	0.012	0.049	0.127
070594c	1711	17	3	F	AR	1	1	Dorsal	0.938	5430744	0.092	0.085	0.085	0.005	0.063	0.116
081194c	1712	17	3	F	AR	1	2	Dorsal	0.715	4149917	0.102	0.095	0.097	0.010	0.052	0.129
081194a	1713	17	3	F	AR	1	3	Dorsal	0.514	2988845	0.088	0.085	0.086	0.013	0.035	0.119
081194a	1754	17	3	F	AR	5	4	Palmar	0.729	4223338	0.099	0.093	0.093	0.008	0.047	0.118
081294f	1755	17	3	F	AR	5	5	Palmar	0.556	3236152	0.090	0.086	0.087	0.012	0.036	0.122
090294b	1756	17	3	F	AR	5	6	Palmar	0.924	5353262	0.104	0.097	0.099	0.012	0.028	0.136
080894b	1714	17	3	F	AR	1	4	Palmar	0.910	5276491	0.091	0.084	0.084	0.003	0.071	0.099
080994b	1715	17	3	F	AR	1	5	Palmar	0.451	2615422	0.092	0.087	0.087	0.008	0.047	0.117
081294a	1716	17	3	F	AR	1	6	Palmar	0.701	4062640	0.104	0.097	0.103	0.012	0.041	0.139
081294f	1758	17	3	F	AR	5	8		0.944	5473223	0.102	0.094	0.092	0.010	0.047	0.130
181494f	1748	17	3	F	AR	4	8		0.965	5590833	0.084	0.079	0.073	0.013	0.033	0.122
082494f	1859	18	9	F	TB	5	9	Central	0.208	1207902	0.067	0.064	0.065	0.009	0.039	0.093
090294c	1851	18	9	F	TB	5	1	Dorsal	0.979	5706473	0.104	0.097	0.099	0.016	0.012	0.136
090294d	1853	18	9	F	TB	5	3	Dorsal	0.979	5673228	0.109	0.101	0.101	0.010	0.044	0.137
080894b	1812	18	9	F	TB	1	2	Dorsal	0.958	5574648	0.090	0.083	0.083	0.004	0.063	0.098
082494f	1854	18	9	F	TB	5	4	Palmar	0.778	4506397	0.105	0.097	0.098	0.008	0.064	0.128
080894c	1855	18	9	F	TB	5	5	Palmar	0.931	5390309	0.120	0.109	0.107	0.009	0.075	0.146
070594e	1814	18	9	F	TB	1	4	Palmar	0.938	5430744	0.092	0.085	0.085	0.005	0.063	0.116
090294e	1815	18	9	F	TB	1	5	Palmar	0.340	2020456	0.106	0.096	0.094	0.004	0.091	0.137
070594f	1816	18	9	TB	F	1	6	Palmar	0.958	5560992	0.085	0.079	0.078	0.003	0.063	0.108
081994g	1847	18	9	F	TB	4	7		0.924	5351402	0.101	0.092	0.092	0.007	0.070	0.134
081994g	2119	21	4	M	QH	1	9	Central	0.354	2053434	0.098	0.091	0.091	0.008	0.060	0.122
052793e	2151	21	4	M	QH	5	1	Dorsal	0.931	5406512	0.048	0.047	0.048	0.005	0.028	0.089
081994b	2152	21	4	M	QH	5	2	Dorsal	0.938	5431716	0.082	0.077	0.077	0.006	0.052	0.104
082494e	2153	21	4	M	QH	5	3	Dorsal	0.646	3739960	0.108	0.100	0.101	0.009	0.059	0.136
080994i	2111	21	4	M	QH	1	1	Dorsal	0.868	5033484	0.101	0.092	0.090	0.009	0.056	0.133
081494d	2112	21	4	M	QH	1	2	Dorsal	0.938	5441316	0.094	0.087	0.087	0.007	0.060	0.130
080894c	2113	21	4	M	QH	1	3	Dorsal	0.910	5272994	0.094	0.087	0.087	0.007	0.055	0.125
081194j	2154	21	4	M	QH	5	4	Palmar	0.861	4988379	0.098	0.090	0.090	0.007	0.059	0.121
080994f	2155	21	4	M	QH	5	5	Palmar	0.910	5282268	0.077	0.072	0.072	0.007	0.037	0.118

Plate	Specime	Horse	Age	Gender	Breed	Proxi	Radia	Supersectors	BV/TV	Bone Pixels	mean ETA	median ETA	Mode ETA	SD ETA	min ETA	max ETA
080994K	1159	11	3	M	TB	5	9	Central	0.424	2473100	0.084	0.083	0.085	0.019	0.018	0.140
081294b	1119	11	3	M	TB	1	9	Central	0.167	965922	0.100	0.094	0.095	0.011	0.049	0.116
080994i	1152	11	3	M	TB	5	2	Dorsal	0.958	5549939	0.098	0.090	0.090	0.007	0.064	0.119
081494c	1153	11	3	M	TB	5	3	Dorsal	0.278	1610857	0.112	0.108	0.116	0.020	0.044	0.134
081994e	1111	11	3	M	TB	1	1	Dorsal	0.938	5431242	0.110	0.099	0.100	0.006	0.084	0.139
080994f	1112	11	3	M	TB	1	2	Dorsal	0.979	5672253	0.097	0.090	0.090	0.008	0.043	0.123
070594a	1113	11	3	M	TB	1	3	Dorsal	0.993	5753915	0.098	0.091	0.091	0.010	0.023	0.140
081994a	1154	11	3	M	TB	5	4	Palmar	0.785	4707517	0.092	0.089	0.021	0.019	0.021	0.125
081294a	1155	11	3	M	TB	5	5	Palmar	0.361	2103137	0.101	0.099	0.104	0.019	0.027	0.133
080994i	1115	11	3	M	TB	1	5	Palmar	0.979	5675215	0.096	0.091	0.090	0.014	0.023	0.124
081994g	1116	11	3	M	TB	1	6	Palmar	0.979	5674870	0.110	0.101	0.102	0.010	0.052	0.146
181494f	1158	11	3	M	TB	5	8		0.236	1372025	0.086	0.080	0.081	0.010	0.051	0.121
081994d	1147	11	3	M	TB	4	7		0.965	5589775	0.101	0.093	0.095	0.009	0.054	0.148
090294b	1419	14	1	F	TB	1	9	Central	0.326	1894013	0.103	0.097	0.099	0.016	0.035	0.139
070594g	1451	14	1	F	TB	5	1	Dorsal	0.618	3625576	0.096	0.088	0.087	0.005	0.078	0.106
081994b	1453	14	1	F	TB	5	3	Dorsal	0.965	5590444	0.128	0.115	0.114	0.009	0.061	0.170
081494e	1412	14	1	F	TB	1	2	Dorsal	0.660	3831446	0.097	0.091	0.091	0.010	0.042	0.121
081494e	1413	14	1	F	TB	1	3	Dorsal	0.576	3343196	0.102	0.095	0.097	0.013	0.040	0.135
081994a	1454	14	1	F	TB	5	4	Palmar	0.840	4868521	0.086	0.080	0.081	0.008	0.044	0.127
081994i	1456	14	1	F	TB	5	6	Palmar	0.938	5432030	0.089	0.083	0.083	0.007	0.054	0.124
081994g	1415	14	1	F	TB	1	5	Palmar	0.625	3626963	0.080	0.075	0.075	0.006	0.047	0.101
081294f	1416	14	1	F	TB	1	6	Palmar	0.694	4025904	0.094	0.087	0.088	0.006	0.068	0.113
080894b	1457	14	1	F	TB	5	7		0.882	5143973	0.080	0.074	0.073	0.004	0.060	0.103
081994d	1458	14	1	F	TB	5	8		0.819	4746987	0.075	0.070	0.069	0.006	0.054	0.112
081294b	1447	14	1	F	TB	4	7		0.924	5349352	0.095	0.087	0.084	0.008	0.064	0.124
080994k	1448	14	1	F	TB	4	8		0.924	5352639	0.084	0.079	0.080	0.010	0.035	0.128
052793e	1559	15	1	F	TB	5	9	Central	0.257	1509177	0.039	0.037	0.036	0.004	0.031	0.059
053193a	1519	15	1	F	TB	1	9	Central	0.889	5149123	0.104	0.096	0.096	0.007	0.056	0.118
081994d	1552	15	1	F	TB	4	2		0.660	3834090	0.078	0.076	0.076	0.012	0.033	0.155
070594c	1512	15	1	F	TB	1	2	Dorsal	0.819	4766521	0.093	0.087	0.085	0.007	0.060	0.133
090294c	1554	15	1	F	TB	5	4	Palmar	0.882	5107380	0.092	0.087	0.088	0.012	0.029	0.134
082494d	1556	15	1	F	TB	5	6	Palmar	0.847	4910587	0.070	0.066	0.068	0.007	0.039	0.113
090294a	1514	15	1	F	TB	1	4	Palmar	0.938	5434116	0.097	0.091	0.092	0.010	0.037	0.144
081994c	1516	15	1	F	TB	1	6	Palmar	0.917	5315844	0.095	0.088	0.088	0.007	0.059	0.123
070594d	1547	15	1	F	TB	4	7		0.819	4766521	0.086	0.081	0.079	0.009	0.053	0.134
090294c	1659	16	15	G	TB	5	9	Central	0.278	1630167	0.076	0.082	0.013	0.027	0.013	0.145
080994c	1651	16	15	G	TB	5	1	Dorsal	0.882	5112040	0.106	0.097	0.097	0.006	0.080	0.122
080894b	1653	16	15	G	TB	5	3	Dorsal	0.507	2935880	0.100	0.092	0.092	0.005	0.072	0.121
081994f	1611	16	15	G	TB	1	1	Dorsal	0.625	3619446	0.095	0.088	0.088	0.005	0.069	0.112

080994f	2155	21	4	M	QH	5	5	Palmar	0.910	5282268	0.077	0.072	0.072	0.007	0.037	0.118
090294f	2156	21	4	M	QH	5	6	Palmar	0.729	4229855	0.085	0.080	0.082	0.011	0.032	0.143
080894a	2114	21	4	M	QH	1	4	Palmar	0.958	5560992	0.084	0.079	0.078	0.003	0.065	0.106
081194e	2115	21	4	M	QH	1	5	Palmar	0.542	3140391	0.111	0.101	0.100	0.007	0.080	0.132
082494c	2116	21	4	M	QH	1	6	Palmar	0.396	2293244	0.092	0.086	0.087	0.010	0.046	0.122
091294a	2147	21	4	M	QH	4	7		0.917	5311428	0.083	0.078	0.079	0.007	0.041	0.099
081194j	2259	22	11	G	AR	5	9	Central	0.215	1248508	0.095	0.093	0.094	0.017	0.037	0.145
081194b	2219	22	11	G	AR	1	9	Central	0.278	1621509	0.098	0.092	0.092	0.010	0.054	0.120
070594e	2251	22	11	G	AR	5	1	Dorsal	0.674	3912467	0.093	0.087	0.087	0.005	0.068	0.126
053193a	2213	22	11	G	AR	1	3	Dorsal	0.938	5429203	0.094	0.087	0.087	0.004	0.072	0.112
091294a	2254	22	11	AR	G	5	4	Palmar	0.625	3619446	0.094	0.087	0.087	0.005	0.068	0.110
081994i	2255	22	11	G	AR	5	5	Palmar	0.972	5632832	0.097	0.090	0.090	0.008	0.049	0.119
090294e	2256	22	11	G	AR	5	6	Palmar	0.944	5474406	0.086	0.081	0.082	0.010	0.027	0.122
081494c	2214	22	11	G	AR	1	4	Palmar	0.806	4669183	0.106	0.099	0.100	0.012	0.033	0.132
081294d	2216	22	11	G	AR	1	6	Palmar	0.986	5742487	0.088	0.083	0.086	0.012	0.021	0.114
081494b	2257	22	11	G	AR	5	7		0.951	5511239	0.102	0.093	0.093	0.007	0.069	0.126
081194a	2258	22	11	G	AR	5	8		0.611	3544634	0.085	0.079	0.079	0.007	0.049	0.108
080994a	2247	22	11	G	AR	4	7		0.965	5590422	0.102	0.094	0.095	0.009	0.044	0.126
081494a	2248	22	11	G	AR	4	8		0.931	5396723	0.100	0.092	0.093	0.008	0.074	0.124
081494a	2359	23	20	F	TB	5	9	Central	0.292	1691440	0.099	0.099	0.104	0.022	0.030	0.135
081294e	2349	23	20	F	TB	4	9		0.924	5358283	0.109	0.102	0.102	0.015	0.029	0.134
081294d	2353	23	20	F	TB	5	3	Dorsal	0.931	5389001	0.098	0.091	0.099	0.009	0.047	0.167
090294d	2311	23	20	F	TB	1	1	Dorsal	0.903	5229203	0.100	0.092	0.094	0.008	0.056	0.140
053193a	2312	23	20	F	TB	1	2	Dorsal	0.250	2160845	0.068	0.079	0.027	0.028	0.027	0.108
080994g	2313	23	20	F	TB	1	3	Dorsal	0.514	2984873	0.075	0.071	0.071	0.008	0.041	0.133
081294d	2356	23	20	F	TB	5	6	Palmar	0.417	2415136	0.107	0.102	0.104	0.014	0.041	0.167
081194h	2314	23	20	F	TB	1	4	Palmar	0.924	5350288	0.103	0.095	0.095	0.007	0.059	0.124
081194h	2316	23	20	F	TB	1	6	Palmar	0.944	5469180	0.100	0.093	0.093	0.007	0.057	0.134
081994f	2347	23	20	F	TB	4	7		0.958	5556113	0.103	0.095	0.095	0.007	0.063	0.138
081194e	2559	25	0.42	M	TB	5	9	Central	0.271	1579500	0.078	0.075	0.073	0.013	0.026	0.126
081194b	2519	25	0.42	M	TB	1	9	Central	0.236	1370703	0.093	0.086	0.085	0.004	0.077	0.137
070594b	2511	25	0.42	M	TB	1	1	Dorsal	0.354	2058099	0.085	0.080	0.081	0.006	0.057	0.111
090294f	2556	25	0.42	M	TB	5	6	Palmar	0.292	1691527	0.089	0.086	0.088	0.017	0.021	0.142
080994g	2514	25	0.42	M	TB	1	4	Palmar	0.333	1931685	0.082	0.078	0.078	0.009	0.044	0.124
053193b	2516	25	0.42	M	TB	1	6	Palmar	0.986	5783473	0.091	0.086	0.087	0.011	0.028	0.138

Appendix E: SPSS Mixed Model Instructions

SPSS Mixed Model Instructions

When analyzing a repeated measures data set using a linear mixed model, structure the data such that each data point is a case (i.e., row). An example mixed model with repeated measures data set is provided in Table 2. The same data set in the RM ANOVA structure is shown in Table 1.

Table 1: Repeated Measures ANOVA data set

Nutrition	**Cr_9_c**	**Cd_9_c**
Control	332.5	263.45
Control	390.3883261	298.0639952
Control	284.1097812	223.7992776
Control	266.4999755	226.42
Control	338.5483894	252.8979422
Control	324.165	237.4984528
Control	474.897405	352.7336918
Control	260.9154317	233.7995279
Control	456.5245098	316.0785278
Control	330.4999871	288
Control	268.5757858	213.9272315
Control	389.1750899	326.316
MA	270.985	221.0312746
MA	258.9993113	217.0321148
MA	254.005	231.6383368

MA	311.6900551	264.4983112
MA	327.585	292.2499804
MA	240.505	161.7951724
MA	288.1997608	210.235
MA	169.1	134.601
MA	235.9996787	191.6298914
MA	272.9999492	232.2698613
MA	524.721773	415.04
MA	414.3228928	346.74

Table 2: Mixed Model Data Set

ID	Nutrition	Side	c_9 Hz
1	Control	Cr	332.5
2	Control	Cr	390.3883
3	Control	Cr	284.1098
4	Control	Cr	266.5
5	Control	Cr	338.5484
6	Control	Cr	324.165
7	Control	Cr	474.8974
8	Control	Cr	260.9154
9	Control	Cr	456.5245
10	Control	Cr	330.5
11	Control	Cr	268.5758
12	Control	Cr	389.1751
13	MA	Cr	270.985

14	MA	Cr	258.9993
15	MA	Cr	254.005
16	MA	Cr	311.6901
17	MA	Cr	327.585
18	MA	Cr	240.505
19	MA	Cr	288.1998
20	MA	Cr	169.1
21	MA	Cr	235.9997
22	MA	Cr	272.9999
23	MA	Cr	524.7218
24	MA	Cr	414.3229
1	Control	Cd	263.45
2	Control	Cd	298.064
3	Control	Cd	223.7993
4	Control	Cd	226.42
5	Control	Cd	252.8979
6	Control	Cd	237.4985
7	Control	Cd	352.7337
8	Control	Cd	233.7995
9	Control	Cd	316.0785
10	Control	C	288

		d	
11	Control	Cd	213.9272
12	Control	Cd	326.316
13	MA	Cd	221.0313
14	MA	Cd	217.0321
15	MA	Cd	231.6383
16	MA	Cd	264.4983
17	MA	Cd	292.25
18	MA	Cd	161.7952
19	MA	Cd	210.235
20	MA	Cd	134.601
21	MA	Cd	191.6299
22	MA	Cd	232.2699
23	MA	Cd	415.04
24	MA	Cd	346.74

To analyze the Table 2 data set in SPSS:

Analyze → Mixed Models → Linear...

The initial user interface (Figure 1) prompts the user to specify subjects and repeated variables. ID will be listed in the left box. Highlight ID and click the blue arrow next to the Subjects box to specify it as the Subject variable. Click continue.

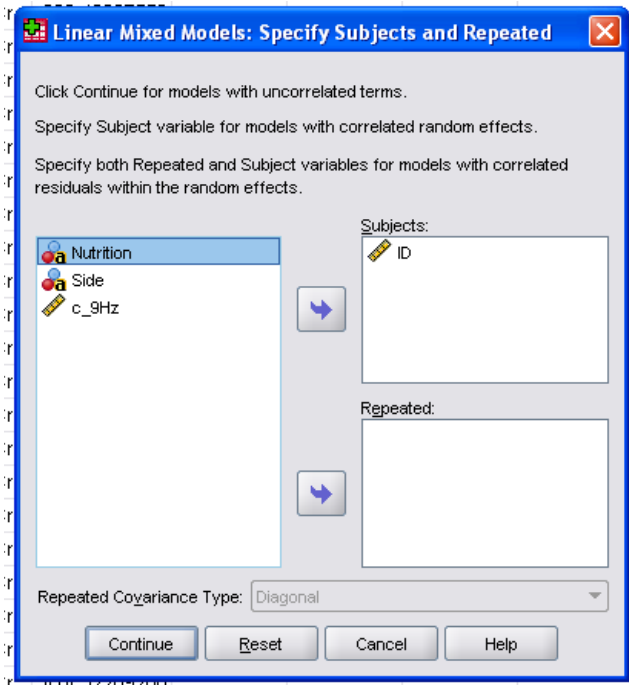


Figure 1: Specify Subjects and Repeated variables

The next interface (Figure 2) is the main interface for the Linear Mixed Models setup. From the left variables box, highlight the dependent variable (in this case, “c_9Hz”) and click the blue arrow to the left of the Dependent Variable box. Highlight the factor(s) (in this example, “Nutrition” and “Side”) and click the

blue arrow to the left of the Factor(s) box to select the variable(s). Next, select the “Fixed...” button on the right to designate factor(s) as fixed.

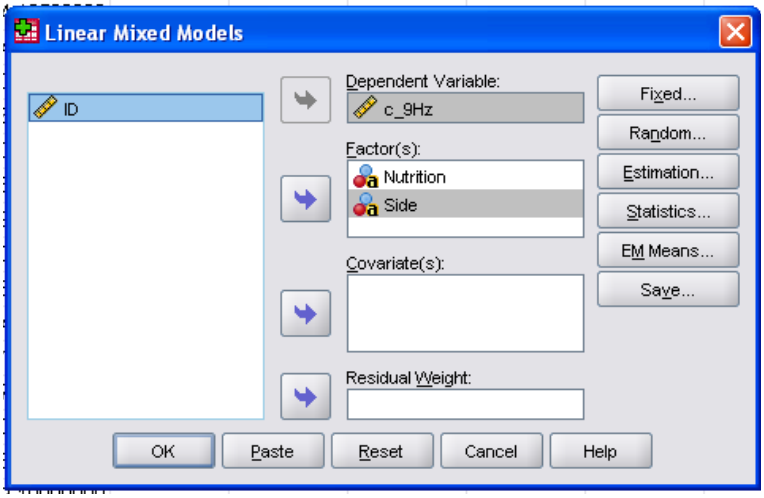


Figure 2: Linear Mixed Models main interface

Figure 3 displays the interface in which the user defines the fixed effects. All of the factors and covariates designated in the main menu will be listed in the “Factors and Covariates” box on the left. Highlight only those to be fixed effects and click the Add button beneath the model box. In this example, it happens that both factors are fixed effects. Click the Continue button.

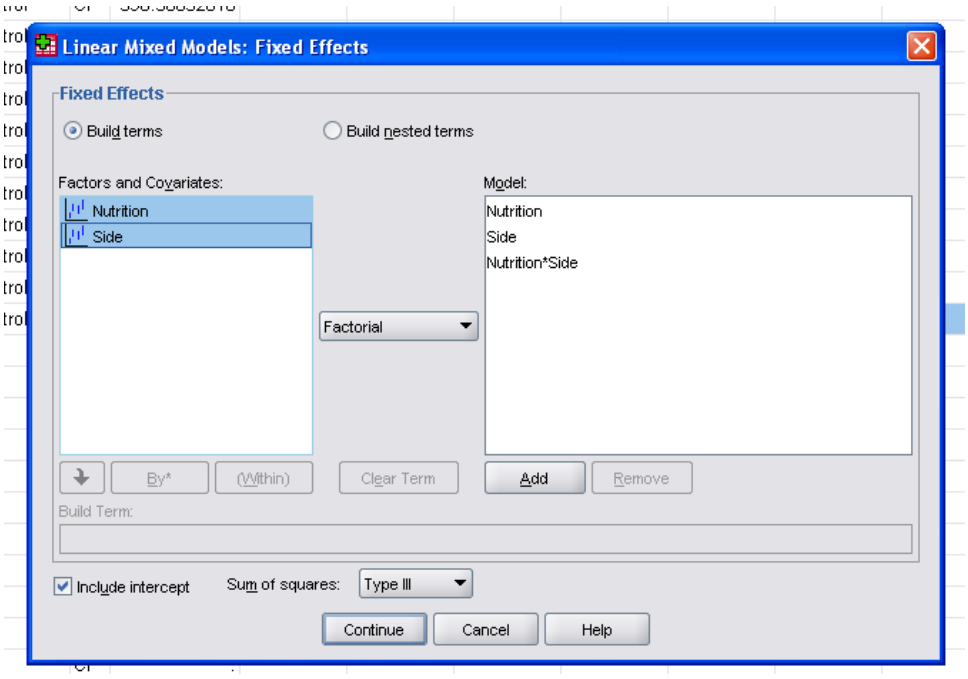


Figure 3: Fixed Effects interface

Clicking the “Random...” button in the main interface will direct the user to the Random Effects interface (Figure 4). Highlight ID within the Subject box in the bottom left of the interface and click the blue button to designate as a combination. Click the dropdown menu next to Covariance Type in the upper center of the interface and select “Compound Symmetry.” Check the checkbox next to “Include intercept.” Click the Continue button.

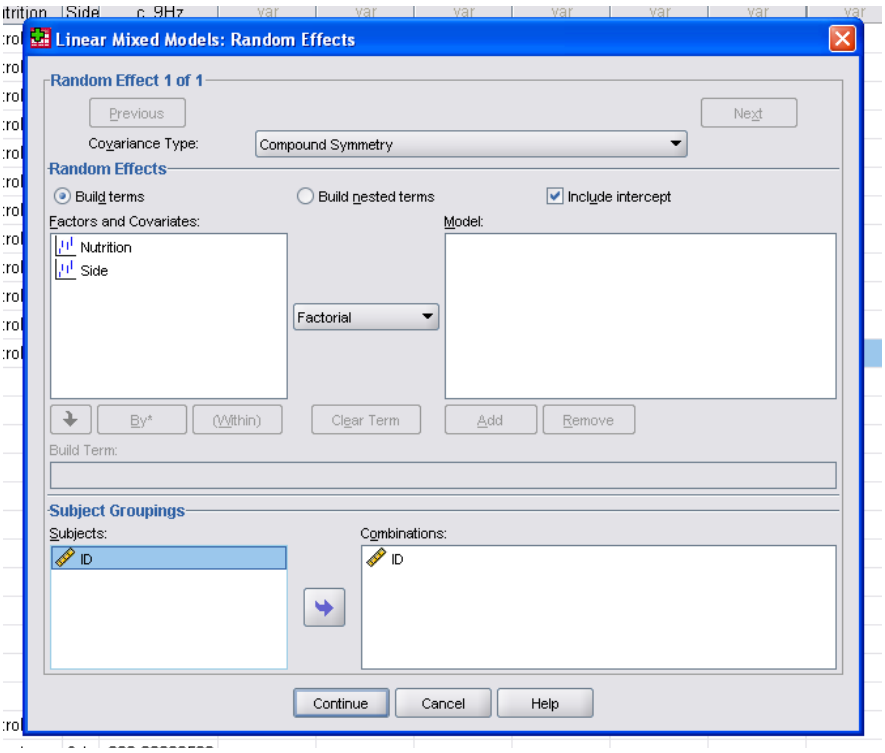


Figure 4: Random Effects interface

Clicking the “Estimation...” button will direct the user to the Estimation interface. Beneath “Method”, choose the “Maximum Likelihood (ML)” method. Click Continue.

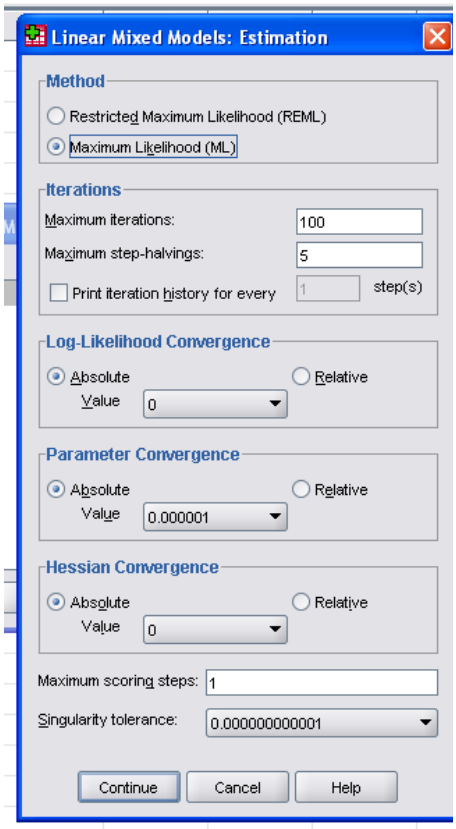


Figure 5: Estimation interface

If additional tests, estimates, or descriptive statistics are desired, the options are located through the “Statistics...” button.

Click OK in the main interface to run the linear mixed model.

Appendix F: Specimen Inventory

Level 1 Inventory

Horse	1	2	3	4	5	6	7	8	9
11	x	x	x	x	x	x			x
14		x	x		x	x			x
15				x		x			x
16	x	x	x	x		x			
17		x	x	x	x	x			
18		x		x	x				
21	x	x	x	x	x	x			x
22			x	x		x			x
23	x	x	x	x		x			
25	x			x		x			x

Level 5 Inventory

Horse	1	2	3	4	5	6	7	8	9
11		x	x	x	x			x	x
14	x		x	x		x	x	x	
15				x		x			
16	x		x	x	x		x	x	x
17				x	x	x		x	x
18	x		x	x	x				x
21	x	x	x	x	x	x			
22	x				x	x	x	x	x
23			x			x			x
25						x			x

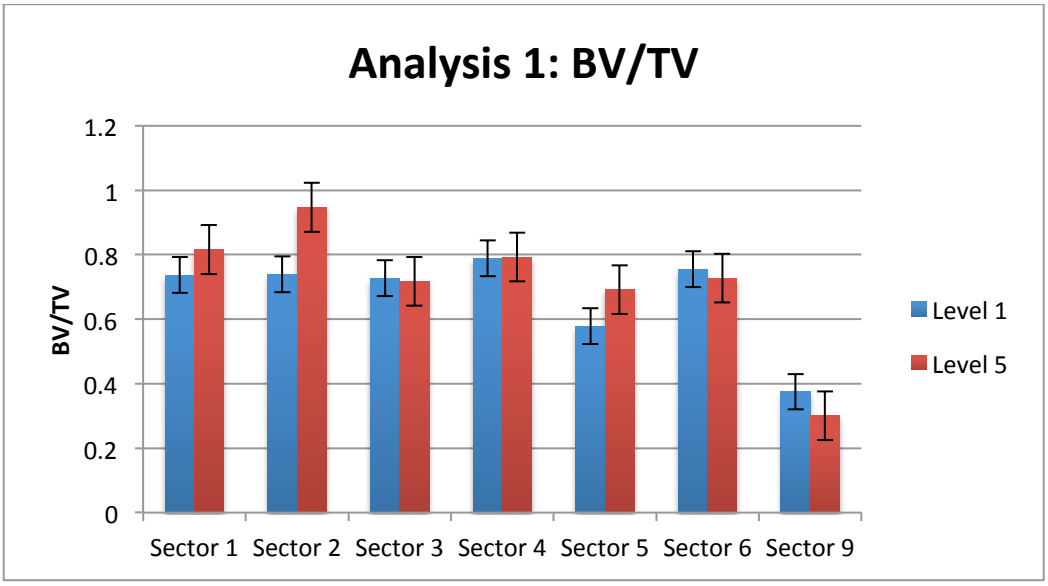
Level 4 Inventory

Horse	1	2	3	4	5	6	7	8	9
11						x			
14						x	x		
15		x				x			
16						x			
17							x		
18						x			
21						x			
22						x	x		
23						x		x	

Appendix G: Densitometry Output For All Specimens

	Plate	Specime	Horse	Age	Gender	Breed	Proxi	Radia	Supersectors	BV/TV	Bone Pixels	mean ETA	median ETA	Mode ETA	SD ETA	min ETA	max ETA
2	080994K	1159	11	3	M	TB	5	9	Central	0.42361111	2473100	0.08387595	0.083227	0.08532059	0.01905647	0.0182529	0.13968572
3	081294b	1119	11	3	M	TB	1	9	Central	0.16666667	965922	0.09959411	0.09366095	0.0954007	0.01125832	0.04895111	0.11552768
4	080994i	1152	11	3	M	TB	5	2	Dorsal	0.95833333	5549939	0.09751141	0.08990108	0.08990108	0.00654751	0.06397071	0.11891859
5	081494c	1153	11	3	M	TB	5	3	Dorsal	0.27777778	1610857	0.11200011	0.10759462	0.11608969	0.02000037	0.04412154	0.13430326
6	081994e	1111	11	3	M	TB	1	1	Dorsal	0.9375	5431242	0.10976562	0.09891029	0.09986771	0.0057787	0.08351173	0.1393506
7	080994f	1112	11	3	M	TB	1	2	Dorsal	0.97916667	5672253	0.09737472	0.09021578	0.08980065	0.00841013	0.0428253	0.12298672
8	070594a	1113	11	3	M	TB	1	3	Dorsal	0.99305556	5753915	0.09788617	0.09127641	0.09127641	0.00964421	0.02317632	0.14038855
9	081994a	1154	11	3	M	TB	5	4	Palmar	0.78472222	4707517	0.09181563	0.08920569	0.02099524	0.0187874	0.02099524	0.12459782
10	081294a	1155	11	3	M	TB	5	5	Palmar	0.36111111	2103137	0.10069556	0.09891029	0.10382019	0.01918012	0.02710038	0.13309074
11	080994i	1115	11	3	M	TB	1	5	Palmar	0.979167	5675215	0.095791	0.090503	0.090202	0.013709	0.023146	0.12447
12	081994g	1116	11	3	M	TB	1	6	Palmar	0.97916667	5674870	0.10992927	0.10128158	0.10178906	0.00982739	0.05157899	0.14578217
13	181494f	1158	11	3	M	TB	5	8		0.23611111	1372025	0.08573687	0.0802748	0.08068132	0.01046873	0.05103346	0.12050833
14	081994d	1147	11	3	M	TB	4	7		0.96527778	5589775	0.10069895	0.09340806	0.09488387	0.00862069	0.05409798	0.14792635
15	090294b	1419	14	1	F	TB	1	9	Central	0.32638889	1894013	0.10253503	0.09748387	0.09928273	0.01626229	0.03535957	0.13855418
16	070594g	1451	14	1	F	TB	5	1	Dorsal	0.61805556	3625576	0.09581822	0.08841796	0.08734524	0.00464173	0.07780328	0.10643645
17	081994b	1453	14	1	F	TB	5	3	Dorsal	0.96527778	5590444	0.12770273	0.11520411	0.11409553	0.00900199	0.0606718	0.16987014
18	081494e	1412	14	1	F	TB	1	2	Dorsal	0.65972222	3831446	0.09683326	0.09076147	0.09105654	0.01025391	0.04226095	0.12065891
19	081494e	1413	14	1	F	TB	1	3	Dorsal	0.57638889	3343196	0.10163042	0.09531216	0.09747973	0.01348695	0.04029103	0.13487999
20	081994a	1454	14	1	F	TB	5	4	Palmar	0.84027778	4868521	0.08550765	0.08028941	0.08120506	0.0082909	0.04373952	0.12688828
21	081994i	1456	14	1	F	TB	5	6	Palmar	0.9375	5432030	0.08895748	0.08274757	0.08339789	0.00735226	0.05352911	0.12414344
22	081994g	1415	14	1	F	TB	1	5	Palmar	0.625	3626963	0.08024589	0.07509751	0.07465021	0.00607396	0.04705324	0.10128158
23	081294f	1416	14	1	F	TB	1	6	Palmar	0.69444444	4025904	0.09425056	0.08719836	0.08844347	0.00648375	0.06776435	0.11317869
24	080894b	1457	14	1	F	TB	5	7		0.88194444	5143973	0.07956217	0.07437108	0.07339737	0.00418157	0.06008906	0.10260401
25	081994d	1458	14	1	F	TB	5	8		0.81944444	4746987	0.07540068	0.07027247	0.0692454	0.00595674	0.05365863	0.11186399
26	081294b	1447	14	1	F	TB	4	7		0.92361111	5349352	0.09528031	0.087066	0.08434134	0.00831622	0.06372968	0.1238643
27	080994k	1448	14	1	F	TB	4	8		0.92361111	5352639	0.08424751	0.07920876	0.07995927	0.01012748	0.0347484	0.12773885
28	052793e	1559	15	1	F	TB	5	9	Central	0.25694444	1509177	0.03949762	0.03737867	0.03608927	0.00411023	0.03057231	0.05896385
29	053193a	1519	15	1	F	TB	1	9	Central	0.88888889	5149123	0.1037782	0.09574571	0.096054	0.00650315	0.05610979	0.11809054
30	081994d	1552	15	1	F	TB	4	2		0.65972222	3834090	0.07802737	0.07550512	0.0762161	0.01173497	0.03263482	0.15489719
31	070594c	1512	15	1	F	TB	1	2	Dorsal	0.81944444	4766521	0.09311003	0.08692739	0.08537164	0.00738461	0.05988713	0.13260121
32	090294c	1554	15	1	F	TB	5	4	Palmar	0.88194444	5107380	0.09232071	0.08718207	0.08847942	0.01201497	0.02908965	0.13406748
33	082494d	1556	15	1	F	TB	5	6	Palmar	0.84722222	4910587	0.07027216	0.06649487	0.06774663	0.0071454	0.03856133	0.11271949
34	090294a	1514	15	1	F	TB	1	4	Palmar	0.9375	5434116	0.09655914	0.09083825	0.09194229	0.01020506	0.03721475	0.14360377
35	081994c	1516	15	1	F	TB	1	6	Palmar	0.91666667	5315844	0.09456172	0.08821548	0.08821548	0.00735086	0.05929192	0.12319488
36	070594d	1547	15	1	F	TB	4	7		0.81944444	4766521	0.08624308	0.08102968	0.07912489	0.00868438	0.05271619	0.13415773
37	090294c	1659	16	15	G	TB	5	9	Central	0.27777778	1630167	0.0755977	0.0822366	0.01312184	0.0270331	0.01312184	0.14468042
38	080994c	1651	16	15	G	TB	5	1	Dorsal	0.88194444	5112040	0.10565521	0.09675574	0.09708798	0.00570128	0.07959989	0.12175621
39	080894b	1653	16	15	G	TB	5	3	Dorsal	0.50694444	2935880	0.09990915	0.09222925	0.09167411	0.00513106	0.07234141	0.12127425
40	081994f	1611	16	15	G	TB	1	1	Dorsal	0.625	3619446	0.09503296	0.0878072	0.08822833	0.00519266	0.06866972	0.11165999
41	082494e	1612	16	15	G	TB	1	2	Dorsal	0.67361111	3907335	0.0990508	0.09497946	0.09639768	0.01660691	0.02333486	0.12609592
42	181494f	1613	16	15	G	TB	1	3	Dorsal	0.64583333	3743115	0.08369871	0.07945407	0.0802748	0.0127333	0.03176713	0.13897396
43	081494e	1654	16	15	G	TB	5	4	Palmar	0.68055556	3941845	0.10426526	0.09640306	0.09774691	0.00875998	0.05465701	0.12850843
44	081294a	1655	16	15	G	TB	5	5	Palmar	0.42361111	2464727	0.09021924	0.08867381	0.09467781	0.01635105	0.04052448	0.12591759
45	081194g	1614	16	15	G	TB	1	4	Palmar	0.51388889	2977030	0.09433422	0.08699236	0.08584826	0.00784299	0.06024431	0.12343166
46	081994c	1616	16	15	G	TB	1	6	Palmar	0.19444444	1126352	0.09914005	0.09182649	0.09133373	0.0081619	0.06702883	0.12319488
47	080894a	1657	16	15	G	TB	5	7		0.92361111	5355342	0.1007216	0.09244169	0.09194776	0.00380647	0.07688358	0.1221636
48	080994h	1658	16	15	G	TB	5	8		0.5625	3258269	0.09427184	0.08798946	0.08931021	0.01037254	0.05178661	0.11888413
49	081494a	1647	16	15	G	TB	4	7		0.93055556	5391493	0.1047614	0.09719328	0.10132808	0.01022181	0.0523253	0.1328616
50	080994j	1759	17	3	F	AR	5	9	Central	0.41666667	2414371	0.09549462	0.0906451	0.09193175	0.01160932	0.04899536	0.12717987

50	080994j	1759	17	3	F	AR	5	9	Central	0.41666667	2414371	0.09549462	0.0906451	0.09193175	0.01160932	0.04899536	0.12717987
51	070594c	1711	17	3	F	AR	1	1	Dorsal	0.9375	5430744	0.09205864	0.08524188	0.08524188	0.00496562	0.06307921	0.11606013
52	081194c	1712	17	3	F	AR	1	2	Dorsal	0.71527778	4149917	0.10181544	0.0953255	0.0967764	0.00984008	0.05235584	0.12915367
53	081194a	1713	17	3	F	AR	1	3	Dorsal	0.51388889	2988845	0.08817974	0.08463719	0.08635992	0.01251061	0.03460603	0.11850902
54	081194a	1754	17	3	F	AR	5	4	Palmar	0.72916667	4223338	0.09946547	0.09265196	0.09313897	0.00847096	0.04702843	0.11820795
55	081294f	1755	17	3	F	AR	5	5	Palmar	0.55555556	3236152	0.09041423	0.08590434	0.08719836	0.01206136	0.035665	0.12218812
56	090294b	1756	17	3	F	AR	5	6	Palmar	0.92361111	5353262	0.10425548	0.09748387	0.09868487	0.0124845	0.0284566	0.13628477
57	080894b	1714	17	3	F	AR	1	4	Palmar	0.90972222	5276491	0.09116314	0.0843698	0.0843698	0.00327825	0.07118714	0.09916194
58	080994b	1715	17	3	F	AR	1	5	Palmar	0.45138889	2615422	0.0921671	0.08683333	0.08735385	0.00848349	0.04671842	0.11701137
59	081294a	1716	17	3	F	AR	1	6	Palmar	0.70138889	4062640	0.10368013	0.09689104	0.10340982	0.01180439	0.04052448	0.13938958
60	081294f	1758	17	3	F	AR	5	8		0.94444444	5473223	0.10243712	0.09405475	0.0924674	0.01012061	0.04680167	0.12987421
61	181494f	1748	17	3	F	AR	4	8		0.96527778	5590833	0.08443598	0.07862267	0.07337702	0.01252587	0.03302264	0.12220732
62	082494f	1859	18	9	F	TB	5	9	Central	0.20833333	1207902	0.06737779	0.06383091	0.06460329	0.00907103	0.03863605	0.09309415
63	090294c	1851	18	9	F	TB	5	1	Dorsal	0.97916667	5706473	0.10364839	0.09703117	0.0988421	0.01599295	0.01212527	0.13643327
64	090294d	1853	18	9	F	TB	5	3	Dorsal	0.97916667	5673228	0.10922191	0.1006411	0.10106087	0.01043637	0.04379943	0.13653019
65	080894b	1812	18	9	F	TB	1	2	Dorsal	0.95833333	5574648	0.08971097	0.08345292	0.08345292	0.00418032	0.06280737	0.0979273
66	082494f	1854	18	9	F	TB	5	4	Palmar	0.77777778	4506397	0.10540608	0.09696766	0.09790744	0.00836832	0.06383091	0.12795191
67	080894c	1855	18	9	F	TB	5	5	Palmar	0.93055556	5390309	0.12000429	0.10867029	0.10746887	0.00883234	0.07516143	0.1455902
68	070594e	1814	18	9	F	TB	1	4	Palmar	0.9375	5430744	0.09220217	0.08537164	0.08537164	0.00500763	0.06293211	0.11633274
69	090294e	1815	18	9	F	TB	1	5	Palmar	0.34027778	2020456	0.10597758	0.09615135	0.09358627	0.00379951	0.09142186	0.13665399
70	070594f	1816	18	9	TB	F	1	6	Palmar	0.95833333	5560992	0.08460669	0.0790323	0.07836249	0.00343314	0.06280737	0.10770282
71	081994g	1847	18	9	F	TB	4	7		0.92361111	5351402	0.10093365	0.09243179	0.09188328	0.00653812	0.07021597	0.13412367
72	081994g	2119	21	4	M	QH	1	9	Central	0.35416667	2053434	0.09790654	0.09077085	0.09132976	0.00837	0.06010325	0.12152974
73	052793e	2151	21	4	M	QH	5	1	Dorsal	0.93055556	5406512	0.04766427	0.04666802	0.04772055	0.00538431	0.02756548	0.08901468
74	081994b	2152	21	4	M	QH	5	2	Dorsal	0.9375	5431716	0.08210122	0.0768307	0.07731742	0.00578538	0.05153579	0.10382846
75	082494e	2153	21	4	M	QH	5	3	Dorsal	0.64583333	3739960	0.10835636	0.10005008	0.1013764	0.00918088	0.05860557	0.13562972
76	080994i	2111	21	4	M	QH	1	1	Dorsal	0.86805556	5033484	0.10066892	0.09162054	0.09028544	0.00936022	0.05607546	0.13288671
77	081494d	2112	21	4	M	QH	1	2	Dorsal	0.9375	5441316	0.0942029	0.08740399	0.08680998	0.00682512	0.06034307	0.1299557
78	080894c	2113	21	4	M	QH	1	3	Dorsal	0.90972222	5272994	0.09384379	0.08716432	0.08716432	0.00690194	0.05513138	0.12485463
79	081194j	2154	21	4	M	QH	5	4	Palmar	0.86111111	4988379	0.09809634	0.09020662	0.08952088	0.00655176	0.05898659	0.12147288
80	080994f	2155	21	4	M	QH	5	5	Palmar	0.90972222	5282268	0.07664381	0.07228382	0.07151713	0.00741896	0.03716259	0.11840056
81	090294f	2156	21	4	M	QH	5	6	Palmar	0.72916667	4229855	0.08464634	0.08049626	0.0819987	0.01084805	0.03203347	0.14250834
82	080894a	2114	21	4	M	QH	1	4	Palmar	0.95833333	5560992	0.08444767	0.07881631	0.0782039	0.00309424	0.06482723	0.10611562
83	081194e	2115	21	4	M	QH	1	5	Palmar	0.54166667	3140391	0.11086196	0.10078655	0.10045985	0.00684668	0.07954807	0.13223874
84	082494c	2116	21	4	M	QH	1	6	Palmar	0.39583333	2293244	0.09215482	0.08649965	0.08720025	0.01042278	0.04583523	0.12238939
85	091294a	2147	21	4	M	QH	4	7		0.91666667	5311428	0.08313702	0.07836409	0.07886426	0.00678741	0.04132992	0.09850239
86	081194j	2259	22	11	G	AR	5	9	Central	0.21527778	1248508	0.0950214	0.09255073	0.09417871	0.01686199	0.03667709	0.1451986
87	081194b	2219	22	11	G	AR	1	9	Central	0.27777778	1621509	0.09765976	0.09196968	0.09228092	0.00993921	0.05444202	0.11964072
88	070594e	2251	22	11	G	AR	5	1	Dorsal	0.67361111	3912467	0.09343059	0.08692739	0.08692739	0.00462639	0.06778893	0.12639954
89	053193a	2213	22	11	G	AR	1	3	Dorsal	0.9375	5429203	0.09430987	0.08710121	0.08666954	0.00402061	0.07165508	0.11182986
90	091294a	2254	22	11	AR	G	5	4	Palmar	0.625	3619446	0.09405813	0.08697838	0.08739573	0.00514918	0.06796124	0.11042762
91	081994i	2255	22	11	G	AR	5	5	Palmar	0.97222222	5632832	0.09700554	0.08993804	0.08993804	0.00761241	0.04902248	0.11899837
92	090294e	2256	22	11	G	AR	5	6	Palmar	0.94444444	5474406	0.0862151	0.08147589	0.08241625	0.00994154	0.02746901	0.12226308
93	081494c	2214	22	11	G	AR	1	4	Palmar	0.80555556	4669183	0.10600203	0.09867745	0.09966263	0.01201655	0.03281652	0.1317968
94	081294d	2216	22	11	G	AR	1	6	Palmar	0.98611111	5742487	0.087526	0.08281235	0.08588354	0.01183819	0.02114489	0.11411803
95	081494b	2257	22	11	G	AR	5	7		0.95138889	5511239	0.10173797	0.09322612	0.09291793	0.00692932	0.069442	0.12591797
96	081194a	2258	22	11	G	AR	5	8		0.61111111	3544634	0.084555	0.07901291	0.07901291	0.00706413	0.04850716	0.10839742
97	080994a	2247	22	11	G	AR	4	7		0.96527778	5590422	0.10173043	0.0943006	0.09482195	0.00928066	0.04389383	0.12592787
98	081494a	2248	22	11	G	AR	4	8		0.93055556	5396723	0.10048131	0.09202931	0.09265889	0.00803682	0.07357869	0.12355608
99	081494a	2359	23	20	F	TB	5	9	Central	0.29166667	1691440	0.09948509	0.09854086	0.10351607	0.02209516	0.02991242	0.13478975
100	081294e	2349	23	20	F	TB	4	9		0.92361111	5358283	0.10934671	0.10158386	0.10188499	0.01511949	0.02918553	0.13366807
101	081294d	2353	23	20	F	TB	5	3	Dorsal	0.93055556	5389001	0.09824379	0.09078368	0.09904081	0.00942688	0.04655839	0.16699068
102	090294d	2311	23	20	F	TB	1	1	Dorsal	0.90277778	5229203	0.10004248	0.09245918	0.09378056	0.00805628	0.05614292	0.13982536
103	053193a	2312	23	20	F	TB	1	2	Dorsal	0.25	2160845	0.06796579	0.07897037	0.02698253	0.02797573	0.02698253	0.10801948
104	080994g	2313	23	20	F	TB	1	3	Dorsal	0.51388889	2984873	0.07522847	0.07121451	0.07121451	0.00833917	0.04143183	0.13299903
105	081294d	2356	23	20	F	TB	5	6	Palmar	0.41666667	2415136	0.10729194	0.10182885	0.10389152	0.0140044	0.04141746	0.16699068
106	081194h	2314	23	20	F	TB	1	4	Palmar	0.92361111	5350288	0.10294392	0.09454555	0.09494351	0.00679956	0.05863355	0.12387837
107	081194h	2316	23	20	F	TB	1	6	Palmar	0.94444444	5469180	0.10001797	0.09251164	0.09292458	0.00718135	0.05653167	0.13394122
108	081994f	2347	23	20	F	TB	4	7		0.95833333	5556113	0.10317803	0.0949877	0.0949877	0.00658753	0.06349461	0.13834313
109	081194e	2559	25	0.42	M	TB	5	9	Central	0.27083333	1579500	0.07791437	0.07468537	0.07334453	0.01348772	0.02592318	0.1261086
110	081194b	2519	25	0.42	M	TB	1	9	Central	0.23611111	1370703	0.09316958	0.0856585	0.08525627	0.00432804	0.07693585	0.13669339
111	070594b	2511	25	0.42	M	TB	1	1	Dorsal	0.35416667	2058099	0.08483918	0.08040967	0.08128059	0.00604911	0.0573258	0.11147678
112	090294f	2556	25	0.42	M	TB	5	6	Palmar	0.29166667	1691527	0.08868607	0.08611365	0.08761068	0.01725576	0.02099582	0.14154368
113	080994g	2514	25	0.42	M	TB	1	4	Palmar	0.33333333	1931685	0.0822523	0.0777826	0.0777826	0.00901744	0.04350155	0.12363056
114	053193b	2516	25	0.42	M	TB	1	6	Palmar	0.98611111	5783473	0.09108116	0.08574483	0.0865953	0.01061019	0.02824495	0.13803521

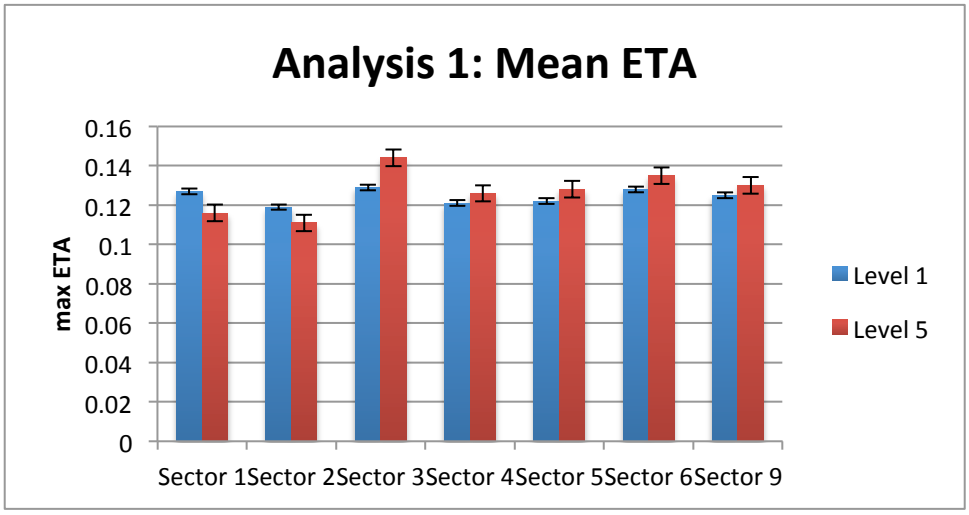


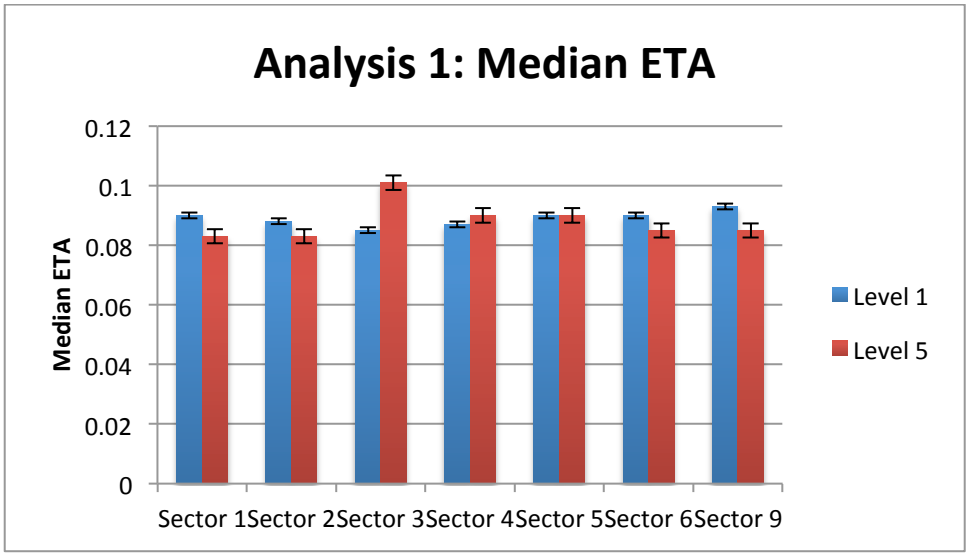
All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

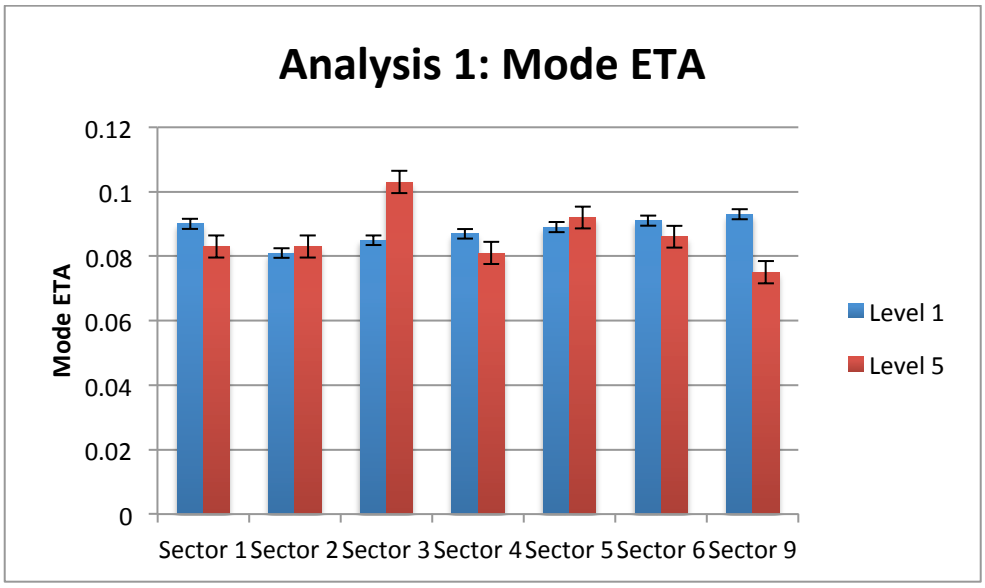
Comparisons for factor: **Sector**

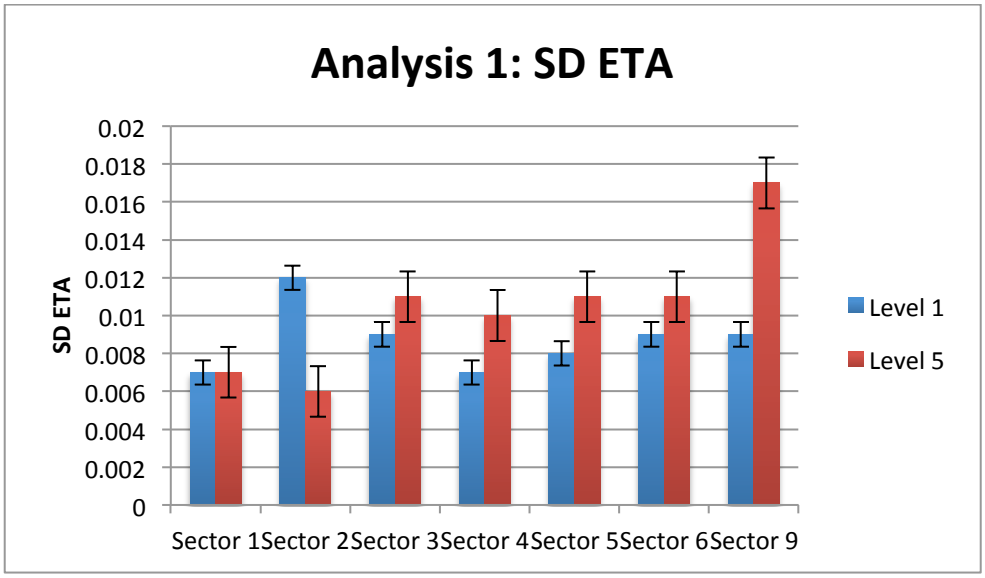
Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
2.000 vs. 9.000	0.494	0.283	0.002	Yes
2.000 vs. 6.000	0.314	0.283	0.032	Yes
2.000 vs. 5.000	0.294	0.264	0.031	Yes
2.000 vs. 3.000	0.242	0.238	0.047	Yes
2.000 vs. 1.000	0.238	0.264	0.074	No
2.000 vs. 4.000	0.118	0.284	0.392	Do Not Test
4.000 vs. 9.000	0.376	0.283	0.012	Yes
4.000 vs. 6.000	0.195	0.283	0.164	No

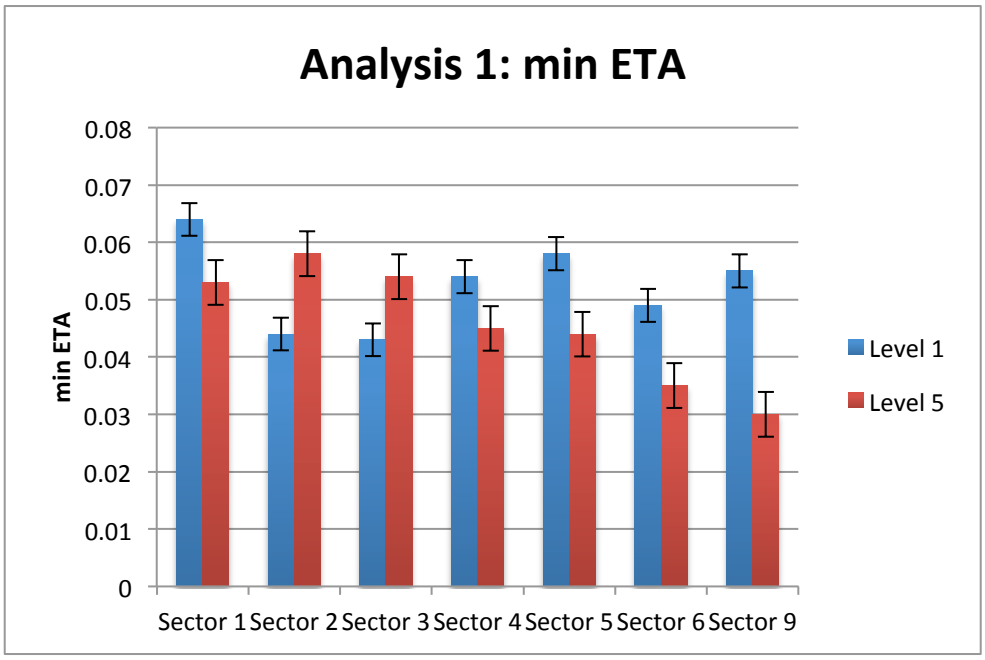
4.000 vs. 5.000	0.176	0.264	0.179	Do Not Test
4.000 vs. 3.000	0.123	0.238	0.291	Do Not Test
4.000 vs. 1.000	0.12	0.264	0.354	Do Not Test
1.000 vs. 9.000	0.256	0.263	0.056	No
1.000 vs. 6.000	0.0758	0.263	0.552	Do Not Test
1.000 vs. 5.000	0.0561	0.242	0.632	Do Not Test
1.000 vs. 3.000	0.00364	0.214	0.972	Do Not Test
3.000 vs. 9.000	0.252	0.237	0.038	Do Not Test
3.000 vs. 6.000	0.0722	0.237	0.53	Do Not Test
3.000 vs. 5.000	0.0525	0.214	0.612	Do Not Test
5.000 vs. 9.000	0.2	0.263	0.127	Do Not Test
5.000 vs. 6.000	0.0197	0.263	0.877	Do Not Test
6.000 vs. 9.000	0.18	0.282	0.196	Do Not Test

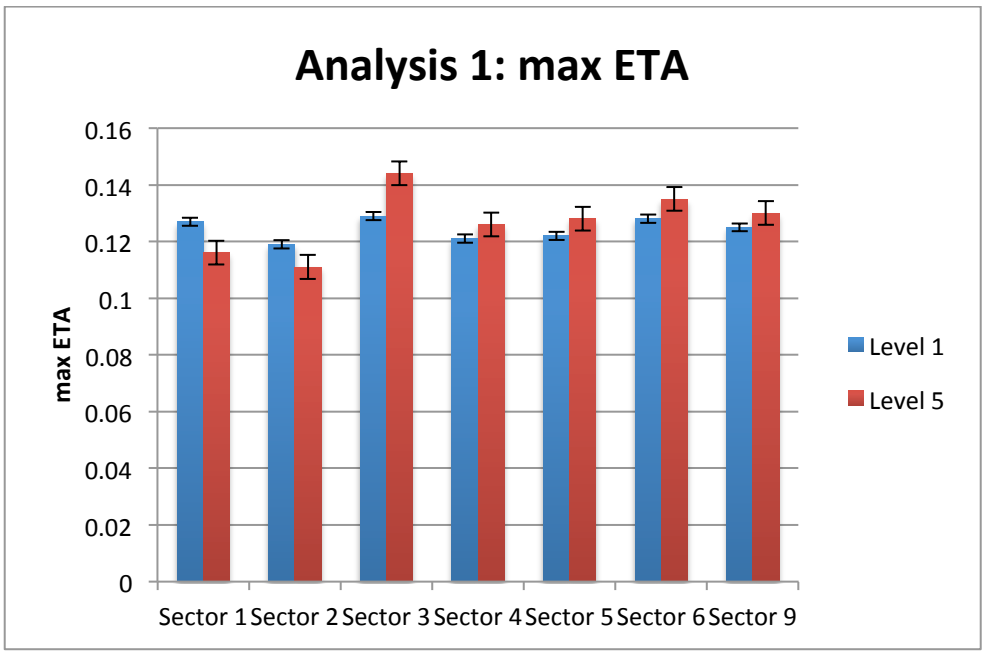



Analysis 1: Median ETA
Median ETA
0.12
0.1
0.08
0.06
0.04
0.02
0
Sector 1
Sector 2
Sector 3
Sector 4
Sector 5
Sector 6
Sector 9
Level 1
Level 5


Analysis 1: Mode ETA
Mode ETA
0.12
0.1
0.08
0.06
0.04
0.02
0
Sector 1
Sector 2
Sector 3
Sector 4
Sector 5
Sector 6
Sector 9
Level 1
Level 5


Analysis 1: SD ETA
SD ETA
0.02
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
Sector 1
Sector 2
Sector 3
Sector 4
Sector 5
Sector 6
Sector 9
Level 1
Level 5


Analysis 1: min ETA
min ETA
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
Sector 1
Sector 2
Sector 3
Sector 4
Sector 5
Sector 6
Sector 9
Level 1
Level 5

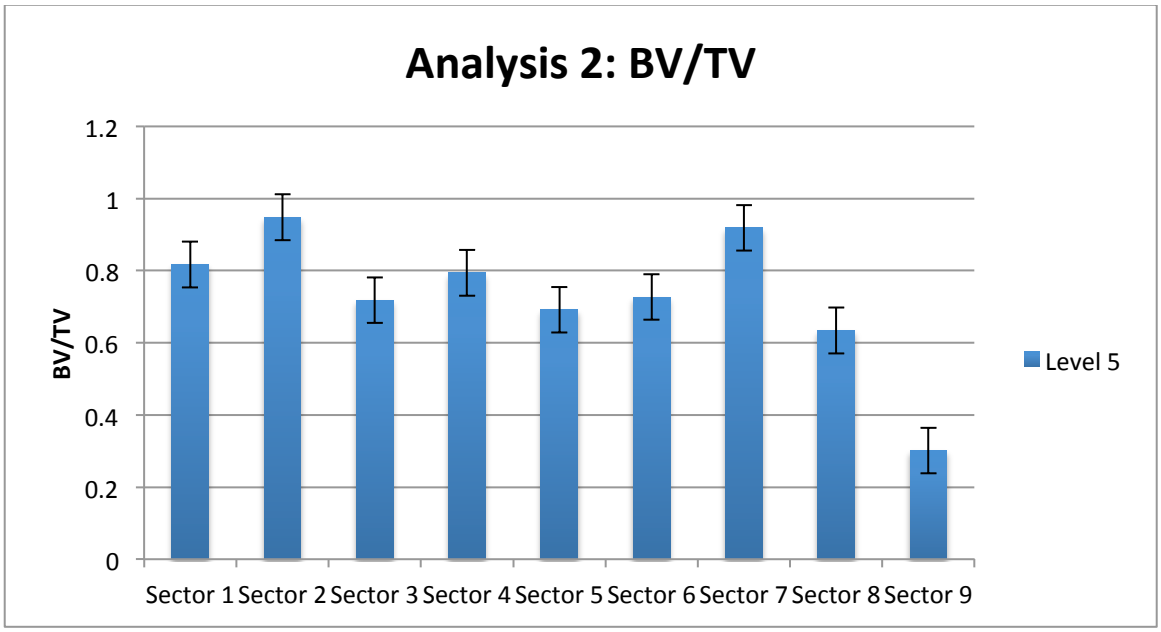


All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Sector**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
3.000 vs. 4.000	0.0209	0.0158	0.013	Yes
3.000 vs. 1.000	0.0208	0.0142	0.007	Yes
3.000 vs. 2.000	0.0181	0.0158	0.028	Yes
3.000 vs. 5.000	0.0148	0.0142	0.042	Yes
3.000 vs. 6.000	0.00473	0.0157	0.536	No
3.000 vs. 9.000	0.000296	0.0157	0.969	Do Not Test
9.000 vs. 4.000	0.0206	0.0188	0.034	Yes
9.000 vs. 1.000	0.0205	0.0175	0.024	Yes
9.000 vs. 2.000	0.0178	0.0188	0.063	No

9.000 vs. 5.000	0.0146	0.0175	0.097	Do Not Test
9.000 vs. 6.000	0.00443	0.0187	0.625	Do Not Test
6.000 vs. 4.000	0.0161	0.0188	0.088	No
6.000 vs. 1.000	0.016	0.0175	0.07	Do Not Test
6.000 vs. 2.000	0.0133	0.0188	0.154	Do Not Test
6.000 vs. 5.000	0.0101	0.0175	0.239	Do Not Test
5.000 vs. 4.000	0.00601	0.0175	0.481	Do Not Test
5.000 vs. 1.000	0.00591	0.0161	0.451	Do Not Test
5.000 vs. 2.000	0.00322	0.0175	0.705	Do Not Test
2.000 vs. 4.000	0.00279	0.0189	0.76	Do Not Test
2.000 vs. 1.000	0.0027	0.0175	0.75	Do Not Test
1.000 vs. 4.000	0.0000923	0.0175	0.991	Do Not Test



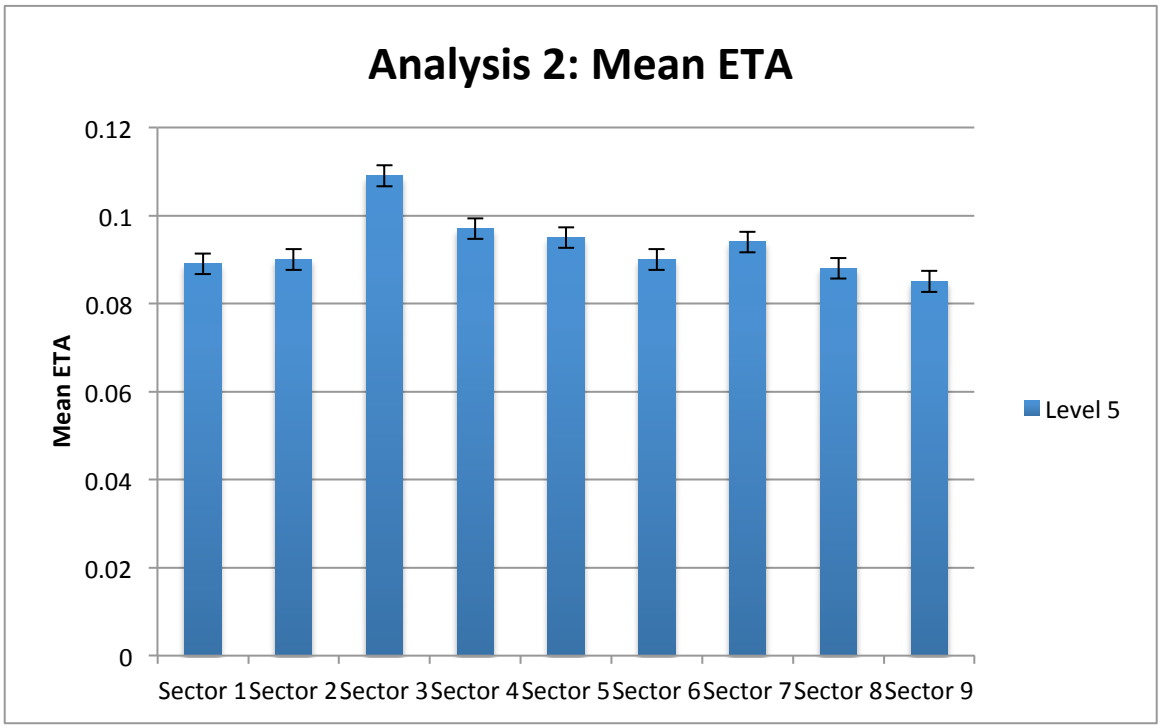
All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

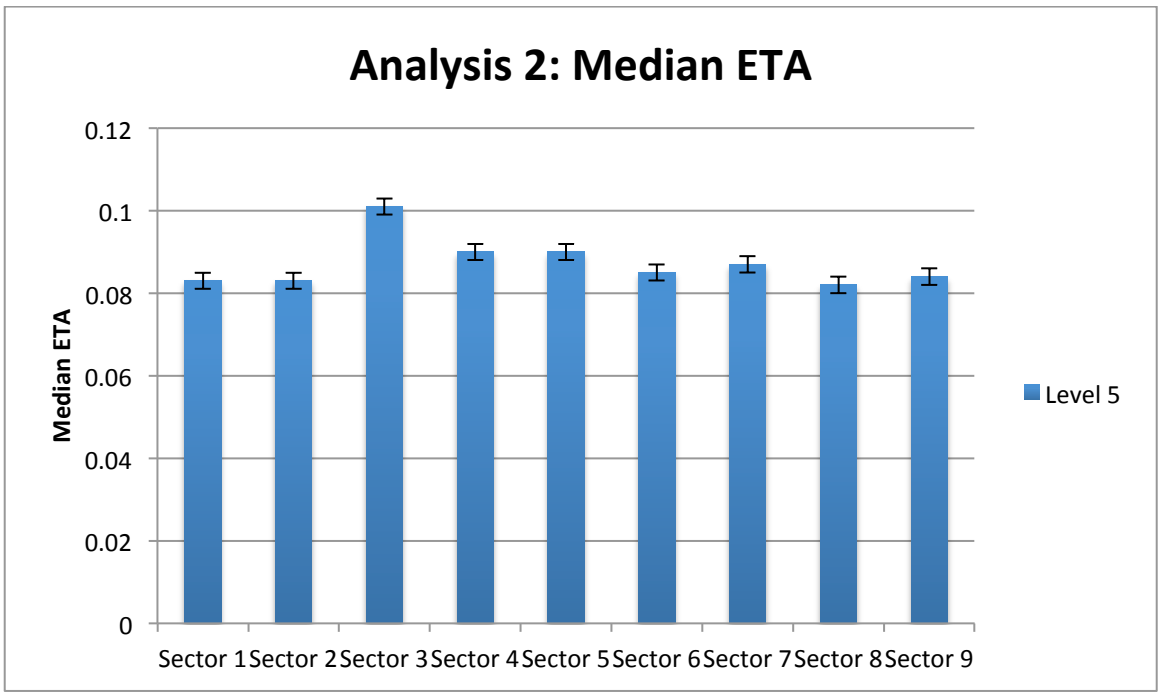
Comparisons for factor: **Sector**

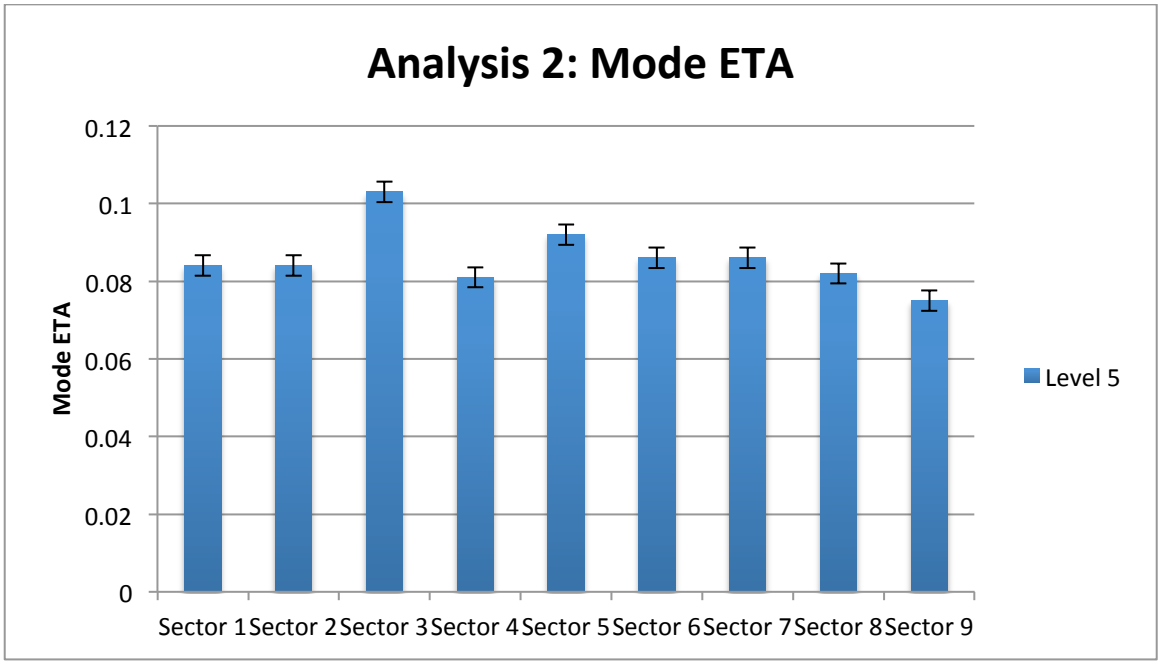
Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
2.000 vs. 9.000	0.669	0.345	<0.001	Yes
2.000 vs. 8.000	0.371	0.361	0.045	Yes
2.000 vs. 5.000	0.328	0.352	0.067	No
2.000 vs. 6.000	0.304	0.344	0.081	Do Not Test
2.000 vs. 3.000	0.288	0.352	0.106	Do Not Test
2.000 vs.	0.25	0.345	0.15	Do Not

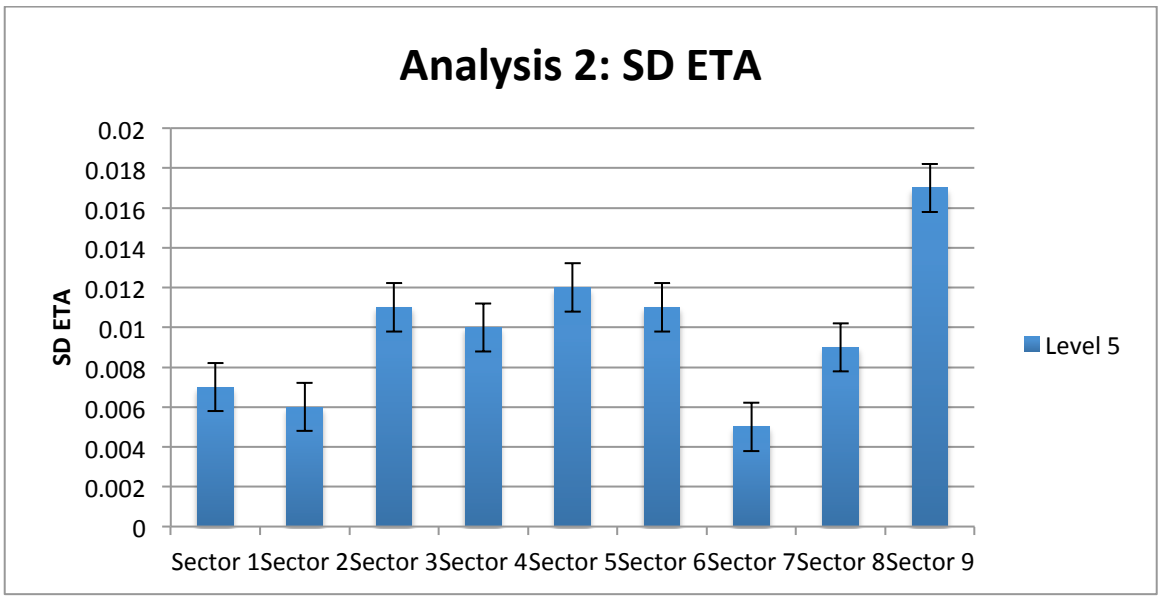
4.000				Test
2.000 vs. 1.000	0.245	0.361	0.177	Do Not Test
2.000 vs. 7.000	0.116	0.395	0.554	Do Not Test
7.000 vs. 9.000	0.553	0.295	<0.001	Yes
7.000 vs. 8.000	0.255	0.314	0.108	No
7.000 vs. 5.000	0.213	0.304	0.163	Do Not Test
7.000 vs. 6.000	0.188	0.294	0.201	Do Not Test
7.000 vs. 3.000	0.172	0.303	0.257	Do Not Test
7.000 vs. 4.000	0.134	0.295	0.361	Do Not Test
7.000 vs. 1.000	0.129	0.314	0.409	Do Not Test
1.000 vs. 9.000	0.424	0.248	0.001	Yes
1.000 vs. 8.000	0.126	0.271	0.349	Do Not Test
1.000 vs. 5.000	0.0838	0.259	0.513	Do Not Test
1.000 vs. 6.000	0.0593	0.247	0.628	Do Not Test
1.000 vs. 3.000	0.0429	0.258	0.737	Do Not Test
1.000 vs. 4.000	0.00513	0.248	0.967	Do Not Test
4.000 vs. 9.000	0.419	0.223	<0.001	Yes
4.000 vs. 8.000	0.121	0.248	0.328	Do Not Test
4.000 vs. 5.000	0.0787	0.235	0.5	Do Not Test
4.000 vs. 6.000	0.0541	0.222	0.623	Do Not Test
4.000 vs. 3.000	0.0378	0.235	0.745	Do Not Test
3.000 vs. 9.000	0.381	0.234	0.002	Yes
3.000 vs. 8.000	0.0832	0.258	0.515	Do Not Test

3.000 vs. 5.000	0.0409	0.245	0.736	Do Not Test
3.000 vs. 6.000	0.0164	0.233	0.887	Do Not Test
6.000 vs. 9.000	0.365	0.222	0.002	Yes
6.000 vs. 8.000	0.0668	0.247	0.584	Do Not Test
6.000 vs. 5.000	0.0246	0.234	0.831	Do Not Test
5.000 vs. 9.000	0.34	0.234	0.006	Yes
5.000 vs. 8.000	0.0422	0.258	0.741	Do Not Test
8.000 vs. 9.000	0.298	0.248	0.02	Yes




Analysis 2: Median ETA
Median ETA
0.12
0.1
0.08
0.06
0.04
0.02
0
Sector 1
Sector 2
Sector 3
Sector 4
Sector 5
Sector 6
Sector 7
Sector 8
Sector 9
Level 5


Analysis 2: Mode ETA
Mode ETA
0.12
0.1
0.08
0.06
0.04
0.02
0
Sector 1
Sector 2
Sector 3
Sector 4
Sector 5
Sector 6
Sector 7
Sector 8
Sector 9
Level 5



All Pairwise Multiple Comparison Procedures (Fisher LSD Method):

Comparisons for factor: **Sector**

Comparison	Diff of Means	LSD(alpha=0.050)	P	Diff >= LSD
9.000 vs. 2.000	0.0116	0.00729	0.003	Yes
9.000 vs. 7.000	0.00989	0.00623	0.003	Yes
9.000 vs. 1.000	0.00751	0.00524	0.006	Yes
9.000 vs. 8.000	0.00748	0.00523	0.007	Yes
9.000 vs. 3.000	0.00607	0.00495	0.018	Yes
9.000 vs. 4.000	0.00564	0.00472	0.021	Yes
9.000 vs. 5.000	0.00431	0.00496	0.086	No
9.000 vs. 6.000	0.00405	0.00468	0.087	Do Not Test
6.000 vs. 2.000	0.00758	0.00727	0.042	Yes
6.000 vs.	0.00584	0.00621	0.065	No

7.000				
6.000 vs. 1.000	0.00346	0.00522	0.187	Do Not Test
6.000 vs. 8.000	0.00342	0.00522	0.19	Do Not Test
6.000 vs. 3.000	0.00202	0.00493	0.41	Do Not Test
6.000 vs. 4.000	0.00159	0.0047	0.496	Do Not Test
6.000 vs. 5.000	0.000258	0.00494	0.916	Do Not Test
5.000 vs. 2.000	0.00732	0.00745	0.054	No
5.000 vs. 7.000	0.00558	0.00642	0.086	Do Not Test
5.000 vs. 1.000	0.0032	0.00547	0.242	Do Not Test
5.000 vs. 8.000	0.00316	0.00546	0.246	Do Not Test
5.000 vs. 3.000	0.00176	0.00519	0.494	Do Not Test
5.000 vs. 4.000	0.00133	0.00497	0.59	Do Not Test
4.000 vs. 2.000	0.00599	0.0073	0.104	Do Not Test
4.000 vs. 7.000	0.00425	0.00624	0.174	Do Not Test
4.000 vs. 1.000	0.00187	0.00525	0.473	Do Not Test
4.000 vs. 8.000	0.00184	0.00525	0.48	Do Not Test
4.000 vs. 3.000	0.000431	0.00496	0.86	Do Not Test
3.000 vs. 2.000	0.00556	0.00744	0.138	Do Not Test
3.000 vs. 7.000	0.00382	0.00641	0.233	Do Not Test
3.000 vs. 1.000	0.00144	0.00546	0.594	Do Not Test
3.000 vs. 8.000	0.00141	0.00545	0.602	Do Not Test
8.000 vs. 2.000	0.00415	0.00764	0.276	Do Not Test
8.000 vs. 7.000	0.00241	0.00664	0.463	Do Not Test

8.000 vs. 1.000	0.0000347	0.00572	0.99	Do Not Test
1.000 vs. 2.000	0.00412	0.00764	0.28	Do Not Test
1.000 vs. 7.000	0.00238	0.00664	0.47	Do Not Test
7.000 vs. 2.000	0.00174	0.00835	0.674	Do Not Test