MECHANICAL PROPERTIES OF BONE DUE TO SOST EXPRESSION: NANOINDENTATION ASSESSMENT OF MURINE FEMURS

A Thesis

presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Biomedical Engineering

by

Amir Rafie

December 2013

COMMITTEE MEMBERSHIP

TITLE:	Mechanical Properties of Bone Due to SOST Expression: Nanoindentation Assessment of Murine Femurs
AUTHOR:	Amir Rafie
DATE SUBMITTED:	December, 2013
COMMITTEE CHAIR:	Dr. Scott Hazelwood, Biomedical Engineering Associate Professor
COMMITTEE MEMBER:	Dr. Lanny Griffin, Biomedical Engineering Professor & Department Chair
COMMITTEE MEMBER:	Dr. Daniel Walsh, Biomedical Engineering Professor

ABSTRACT

Mechanical Properties of Bone Due to SOST Expression: Nanoindentation Assessment of Murine Femurs

Amir Rafie

In the human genome, the SOST gene codes for a protein sclerostin. Sclerostin is an osteocyte-expressed negative regulator of bone formation. When the SOST gene is not coded, bone formation is reduced in individuals during skeletal maturation. This study utilizes nanoindentation methods to test for the mechanical properties of bones that both express and do not express the SOST gene. 100 transgenic murine femurs were obtained from Lawrence Livermore Labs in the form of 6 and 8 month SOST transgenic mice, 6 and 12 month SOST knockout mice, and wild type control littermates for each of the 4 age groups. Prior to nanoindentation the bones were broken in a previous experiment under three-point bending tests. Samples were embedded in epoxy and polished to a 0.05 micron level before indentation. Results showed significant difference amongst the treatment group effects for maximum load, hardness and elastic modulus. SOST KO mice had significantly higher values for these properties in comparison to the transgenic and wild type littermates. Additionally, side by side limb differences were examined in which there was a significant difference found amongst the treatment groups. Indentations were conducted in the 4 anatomical regions of each femur in expectation of examining any differences amongst them which resulted with no significant findings amongst them. Data from this study will support research which may result in potential new gene therapies targeted for the treatment of bone diseases such as osteoporosis.

Keywords: SOST, sclerostin, nanoindentation, mechanical properties

ACKNOWLEDGMENTS

I would like to begin by thanking Dr. Scott Hazelwood, my advisor on this project, not only for all his help but also for being so patient with me. I would also like to thank my first committee member Dr. Lanny Griffin for allowing me to use his nanoindentation machine as well as all the time he spent training me on it, and my second committee member, Dr. Dan Walsh for his time support. Next I would like to thank my colleague Kainoa Peterson for all his help and for the fateful day we ran into each other in passing and ultimately introducing me to this project. A special thank you to Lawrence Livermore Laboratories for providing us with testing samples and to California Polytechnic State Universities Biomedical Engineering and Materials Engineering department for allowing me to use their labs, and Dr. Kristen Cardinal for helping fund the sample preparation of my project with the Hannah-Forbes Project Funding. Thank you to Bob Hayes from Metals Technology for helping with my sample preparation polishing and to David Laiho for his countless hours of help. Lastly I would like to thank my parents for always their love and support.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

1.1 Bone Physiology and Structure

The skeleton provides a variety of functions such as structural support for the rest of the body, locomotion in conjunction with muscles, protecting vital internal organs and structures, and maintenance of mineral homeostasis [1]. It has been known that bones adapt to mechanical loading - moving and removing bone depending on the various levels of load in a region. Regions exhibiting higher amounts of strain have denser more actively remodeled bone than regions with lower stress levels. This mechanosensory system is what dictates the size, shape and function of bone. [2].

The four general categories of bones are long bones, short bones, flat bones, and irregular bones. Long bones are composed of a hollow diaphysis; cone-shaped metaphyses below the growth plates; and rounded epiphyses above the growth plates. The diaphysis is composed primarily of dense cortical bone, whereas the metaphysis and epiphysis are composed of trabecular bone surrounded by a relatively thin shell of dense cortical bone [3].

1.1.1 Cortical Bone and Trabecular Bone

The adult human skeleton is composed of 80% cortical bone and 20% trabecular bone overall [3]. The various bones and skeletal sites have different ratios of cortical to trabecular bone. This ratio is 50:50 in the femoral head and 95:5 in the radial diaphysis (Figure 1.1) [1]. Cortical bone is characterized by low, 5-10%, porosity and is often used for testing when determining the mechanical properties of bone. [4]

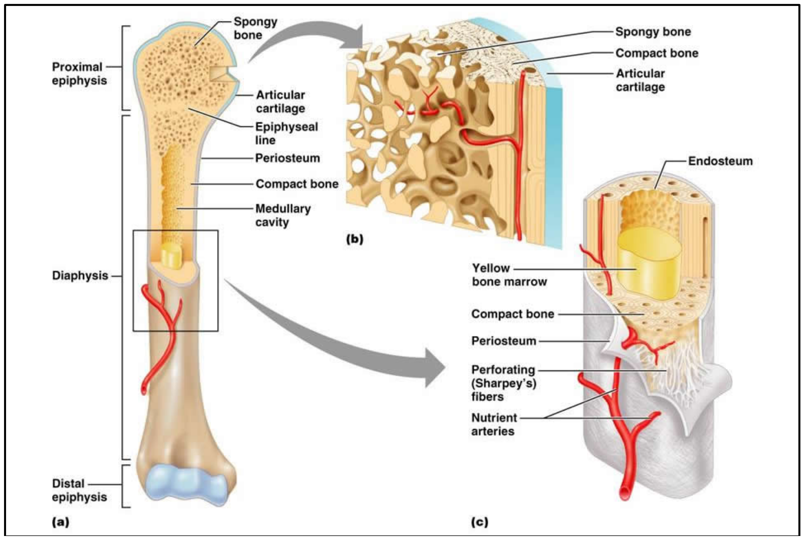


Figure 1.1: Detailed look at the femoral bone and its structure. Cortical bone is often referred to as compact bone and trabecular bone is often referred to as spongy bone. http://classes.midlandstech.edu/carterp/Courses/bio210/chap06/Slide3.JPG

Trabecular bone is characterized by its highly porous network (Figure 1.1). This type of bone is found at both the proximal and distal ends of long bone epiphyses. Trabecular bone's disorganized structure is composed of interconnected pores containing bone marrow and arteries which incorporate approximately 75-95% of trabecular bone volume [4]. Because of its porous structure, trabecular bone is much weaker in its mineral content, tissue density, and mechanical properties (e.g. modulus, hardness) than that of cortical bone [5].

1.1.2 Modeling and Remodeling of Bone

Bone undergoes longitudinal and radial growth development during childhood and adolescence. As the body undergoes various forces and stresses, bone adapts to maintain its functions by two phases: modeling and remodeling. Modeling is the process by which bones change their overall shape in response to physiologic influences or mechanical

forces, leading to gradual adjustment of the skeleton to the forces that it encounters. Bone remodeling is the process by which bone is renewed to maintain bone strength and mineral homeostasis. Therefore, modeling is typically the formation of primary bone as the body adjusts to the strains of growth, while remodeling creates secondary bone which serves to strengthen and repair damaged bone as fatigue and increased stresses are experienced by the bone following skeletal maturity [3-4]. Modeling and remodeling is activated via a mechanosensory system controlled by various bone cells.

1.1.2.1 Bone Cells

Bone cells main function is to either form or resorb bone. The first to act in bone formation are osteoclasts (Figure 1.2) which resorb bone. Activated multinucleated osteoclasts are derived from mononuclear precursor cells of the monocyte-macrophage lineage [3]. These cells respond to stresses (or lack thereof) and remove bone by demineralizing and dissolving the resulting collagen [4].

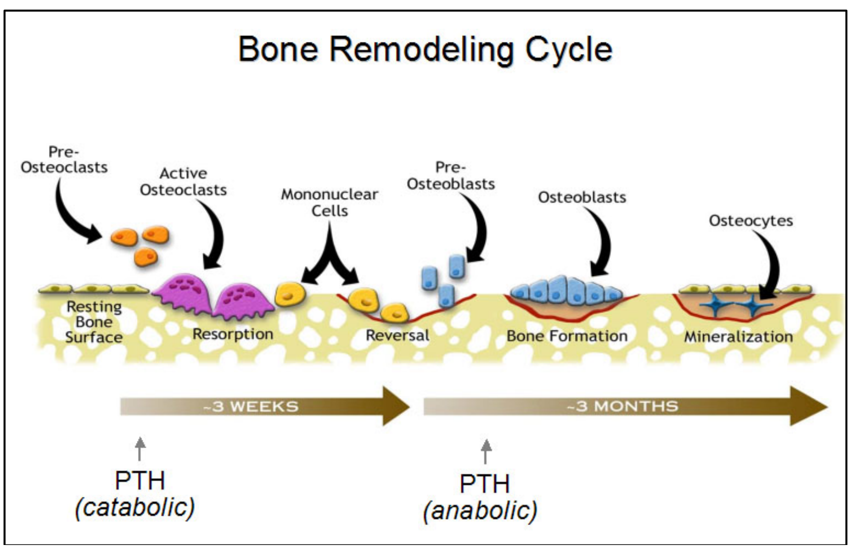


Figure 1.2: Bone cells and their roles in the remodeling cycle. http://www.ns.umich.edu/Releases/2005/Feb05/img/bone.jpg

The voids left behind from the active osteoclast's resorption period are resorption cavities [3]. Following resorption, if in a region of low stress, osteoclasts remain dormant, whereas in regions of higher stress, new bone is laid down by osteoblasts. Osteoblasts (Figure 1.2) are cuboidal mononuclear cells that respond to mechanical stresses. Osteoblasts are differentiated from mesenchymal cells and deposit the organic portion of bone matrix, the osteoid [4].

Once the bone formation period is completed from the osteoblasts, osteocytes (Figure 1.2) are then terminally differentiated cells from osteoblasts. Osteocytes are embedded and surrounded by the osteoid matrix laid down by their precursor osteoblast cells [4]. Osteocytes compose over 90-95% of all bone cells in the adults and are responsible for sensing and initiating mechanical signaling [6].

1.2 Bone Disorders

There are a number of genes that are considered regulators of bone homeostasis, as well as a number of disorders that affect these genes, altering bone's ability to model and remodel functionally. Three such diseases: osteoporosis, Van Buchem disease and Sclerosteosis are all of importance to this project.

1.2.1 Osteoporosis

Osteoporosis occurs when bone remodeling becomes incapable of laying bone down faster then it is being removed and results in "a disease characterized by low bone mass and microarchitectural deterioration of bone tissue" [7]. The clinical significance of

osteoporosis lies in the risk of fracture which is greatly increased when bone density is reduced. The risk of osteoporosis is greater in women than in men because of their naturally smaller bone structures and the reduction of estrogen when women reach menopause [8].

While there have been a number of studies and therapies developed to control the effects of osteoporosis, no treatment to date has had a significant influence on reversing the damaging effects of osteoporosis. Research has begun to examine the connection between osteocytes activity and its gene network, potentially opening doors for new gene therapies to combat osteoporosis [9].

1.2.2 Van Buchem Disease

Van Buchem disease (MIM 239100) is a hereditary sclerosing dysplasia of bone. Both dominant and autosomal recessive modes of transmission have been described. The dominant form tends to be a benign disorder and symptoms are usually confined to those associated with the enlargement of the jaw (Figure 1.3). The recessive forms tend to have a greater morbidity and symptoms arise from pressure on cranial nerves by hyperostotic bone at the base of the skull [10].

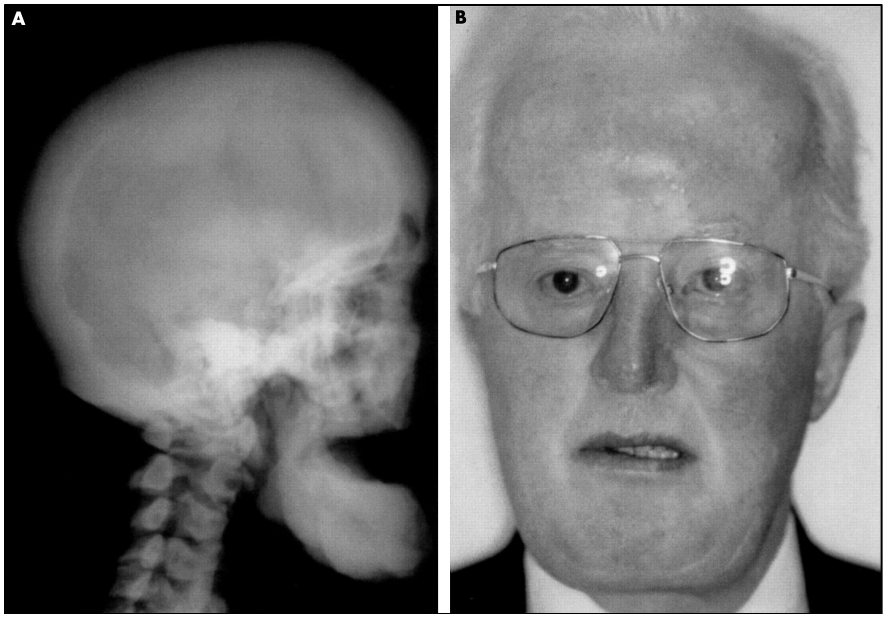


Figure 1.3: Van Buchem Diseases symptoms resulting in an unusual enlargement of the jaw as seen through the x-ray. http://jmg.bmj.com/content/vol39/issue2/images/large/01260.f1.jpeg

This abnormal growth results in greatly increased bone volume and density [10-11]. Van Buchem patients carry a homozygous 52kb non-coding deletion, ~35kb downstream of SOST (described in *Section 1.3*) transcript, and ~10kb upstream of the downstream gene, MEOX1, on human chromosome 17p21 [12-15]. To date, there have been approximately 40 reported cases of Van Buchem disease [15-16].

1.2.3 Sclerosteosis

Sclerosteosis (MIM 269500) belongs to the group of craniotubular bone modeling disorders and is inherited as an autosomal recessive trait [17]. Although Sclerosteosis is phenotypically indistinguishable from Van Buchem disease, it is more severe in its symptoms in that it occasionally displays syndactyly of the digits (Figure 1.4), which is not exhibited in Van Buchem disease [15-16].

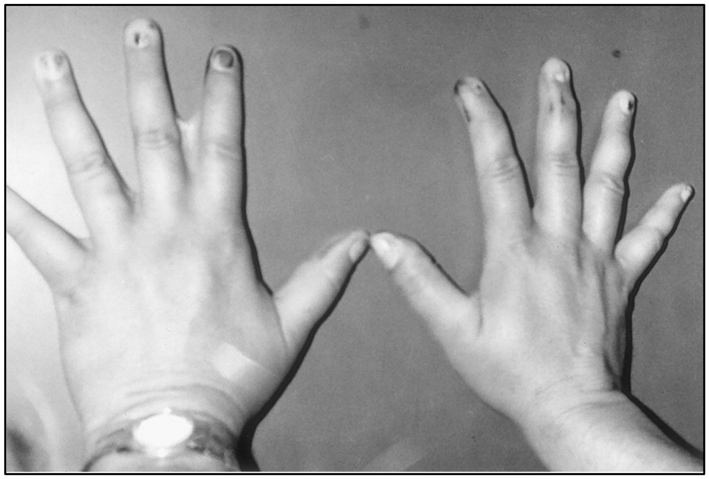


Figure 1.4: Sclerosteosis patients hands exhibiting syndactlies. Balemans 1999.

Sclerosteosis and Van Buchem disease have been hypothesized to be linked due to their clinical similarities and their genetic linkage to the SOST locus on chromosome 17q12, which suggests the two are allelic [14]. Sudden death due to impaction of the brainstem has been described in Sclerosteosis patients [12]. To date, there have been less than 100 reported cases of this disease [13].

1.3 SOST/Sclerostin Gene

Sclerostin is a glycoprotein encoded by the SOST gene. Sclerostin is an inhibitor of bone formation that is expressed by osteocytes in bone. Studies have shown sclerostin is a negative regulator of bone formation that is expressed exclusively in osteocytes that suppresses bone formation by inhibiting the differentiation of osteoblasts, as well as acting as a non-classical bone morphogenic protein (BMP) antagonist. [18]. Sclerostin deficiency has been associated with the two rare sclerosing bone disorders: Sclerosteosis (described in *Section 1.2.3)* and Van Buchem disease (described in *Section 1.2.2)* [11]. In Sclerosteosis, mutations in the SOST gene introduce premature termination codons or

interfere with splicing of the SOST gene, while patients with Van Buchem disease have a 52-kb homozygous deletion 35 kb downstream of the SOST gene believed to be responsible for Sclerostin deficiency [11,16-18].

The localization of SOST expression in bone and the mechanism by which it affects bone formation are still unclear. To better understand its mechanism of action, studies have been done to determine its transcriptional regulation. Sclerostin is a member of the Dan family of glycoproteins of which many members have been reported to antagonize the activity of several growth factors, including BMPs and Wnts [18-19]. Data suggest Wnt/LRP5 (lipoprotein related receptor protein 5/6) signaling is an integral part of the mechanotransduction cascade in normal bone tissue [19]. Recent research provides evidence that sclerostin is an indirect inhibitor of BMP, but specifically antagonizes the Wnt pathway as an antagonist of LRP5 [19].

1.4 Animal Models & Human Bone Disorders

For ethical reasons, the use of human bones for research is limited. In order to further the advancement of bone therapies, more effective methods and models are used. One such method is through the use of animal models, and in the case of bone experiments, murine models. Although there are complications regarding their small size, murine models have many advantages over the use of larger species for skeletal research. Through genetic engineering, transgenic mice with genetically modified skeletal functions have been of great use in research today [20]. The genetic factors that contribute to susceptibility to bone loss are extremely heterogeneous; with the use of genetically engineered mouse models research can be done to provide invaluable insights into the molecular

mechanisms of progressive bone loss in humans [20,14].

Although multiple bones have been used in mouse model experiments, the femur is one of the most highly used due to is relatively non-curved major axis and cortical surface size. For three-point bending and nanoindentation experiments - while the mouse femur is small in comparison to larger animal models, it is the largest long bone in the body, providing a testable region of cortical bone [20-21].

1.5 Hybridized Mice

Genetically engineered mouse models are becoming increasingly more popular in the study of diseases and experiments today [20,22]. Research facilities are continuously developing knockout and transgenic models to explore the genetic variations on skeletal traits such as tissue growth and disease [22]. Data from a study conducted by Li et al. showed increased bone formation and strength with the targeted deletion of the Sclerostin gene. Their results showed that sclerostin deficiency in humans has the same phenotypic outcome regardless of sex and the phenotype of SOST KO mice and that it was effectively the same for both sexes with differences only in degree for some of the observed effects [23].

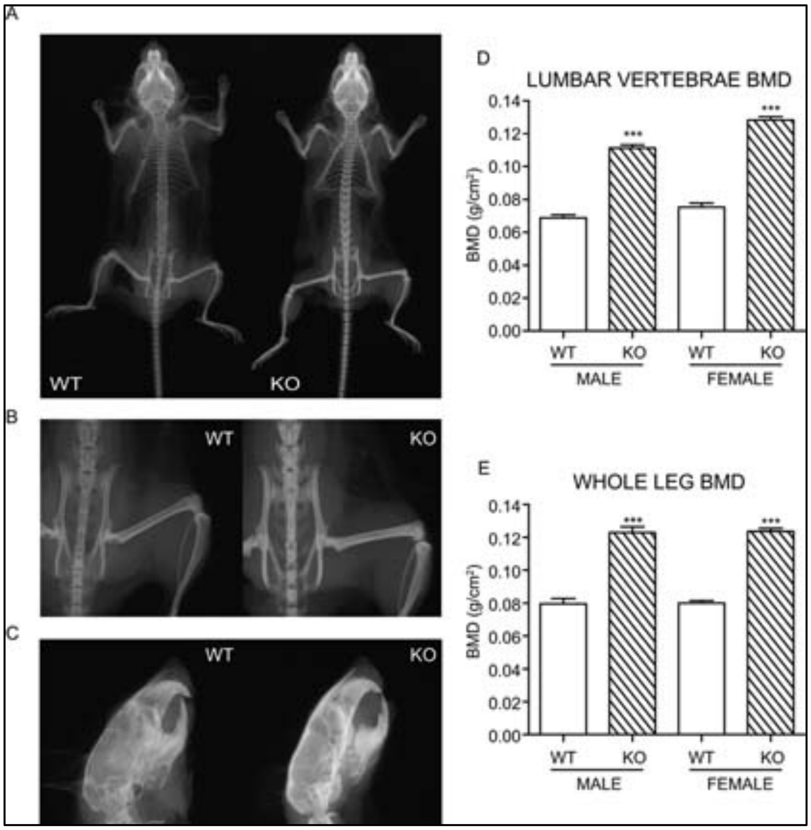


Figure 1.5: Images and data from a study conducted by Li et al. researching the effects of targeted deletion of the SOST gene and its increase on BMD and bone strength in SOST KO mice. (A) Whole body radiographs of 4-mo-old female WT and KO mice showing increased radiodensity throughout the skeleton of KO mice. (B) Enlargement of radiographs showing pelvic region. (C) Enlargement of radiographs showing skull. (D) BMD of lumbar vertebrae and (E) whole leg as assessed by DXA in 5- to 6.5-mo-old male and female WT and KO mice showing increased areal BMD in KO mice. Li et al., 2008.

Li et al. found significant findings of increased radiodensity (BMD) throughout the skeleton (skull, axial skeleton, ribs, pelvis, long bones) of KO mice (Figs. 2A–2C) and increased bone volume in vertebrae, long bone, and calvaria (Figure 1.4) [23]. The effects of sclerostin deficiency on bone seen in the SOST KO mice are the opposite of, and

biologically consistent with, the effects described for sclerostin overexpression in transgenic mice where excess sclerostin production resulted in decreases in BMD, bone volume, osteoblast surface, bone formation rate, and bone strength [14, 23-24].

A study conducted in conjunction with Lawrence Livermore National Labs, located in Livermore, California, has discovered using bacterial artificial chromosome (BAC) recombination and transgenesis to characterize the expression of human sclerostin (SOST) from normal ($SOST^{wt}$) or Van Buchem ($SOST^{vb\Delta}$) alleles [14]. Their study focused on sclerostin during limb development, demonstrating that Van Buchem (VB) disease is caused by the removal of bone-specific distant enhancer elements and is allelic to Sclerosteosis [14]. All lines of the $SOST^{wt}$ transgenic animals faithfully expressed human SOST in the mineralized bone of neonatal and adult mice (skull, rib, femur) while all $SOST^{vb\Delta}$ lines had dramatically reduced levels of human SOST mRNA expression. All genetic lines expressed human SOST in the kidney and heart as well (Figure 1.5) [14].

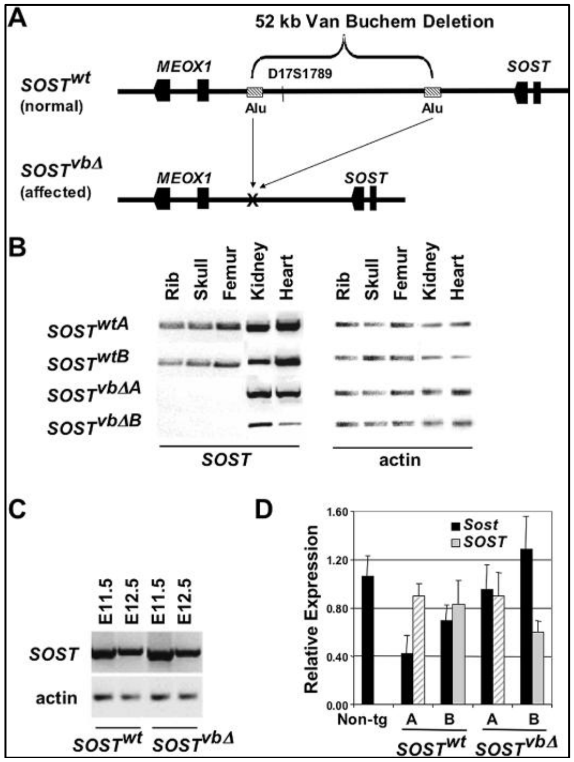


Figure 1.6: Targeted genomic deletion characterization and data from a study conducted by Loots et al. in conjunction with Lawrence Livermore National Labs. (A) A 158-kb human BAC ($SOST^{wt}$) spanning SOST and MEOX1, engineered using in vitro BAC recombination in Escherichia coli by deleting the 52-kb noncoding region missing in VB patients ($SOST^{vb\Delta}$). (B) Embryos analyzed by rtPCR in adult tissues. (C) Quantitative rtPCR in E10.5 embryos (D) from two independent lines of each $SOST^{wt}$ and $SOST^{vb\Delta}$ transgene. Loots et al., 2005.

Data from Loots et al. showed $SOST^{wt}$ mice displayed a decrease in BMD at skeletal maturity. The $SOST^{vb\Delta}$ mice were indistinguishable from their non-transgenic littermates and the observed osteopenia was gene-dose dependent [14]. Through the hybridization of these mice, it is apparent that the SOST gene has an effect on bone formation with overexpression of human SOST negatively effecting BMD at skeletal maturity and under-expression resulting in an increase in BMD. Their data supports the evidence that the lack of human SOST bone expression in $SOST^{vb\Delta}$ animals is dependent on the 52-kb noncoding deletion, rather than an artifact due to transgene copy [14].

1.5.1 Lawrence Livermore National Labs Mouse Treatment Groups

For this experiment, genetically engineered murine femur samples obtained from Lawrence Livermore National Labs. The murine femurs were classified into one of two genotypes; SOST KO (knockout) or SOST TG (transgenic). SOST TG mice carried a BAC 209M4, resulting in an overexpression of human SOST.

Grouping within the SOST TG genotypes were as follows: sub-breakdowns (by age) of TG, animals with limb defects (DEF), and littermate wild type (WT) controls. Classification between TG and DEF was based on the presence of limb defects, presumably caused by higher levels of SOST expression. SOST TG mice were hemizygous, signifying the integration of the BAC at a single site, while SOST DEF mice were offspring from the mating of two SOST TG mice. In turn, the SOST DEF mice were believed to have twice the amount of human SOST expression in comparison to the SOST TG mice.

The SOST KO genotype grouping was as followed: a sub-breakdown (by age) consisting of KO and littermate WT controls. The SOST KO mice were homozygous for SOST deficiency and displayed high bone mass as previously discussed in Section 1.4. The wild type mice were used as controls - non-transgenic littermates of the alleles expressing normal levels of mouse SOST expression.

1.6 Mechanical Testing of Bone

Bone has a diverse arrangement of material structures working together to perform diverse mechanical functions such as structural support and mineral ion homeostasis. There are a number of tests used to determining the mechanical properties of bone

including bending, torsional, tensile, compression and shear stress tests [25-26]. Bone also does have biological and chemical functions that are not tested in in vitro biomechanical tests, the overall goal of which is to test the skeletal structure in loading and unloading environments [27]. Due to their small size, determining the material properties of mouse bone has proven difficult to measure directly. Much of the mechanical property measurements have been based on theory and measurements of whole bone mechanical properties and cross sectional geometry. Because murine bones do not meet the exact specifications of engineering theory in regards to constant geometric and material properties through the cortical bone, there is room for error in estimations of their mechanical properties [28]. Studies have shown more accurate results with biomechanical bench top testing such as three point bending and nanoindentation for determining the mechanical properties of mouse bones.

1.6.1 Three-Point Bending Experiment

In an experiment conducted at California Polytechnic State University (San Luis Obispo) by Kainoa Peterson, the mice samples obtained from Lawrence Livermore National Labs were mechanically tested via three-point bending tests. 100 femurs were excised from the mice and loaded until failure. In this experiment Kainoa examined significant differences in various properties such as cross sectional area, area moment of inertia, elastic modulus, yield force, ultimate force, post yield displacement, yield stress and ultimate stress. Results showed significant differences in treatment group effects for cross sectional area, yield force, and ultimate force as well as that SOST knockout (KO) mice were found to have significantly higher values for these properties in comparison to transgenic (TG) and

wildtype (WT) littermates. The experiment's secondary goal was to note any affects on axis of loading in which the results showed a noted effect dependent on the primary axis of loading, anterior-posterior versus cranial-caudal. Data from this experiment also supported the existing hypothesis that there is no systematic (left or right limb) difference in bone formation [29]. After the experiment, the broken femur halves were embedded in epoxy for further testing for this experiment using nanoindentation to study the mechanical properties of the SOST mice.

1.6.2 Nanoindentation

Nanoindentation has been applied in recent years to measure elastic properties of bone tissue at a microscopic length scale, overcoming the limitations of traditional mechanical testing techniques for small samples [30]. Nanoindentation uses a pendulum pivoted on frictionless bearings to measure the movement of a stylus in contact with a surface.

For indentation measurements, the stylus is impressed into the surface under the influence of an increasing load; after reaching a pre-determined maximum value, the load is reduced and the penetration depth decreases due to elastic recovery of the deformed material.

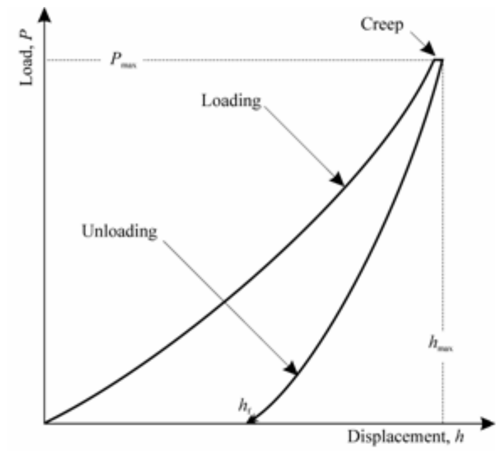


Figure 1.7: Nanoindentation load vs displacement curve. Hardness and modulus values are determined based on these graphs. http://www.xray.cz/xray/csca/kol2005/abst/ctvrtlik.htm

The depth and load are monitored continuously, which allows both hardness and elastic modulus data to be derived (Figure 1.7) [31]. Styluses come in many types for different testing applications, the best of which for bone testing purposes is a Berkovich indenter.

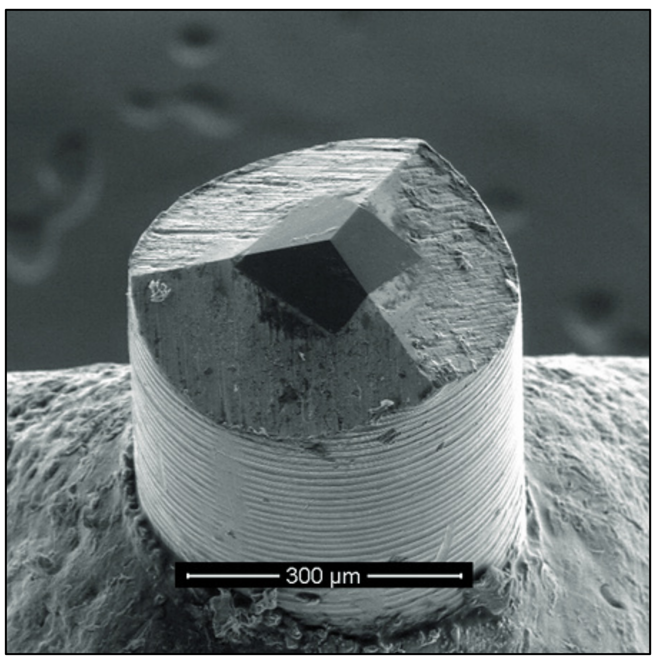


Figure 1.8: An SEM image of the Berkovich indenter, a three faced pyramid with a tip radius of 100-500nm. www.engineering.unl.edu/research/bm3/graphics/nanoindenter_Berkovich_tip_01.jpg

Nanoindentation provides a measure of the modulus of the solid phase of bone at the microstructural scale, with no influence from porosity (porosity including vascular and

resorption but not lacunar or canicular voids) [32]. The nanoindentation method of obtaining mechanical properties is precise and accurate and is well suited for measuring material properties in mouse bones [30]. Results from the nanoindentation tests will yield a maximum depth and load, plastic depth, hardness and elastic modulus values.

1.6.2.1 Rational for Testing

Nanoindentation has been applied in recent years to measure elastic properties of bone tissue at a microscopic length, overcoming the limitations of traditional mechanical testing techniques for small samples. Nanoindentation is precise and accurate with the data collected from testing [32]. Measuring the microscopic mechanical properties of bone tissue is important in support of understanding the etiology and pathogenesis of many bone diseases [33]. Studies suggest that bone reveals different mechanical properties when loading increases from the nano- to the micro-scale range (μ N to N) [34]. There have been no experiments utilizing nanoindentation to determine the effects, if any, of anatomical region on bone mechanical properties.

1.6.2.2 Testing Conditions

Other research groups have used load-control nanoindentation for studying mechanical properties of mice bones as well. Testing conditions are performed in a dry state at room temperature and humidity, and often – like in the case of this study, the testing is inside a temperature control cabinet. Most research groups as well have used the Berkovich tip (Figure 1.7) against a loading direction that is perpendicular to the longitudinal bone axis

[32]. Many research groups have used different methods for testing parameters when nanoindenting. Previous studies have reported using nanoindentation in the mid-diaphysis, rather than site specific, of murine femora and tibiae. Maximum loads and rates have also varied in studies including maximum load and load/unload rate of 6mN and 0.3mN/s as well as 8mN and 400 μ N/s [30]. Both of these sets of maximum loads and load rates generate a 500-700nm indentation in the bone material [30, 32].

1.7 Study Goals

The goals of this study are to further examine any significant mechanical property differences between the various murine treatment groups described in Section 1.5, by method of nanoindentation. Mechanical properties for comparison have been discussed in Section 1.6.2 and include modulus and hardness along with their corresponding maximum depth, plastic depth and max load. Testing was conducted in the 4 anatomical regions of each bone as a secondary goal to determine any effects on the mechanical properties in these regions amongst the different treatment groups. Additional goals include determination of the effect of age and limb on the mechanical properties of the tested murine femurs. Data from this study will aid ongoing research by providing laboratory data regarding the mechanical properties of the mice hybridized by Lawrence Livermore National Labs. Determination of significant differences in the strength of bone for the transgenic mice can support research which may result in potential new gene therapies targeted for the treatment of osteoporosis, Sclerosteosis and Van Buchem disease.

II. MATERIALS AND METHODS

100 murine femurs, from 50 hybridized mice of various SOST genotype and phenotypes (SOST TG/DEF/WT 6 month, SOST TG/DEF/WT 8 month, SOST KO/WT 6 month, SOST KO/WT 12 month), were provided by Lawrence Livermore National Labs (LLNL – located in Livermore, California) for mechanical testing (Table 1; for a full breakdown of femur number and treatment groups refer to Appendix A). SOST transgenic (TG), wild type (WT), and deficient (DEF) mice were hybridized with Friend leukemia virus B (FVB) mixed with C57/Bl/6J, backcrossed 4 generations to C57/Bl/6J. The SOST knockout (KO) and wild types (WT) mice were a mixture of C57/Bl/6J and 129 background, primarily showing body characteristics of the 129 strain.

Table 1: Treatment Group Distribution

Treatment	Mice	Description
SOST TG 6 Month	2	Human SOST
SOST DEF 6 Month	9	Twice the amount of human SOST as TG
WT 1 *	11	6 month old WT expressing SOST TG/DEF
SOST TG 8 Month	2	Human SOST
SOST DEF 8 Month	4	Twice the amount of human SOST as TG
WT 2 *	4	8 month old WT of SOST TG/DEF
SOST KO 6 Month	5	No SOST expression
WT 3 *	6	6 month old WT SOST KO
SOST KO 12 Month	5	No SOST Expression
WT 4	2	12 month old WT SOST KO
TOTAL	**50**	

*** = Faithfully express murine SOST**

Prior to nanoindentation, femur samples were used in an experiment by Kainoa Peterson at California Polytechnic State University in which the mechanical properties of the

femurs were determined using 3-point bending. Complete murine hind limbs were freeze shipped frozen from LLNL and required femurs to be dissected from the remaining portion of the hind limb. Bones were then cleansed of any remaining soft tissue to eliminate effect on the mechanical testing. Bones were wrapped in phosphate buffered saline (PBS) soaked gauze and stored in individually labeled vials in a -20°Cfreezer until the time of 3-point bend testing. Samples were removed from the freezer one at a time and allowed to thaw until ready for testing.

Each bone was loaded until failure with results showing significant differences in treatment group effects for cross sectional area, yield force, and ultimate force. In that experiment, SOST knockout (KO) mice were found to have significantly higher values for these properties in comparison to the transgenic (TG) and wild type (WT) littermates [29]. Following 3-point bending assessment, the two halves of the broken bones were wrapped in PBS soaked gauze and re-frozen until being prepped for epoxy embedding in epoxy allowing for nanoindentation assessment.

2.1 Sample Embedding

The previous 3-point bending experiment primarily resulted in oblique fractures to the bones, leaving a surface which was unable to be nanoindented. For nanoindentation, samples must be flat, embedded in epoxy, and polished to a mirrored surface. Following bending and refreezing, distal femur halves (initially the proximal halves were embedded but after polishing were deemed untestable) were dehydrated in increasing concentrations of alcohol, to remove remaining moisture after thawing. 70% isopropyl alcohol and varying amounts of distilled water were used to create the diluted alcohol concentrations.

Initially, bones were placed in individually labeled vials of 50% isopropyl and 50% distilled water for one hour. This was followed by increasing concentrations of 60%, 70%, 80%, 90%, and 100% (70%) isopropyl each for one hour time spans.

Bone sample placement and grouping for the embedding process was determined based on treatment type and limb. Grouping yielded 20 epoxy “pucks” holding 4 to 6 femurs each (A breakdown of the different epoxy pucks with the corresponding femur sample numbers can be found in Appendix A). Aluminum tubing of 5/32” or 3/16” (inner diameter) was cut using a cylindrical tube cutting tool in order to hold the bones in the epoxy. Upon examining the set of bones being embedded into each puck, tubes were chosen to fit each bone and glued together prior to embedding, thus allowing easy identification and separation of bones.

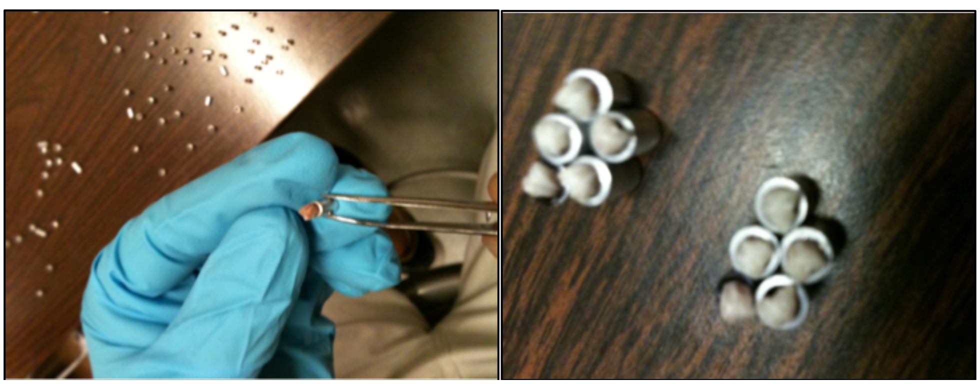


Figure 2.1. Sample bone halves being placed into their respective holding configurations before epoxy embedding.

All tubing configurations (4, 5, and 6 ring) were put together in the same fashion. In order to identify which bone was placed in each tube while looking at the epoxy samples, the number 1 aluminum tube (always the top left ring when looking at the sample from below, the non-testing side) was marked with a black sharpie which can be seen through the top of the epoxy during the sample polishing.

Once all bones were arranged in their designated tube, tube arrangements were set in clean 1.25” inner diameter casting mold cups (Buehler, Lake Bluff, IL) in preparation for epoxy molding. The samples holders were then placed in their respective mounting cups within a vacuum container/pump assembly (Model **#:** UN7260.3, KNF Neuberger, Inc., Trenton, NJ). Samples were cast using EPO-Thin Low Viscosity Epoxy Resin (Part No. 20-8140-032 - Buehler, Lake Bluff, IL) and EPO-Thin Low Viscosity Epoxy Hardener (Part No. 20-8142-064 - Buehler, Lake Bluff, IL), which were combined at a ratio of 100 parts resin to 36 parts hardener by weight. Once the sample holders were in place in the vacuum pump, epoxy was carefully measured out using a scale and was slowly poured into each molding cup until the samples were completely covered by roughly 1 centimeter of epoxy. Following the addition of epoxy, many samples began floating out of their respective mounting tubes. These bones were gently pushed back into place using forceps to regain their initial positioning. This was done to insure that all bone surfaces to be polished and subsequently tested were close in proximity, alleviating the need for repolishing between testing. Once all bones were returned to their correct place, a gentle vacuum was applied for 5 minutes followed by a period of no vacuum, which allowed for verification that all bones were still in the proper place. This process was repeated 4 times resulting in a total time of 20 minutes that samples were exposed to the vacuum.

Figure 2.2. Vacuum and pump assembly used for removing air bubbles during epoxy embedding procedure.

Vacuuming was conducted to remove air bubbles that were present within the epoxy puck. Once this was complete, samples were removed from the pump assembly and allowed to cure for a period of 24-48 hours prior to removal from molding cups.

Due to a bad mix of epoxy samples 12 through 20, after 6 months time, had cured on the testing side but remained partially cured on the bottom side. In order for these samples to be prepared the bottom side that remained uncured was scored with a toothpick to create holes, and inserted back into the epoxy cup holders. A fresh well mixed batch of epoxy was poured over the back end roughly 6mm thick and was once again allowed to harden. Once hardened, epoxy pucks were ready for polishing to a 0.05 micron finish in preparation for nanoindentation.

2.2 Sample Polishing

After embedding, samples were ground down to expose not only the cortical surface of the bones, but to pass any partial and oblique fractures thus exposing a complete (or as close as possible) cross sectional area of the bones. This was done using metallurgic polishing techniques.

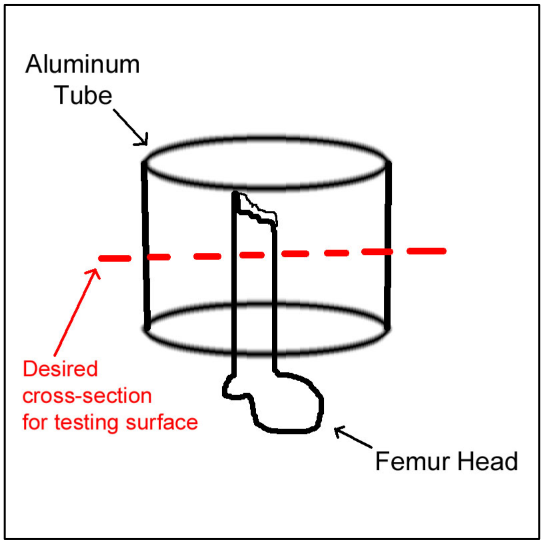


Figure 2.3. Illustration of a sample bone in an aluminum tube after embedding and prior to any polishing.

Initially, following the prior 3-point bending experiment, the proximal halves of the bones were embedded and polished to 0.05 micron by hand using grit paper techniques in order to create a flat, smooth testing surface for nanoindentation. It is pertinent for nanoindentation experiments that the testing surface is not just flat, but flat and parallel on both sides of the puck – which was found to be very difficult to accomplish by hand.

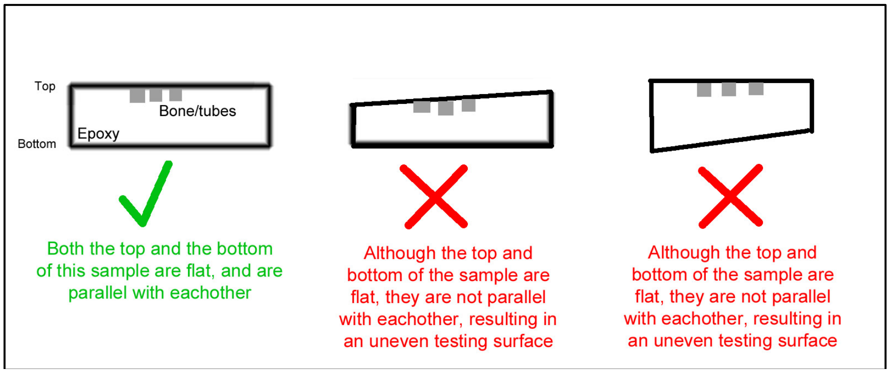


Figure 2.4. Illustration of the properly polished nanoindentation testable sample.

To obtain the desired mirrored surface, multiple grinding and polishing steps were conducted. Pucks were first ground using a 120 grit belt sander. This course grit allowed for exposure of the region of interest, the cortical bone surface. This polishing step resulted in a somewhat uneven surface due to difficulty stabilizing and rotating the puck by hand. Following exposure of the cortical surface, successive sandpaper grits of 240, 320, 400, and 600 were used. Each step was conducted until the previous sanding lines were removed.

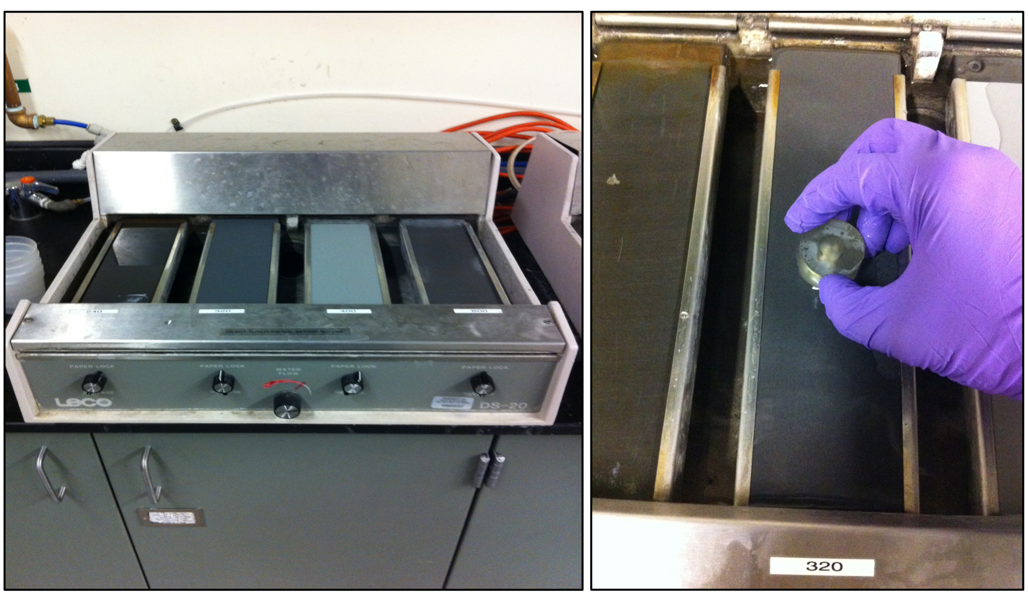


Figure 2.5. Grit paper wetting application pad and the Leco DS-20 grinding system.

This was accomplished with unidirectional strokes along the sandpaper. Each subsequent sanding was done at 90 degrees to the previous grit allowing for identification of when each step was complete. In order to prevent deep scoring of samples, the sandpaper belt was adequately wetted and minimal pressure was applied to the sample during sanding. Grit paper was held in place using a *Leco DS-20* system that automatically disperses water down the length of the grit paper. Visual assessment of the samples was completed using a microscope following rinsing with ethanol to remove particulates.

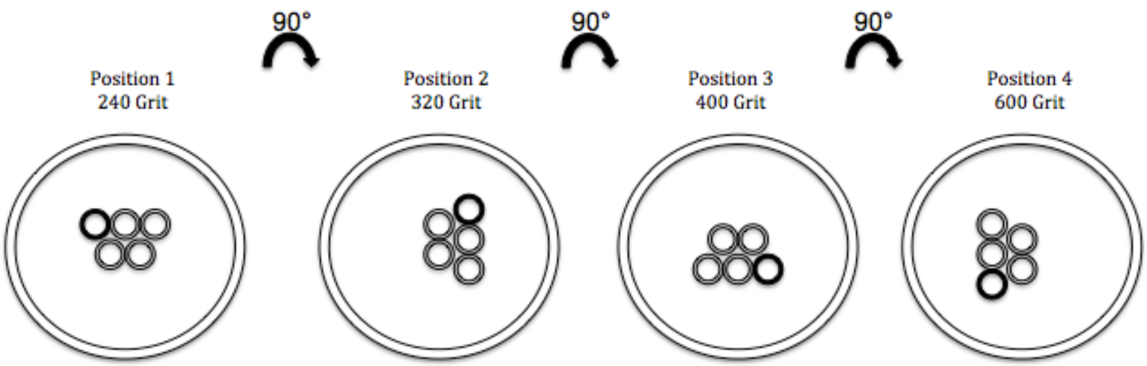


Figure 2.6. Illustration of grit paper polishing directions and the corresponding grit paper value.

Figure 2.7. Image of a murine bone sample successfully ground to through the 600 grit paper. Scratches are in a uniform vertical direction which allows for the sample to be moved to the next step of polishing.

Following adequate sanding, samples were metallographically polished sequentially with a 0.3 micron (Buehler # 40-6249) and a 0.05 micron suspension (Buehler # 40-6377-064) on polishing wheels. Samples were first polished to 0.3 micron on the polishing pads at 200 rpm by using circular and figure eight patterns. Polishing wheel solutions were mixed with 3 drops of 0.3 Silica-Alumina Suspension and 10ml of distilled water, and distributed evenly over the polishing pad. Forgengs solution was also applied to the pad sparingly to maintain smooth polishing rotations. Samples were considered adequate for the next set of polishing after visual assessment of the removal of all 600 grit-sanding lines. Once these lines were completely removed from the cortical bone surface samples were moved to the 0.05 Alumina Oxide micron polishing step. The suspension

formulation and pad preparation were conducted the same as the 0.3 micron level, except the polishing pad (Allied High Tech # 90-500-350) was a magnetic pad, rather than adhesive backed. The sample was polished until there was uniform polishing as determined by visual assessment. The 0.05 polishing step allowed for identification of bone structures and sites of remodeling and is widely recognized as an adequate level of surface roughness for nanoindentation.

This set of polishing resulted in smooth finished surface samples, but the pucks had become non-parallel during the polishing process (traced back to initial belt grinding) and were not able to be tested. As a result, the process was repeated using the distal halves of the broken murine femurs and new techniques were adopted to ensure that both sides of the pucks were flat and parallel.

The embedding process was the same up to the first step of polishing. Because the 120 grit belt grinder was deemed the initial and highest source of sample unevenness, that method was eliminated and an alternate method of using a lathe was adopted. Samples were loaded into a lathe and cut down at 200 rpm until the cortical bone surface was exposed. Using the lathe, both the testing surface and the back of the pucks could be cut so that both sides were flat and parallel. The backside was initially cut flat and then turned over to remove material from the testing side. This process was done very slowly, removing 0.001”-0.003” of material per pass, so as to not damage the embedded bones. Samples were lubricated using aluminum-cutting fluid to ensure clean passes.

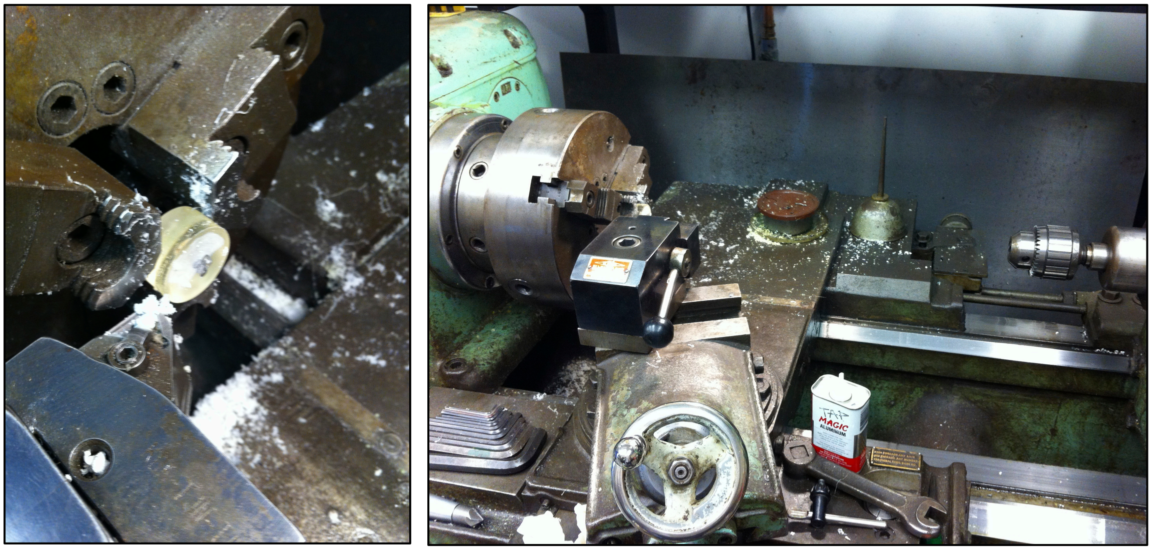


Figure 2.8. An epoxy sample sitting in the chuck of a lathe being cut down till all bones are exposed on the testing surface side.

Following the machine lathe, samples underwent the same successive 240, 320, 400, and 600 grit paper polishing up to the point of metallographic polishing. The metallographic polishing is a long process, and the second time around was outsourced to Metals Technology Inc., a company based in Northridge, California, for polishing by Bob Hayes. Once samples were polished and prepped, they were ready for nanoindentation.

2.3 Determining Anatomical Testing Regions

The anatomical testing regions were determined prior to testing beginning on most of the samples. Because the bones tended to shift during the epoxy embedding process, orientation was varied. To determine which anatomical region was which, the samples were examined under a microscope as well as visually assessed through the sides of the epoxy for identifiable features on the bones such as the lateral and medial condyles. When looking under a microscope the medial shaft wall generally had a wide and more flat outer wall. Between the two assessment methods, all anatomical regions were

determined. This method was adopted shortly after indenting began, while all the indentations made prior to this had been done in the general anatomical regions and reclassified into their respective anatomical regions afterwards.

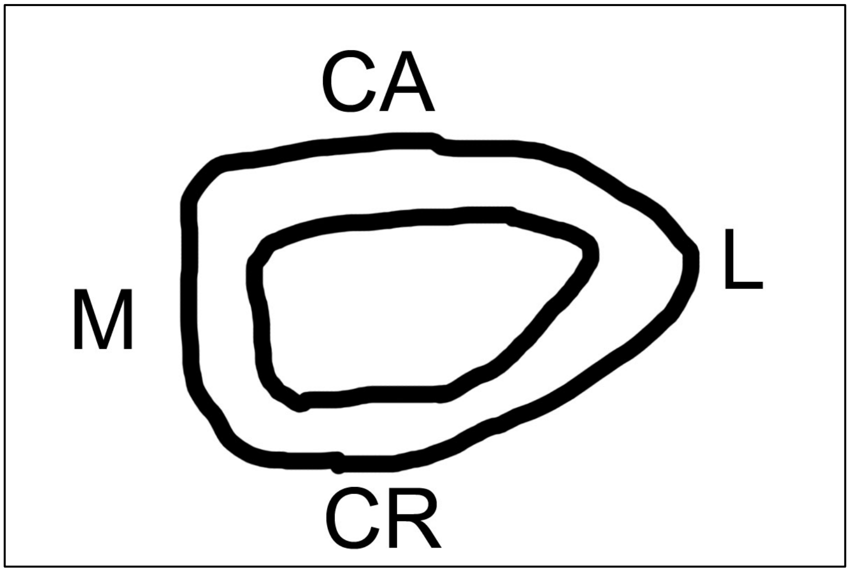


Figure 2.9. Drawing of a left femur cross section depicting the 4 anatomical regions – Medial, Lateral, Cranial and Caudal.

2.4 Nanoindentation

Following sample preparation, nanoindentation training and machine calibration was conducted using a fused silica sample. Nanoindentation was completed on a *Micro Materials Ltd NanoTest* system (Wrexham, United Kingdom). Depth calibrations, conducted once a week, were done on the fused silica test sample enclosed with the nanoindentation system. Nanoindentation was performed using a Berkovich (pyramidal) indenter, which is a three-faced pyramid with tip radius 100-500 nm. The indenter tip was changed from another type to the Berkovich indenter requiring a one-time Sample Stage Calibration. This calibration determines the exact tip of the indenter in reference to the

moving stage. After the proper calibrations were completed, samples were loaded into the stage and experiments were set. Indentation locations were selected using the microscope within the nanoindentation cabinet and a digital xlicap software on the connected computer to pinpoint exact locations of indentations using the crosshairs.

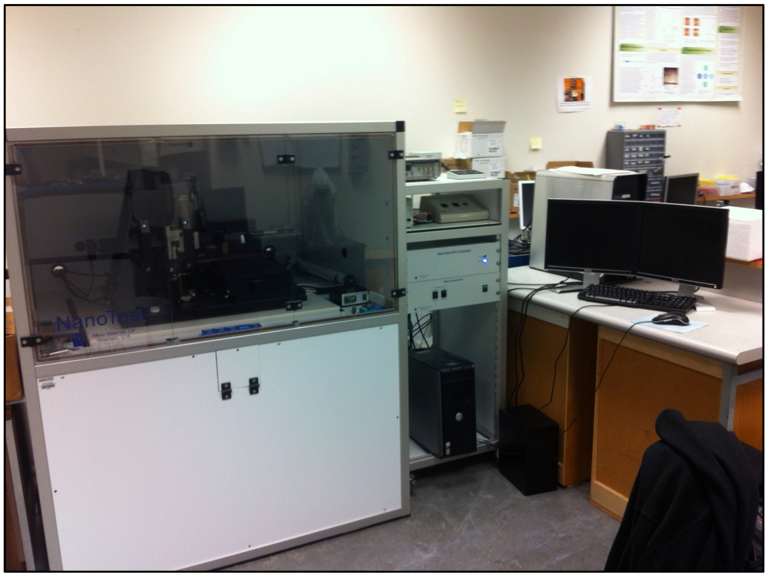


Figure 2.10. Nanoindentation system cabinet and adjacent computer for running experiments and in cabinet microscope control.

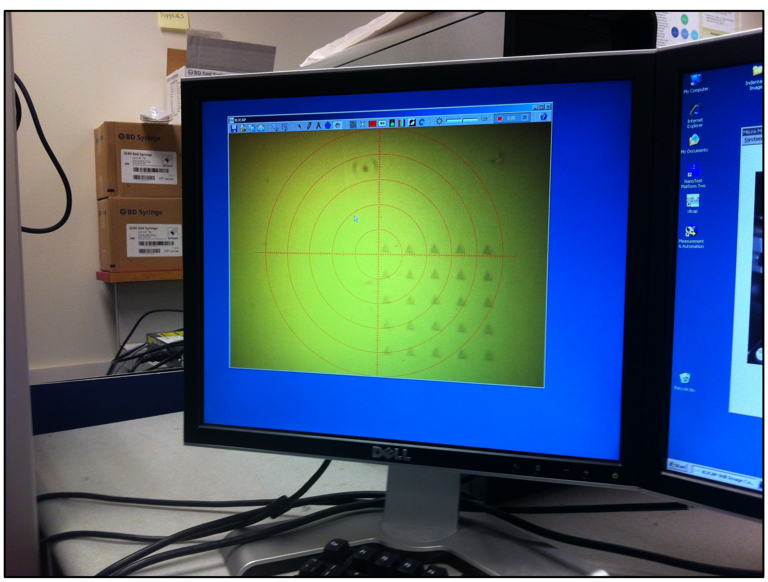


Figure 2.11. Digital image of microscope with the nanoindentation cabinet focusing on a set of indents in a sample.

Indentation conducted in all 4 anatomical regions of each bone – medial, later, cranial, and caudal regions (for human anatomy we use anterior and posterior but for 4 legged animals we use cranial and caudal instead). Regions were scanned for smooth surfaces with little to no voids or lacunae. As a starting test, the first bone on the first puck was tested with 25 indents per anatomical region. After assessing the first set of data points, it was determined that a lesser number of indents would suffice per region. The number of indents was cut from 25 down to 12, and soon after concurrent results were being collected, dropped down to 9 indents per region for the rest of the experiments. Samples had anywhere from 4 to 6 bones in them (some with only partial exposures) resulting in anywhere from 28 to 45 hours to run each experiment (roughly 13 minutes per indent).

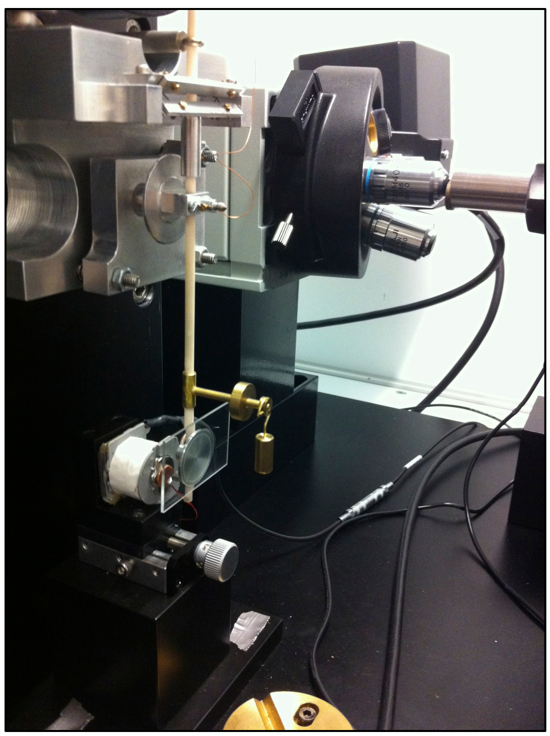


Figure 2.12. Nanoindentation apparatus and Berkovich tip sitting atop the nanoindenter's pendulum distance detection system.

Only one sample could be loaded and indented at once. Samples were loaded in a depth controlled Depth Vs. Load Hysteresis experiment type. A target depth of 1500 nm and maximum load of 100 mN was set with a loading and unloading rate of 0.625 mN/S, a maximum load dwell period for creep of 60 seconds, and a 30 second drift collection dwell period. Retraction distance was set to 30 microns between each indent to ensure tip safety. The resulting data was output graphically via the *Micro Materials LTD, Materials Testing Platform Two* software in graphical Depth vs. Load format.

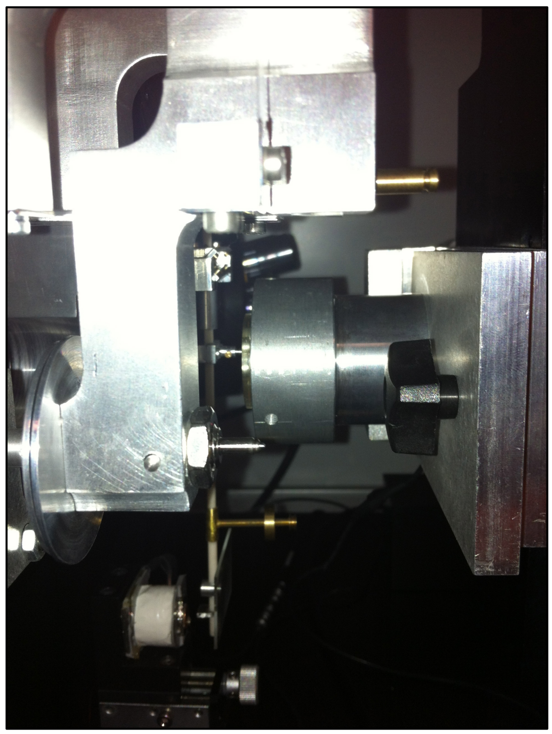


Figure 2.13. Nanoindentation apparatus making contact with a sample creating an indent in the samples testing surface.

2.5 Indentation Imaging

After indentation was completed on a sample, anatomical region indentations were imaged and documented. Having an image of the indentations was useful when interpreting data that did not quite fit the rest of the data points. For example when an

indent landed in a lacunae, the data did not correlate with the rest of the points. However, with an image it was easier to visually assess the cause of this data point outlier. Images were taken using a microscope and an attached Olympus microscope camera with removable SD card. Images of all indentations can be found in Appendix E.

2.6 Data Analysis

Indentation data was directly extracted and analyzed through the NanoTest software. Scheduled data was analyzed using the mean Pre & Post Indentation data, a Berkovich set Beta Factor and was fitted to a general function APd+BPd^2.

2.6.1 Statistical Analysis

Once analyzed on the NanoTest software, the data was then exported into .txt files to be further manipulated in Excel. Nanoindentation experimental results returned a maximum depth, plastic depth, maximum load, hardness and elastic modulus values for each individual indent. Because the experiments were depth controlled, it was considered that analyzing the maximum and plastic depth would prove to be less valuable information because it was controlled. This resulted with using the maximum load, hardness, and elastic modulus for the effects to analyze.

Raw indentation data was compiled into worksheets in Excel and outliers amongst the indents was analyzed for outliers greater than ±2.75 standard deviations. After all the indentation raw data was removed of outliers, the data was grouped by treatment group, sex, limb, and anatomical region in preparation for statistical analysis in Minitab 16 Statistical Software. Data was organized by treatment groups, limb, and the 4 tested

anatomical regions. Each effect - Maximum load, hardness and elastic modulus - was then compared for statistical significance between the treatment groups in Table 1 (i.e. SOST KO 6 month versus SOST TG 6 month versus etc.).

Statistical analysis of the indentation data was done in Minitab 16 Statistical Software through the use of general linear models (GLM). Prior to running a general linear model, a test for equal variances was performed for each effect (max load, hardness, modulus), verifying or discounting the assumption that the variances were equal. Following this test, a three-way interaction GLM was conducted including treatment group, limb (left and right), and anatomical region. Any outliers resulting from the GLM that surpassed ± 2.75 standard deviations were removed as an outlier to maintain normal distribution.

The GLM was run with the inclusion of Tukey post-hoc comparison at a 95% confidence interval to determine the significant effect that treatment type and the secondary variable of either leg or anatomical region had on the desired parameter. A list of the raw data prior to Minitab analysis as well as all Minitab outputs can be found in Appendix C & D, respectively.

Of the 400 testable regions for all the samples, 49 were considered untestable for a number of reasons. For the vast majority, the reason testing was not conducted on a region was because of the lack of little or no exposure of that region of the bone. Depending on the type of fracture during the previous 3 point bending test, or any movements of the bone during the epoxy curing, not all the testing regions for each bone could be exposed while polishing. 10 of the 49 non-tested regions were due to a shift in the epoxy due to bad curing. Those 10 regions were all tested again once, but those tests failed. And to ensure no harm to the indenter tip, those 10 regions were considered

untestable. Because the sample size of testing regions within each treatment group was not affected by these exclusions, the data for the collected regions within the treatment groups sufficed. For a full list of the untested bones and their corresponding untested region, refer to Appendix B.

III. RESULTS

After extraction from the Nanotest Software, data was organized by treatment group, limb and anatomical region, and analyzed using three-way interaction general linear models (GLM) in Minitab as described in Section 2.6. After Minitab analysis, averages and standard deviations were recalculated in excel and histograms were made comparing the Minitab outputs. Error bars in histograms were set to display one standard deviation. For the Minitab outputs, treatment group was referred to as "Genotype+Phenotype" and anatomical region was referred to simply as "region". All Minitab outputs can be found in Appendix D.

3.1 Max Load

Max load was the first parameter examined. The indentation experiments conducted were depth controlled experiments, and hence both maximum depth and plastic depth were considered to show less valuable information. Max load was run through a GLM comparing genotype and phenotype, limb, and anatomical region. Initial GLM returned 2 data points surpassing ±2.75 standard residuals which were excluded from subsequent analysis (9294/Right/Cranial, 9294/Right/Lateral). One value was considered to have large leverage over the data, but was included in analysis due to the small sample size of its treatment group. Figure 3.1 shows the associated p-values for the general linear model (GLM) analysis for this data set. Figure 3.2, Figure 3.3, Figure 3.4, Figure 3.5 and Figure 3.6 display the different groupings and their associated means and significantly different groupings, as determined by a Tukey's comparison with a 95% confidence interval for

treatment type, limb, anatomical region, the interaction between treatment type and limb, and the interaction between treatment type and region, respectively.

Analysis of Variance for Max Load (mN)_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	6600.54	6388.84	709.87	12.80	0.000
Limb	1	331.46	81.72	81.72	1.47	0.226
Region	3	114.52	61.80	20.60	0.37	0.774
Genotype+Phenotype*Limb	9	6820.65	6667.28	740.81	13.36	0.000
Genotype+Phenotype*Region	27	986.26	1039.06	38.48	0.69	0.872
Limb*Region	3	56.98	100.48	33.49	0.60	0.613
Genotype+Phenotype*Limb*Region	27	691.32	691.32	25.60	0.46	0.991
Error	259	14366.86	14366.86	55.47		
Total	338	29968.59				

Figure 3.1: GLM of maximum load across treatment groups and associated p-values. Data shown reflects statistical analysis and excludes 2 outlier values.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	N	Mean	Grouping
SOST KO 12 Month KO	34	61.1	A
SOST KO 12 Month WT	16	53.7	B
SOST TG 6 Month TG	16	53.3	B
SOST TG 6 Month WT	77	52.4	B
SOST KO 6 Month WT	36	51.9	B
SOST KO 6 Month KO	36	51.7	B
SOST TG 6 Month DEF	58	49.8	B
SOST TG 8 Month WT	23	46.3	B C
SOST TG 8 Month TG	15	45.7	B C
SOST TG 8 Month DEF	28	43.2	C

Means that do not share a letter are significantly different.

Figure 3.2: GLM output of treatment group means and significantly different value groupings for max load. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
R	160	51.5	A
L	179	50.3	A

Means that do not share a letter are significantly different.

Figure 3.3: GLM output of limb means and significantly different value groupings for max load. Data shown reflects statistical analysis and excludes 2 outlier values.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
M	86	51.5	A
L	85	51.0	A
CA	85	51.0	A
CR	83	50.1	A

Means that do not share a letter are significantly different.

Figure 3.4: GLM output of anatomical region means and significantly different value groupings for max load. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Data shown reflects statistical analysis and excludes 2 outlier values.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	69.2	A
SOST TG 6 Month WT	R	37	56.2	B
SOST KO 12 Month WT	L	8	55.9	B C
SOST TG 6 Month DEF	R	24	54.4	B C
SOST TG 6 Month TG	R	8	54.2	B C D
SOST KO 6 Month WT	L	21	53.6	B C D
SOST TG 8 Month TG	R	8	53.0	B C D
SOST KO 12 Month KO	R	17	52.9	B C D
SOST KO 6 Month KO	L	16	52.7	B C D
SOST TG 6 Month TG	L	8	52.4	B C D
SOST KO 12 Month WT	R	8	51.5	B C D E
SOST KO 6 Month KO	R	20	50.7	B C D E
SOST TG 8 Month WT	L	13	50.7	B C D E
SOST KO 6 Month WT	R	15	50.2	B C D E
SOST TG 8 Month DEF	R	13	49.6	B C D E
SOST TG 6 Month WT	L	40	48.7	C D E
SOST TG 6 Month DEF	L	34	45.2	D E
SOST TG 8 Month WT	R	10	42.0	D E F
SOST TG 8 Month TG	L	7	38.3	E F
SOST TG 8 Month DEF	L	15	36.8	F

Means that do not share a letter are significantly different.

Figure 3.5: GLM output of the interaction between treatment group and limb and their associated means and significantly different value groupings for max load. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	CA	8	63.5	A
SOST KO 12 Month KO	M	8	63.4	A
SOST KO 12 Month KO	L	9	60.5	A B
SOST KO 12 Month WT	L	4	58.8	A B C
SOST KO 12 Month KO	CR	9	57.0	A B C
SOST KO 6 Month WT	CA	8	56.0	A B C
SOST TG 6 Month TG	CA	4	55.1	A B C D
SOST TG 6 Month TG	M	4	54.5	A B C D
SOST TG 6 Month WT	L	20	54.2	A B C D
SOST KO 12 Month WT	CR	4	53.6	A B C D
SOST TG 6 Month TG	L	4	53.2	A B C D
SOST KO 6 Month WT	M	8	52.8	A B C D
SOST TG 6 Month WT	M	21	52.4	A B C D
SOST KO 6 Month KO	CA	9	52.2	A B C D
SOST KO 12 Month WT	CA	4	52.1	A B C D
SOST KO 6 Month KO	M	9	52.1	A B C D
SOST TG 6 Month WT	CA	19	51.9	A B C D
SOST KO 6 Month KO	L	9	51.5	A B C D
SOST TG 6 Month WT	CR	17	51.4	A B C D
SOST KO 6 Month KO	CR	9	51.1	A B C D
SOST KO 6 Month WT	CR	10	50.8	A B C D
SOST TG 6 Month DEF	CA	16	50.5	B C D
SOST KO 12 Month WT	M	4	50.4	A B C D
SOST TG 6 Month DEF	M	15	50.4	B C D
SOST TG 6 Month TG	CR	4	50.4	A B C D
SOST TG 6 Month DEF	CR	14	49.9	B C D
SOST TG 8 Month WT	M	6	49.0	A B C D
SOST TG 6 Month DEF	L	13	48.3	B C D
SOST KO 6 Month WT	L	10	48.0	B C D
SOST TG 8 Month WT	CR	4	47.3	A B C D
SOST TG 8 Month TG	M	4	47.0	A B C D
SOST TG 8 Month TG	CR	4	46.3	A B C D
SOST TG 8 Month DEF	L	7	46.3	B C D
SOST TG 8 Month WT	L	6	45.8	B C D
SOST TG 8 Month TG	CA	4	45.6	B C D
SOST TG 8 Month TG	L	3	43.7	A B C D
SOST TG 8 Month DEF	CR	8	43.6	C D
SOST TG 8 Month WT	CA	7	43.3	C D
SOST TG 8 Month DEF	M	7	42.9	C D
SOST TG 8 Month DEF	CA	6	40.0	D

Means that do not share a letter are significantly different.

Figure 3.6: GLM output of the interaction between treatment group and anatomical region and their associated means and significantly different value groupings for max load. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Max load was found to be significantly affected by treatment group (p=0.000) as well as both treatment group and limb together (p=0.000), but interestingly not by limb tested

alone (p=0.226, Figure 3.14). There were also no significant findings amongst testing in the different anatomical regions (Figure 3.22).

Observations from these results showed max load for 12 month SOST KO mice to be significantly higher than all other treatment groups, including its own control littermate group (WT 4). No significant differences were seen between the interaction of treatment type and limb for 6 month old SOST TG and their control littermate (WT 1) but a difference in the left and right limb of the SOST DEF (Figure 3.15). Differences were seen in the limbs of the 8 month SOST TG and DEF but not in its control littermate (WT 2) mice (Figure 3.16). Observations from these results showed a significant difference between the 12 month SOST KO and its control littermate (WT 4) mice (Figure 3.10). There were no noted significant differences for max load amongst the 4 anatomical regions alone nor at any combination of region tested and treatment group or limb.

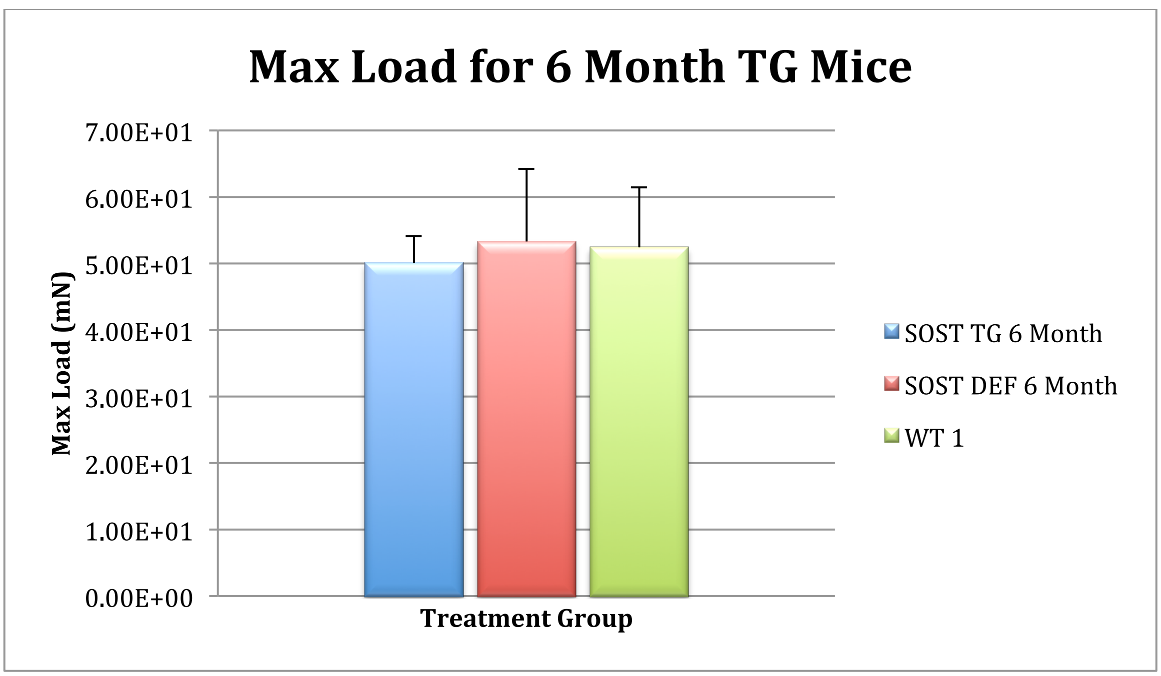


Figure 3.7: Histogram comparison of max load for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

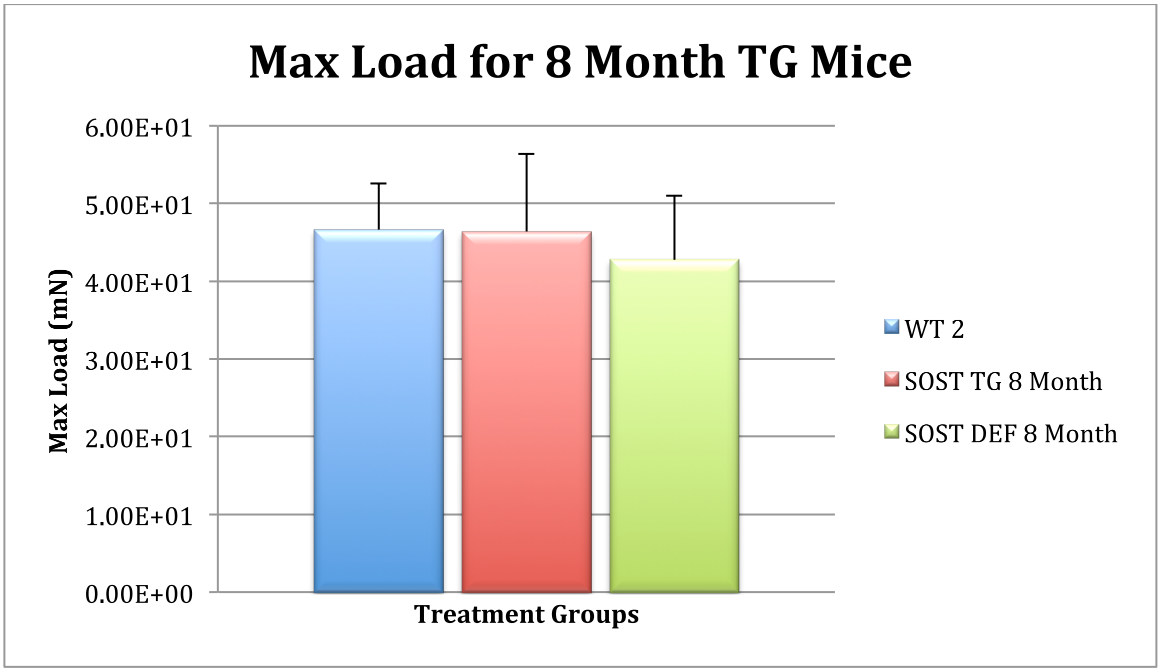


Figure 3.8: Histogram comparison of max load for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

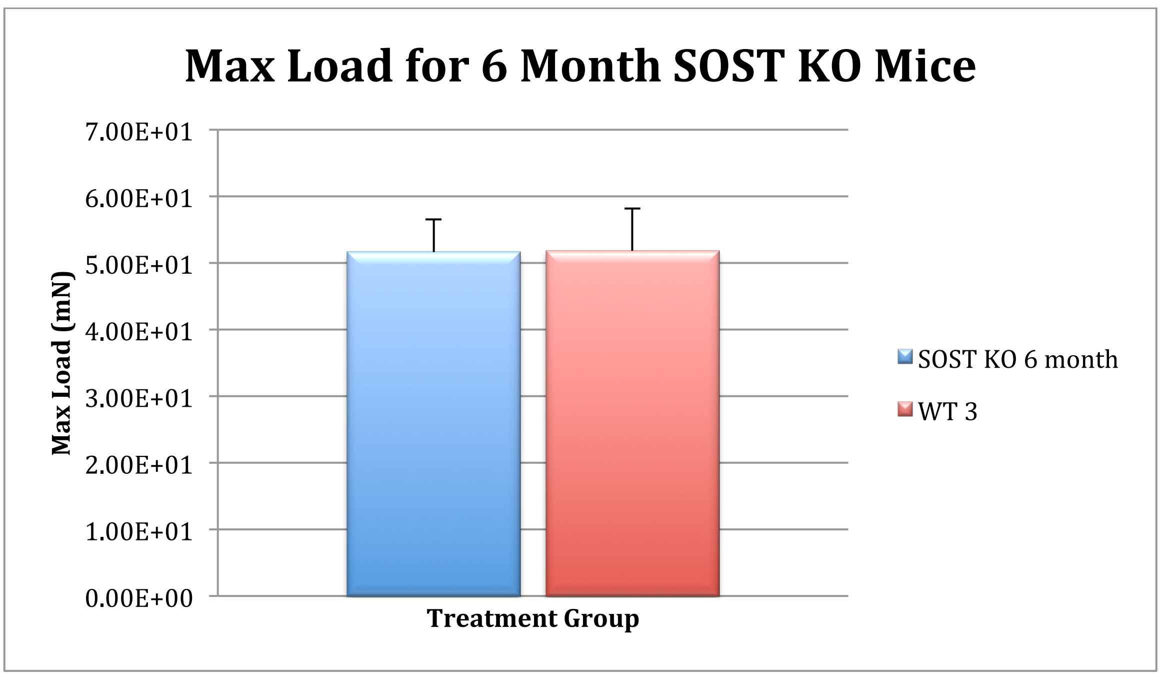


Figure 3.9: Histogram comparison of max load for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

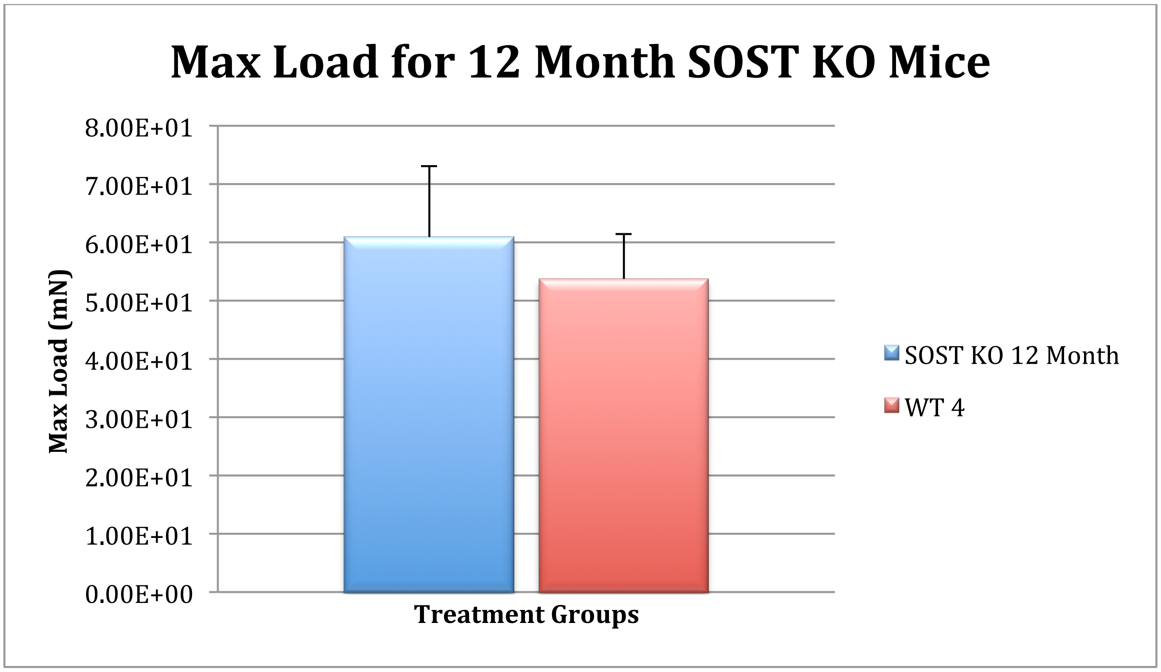


Figure 3.10: Histogram comparison of max load for 12 month old SOST knockout mice. WT 4 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

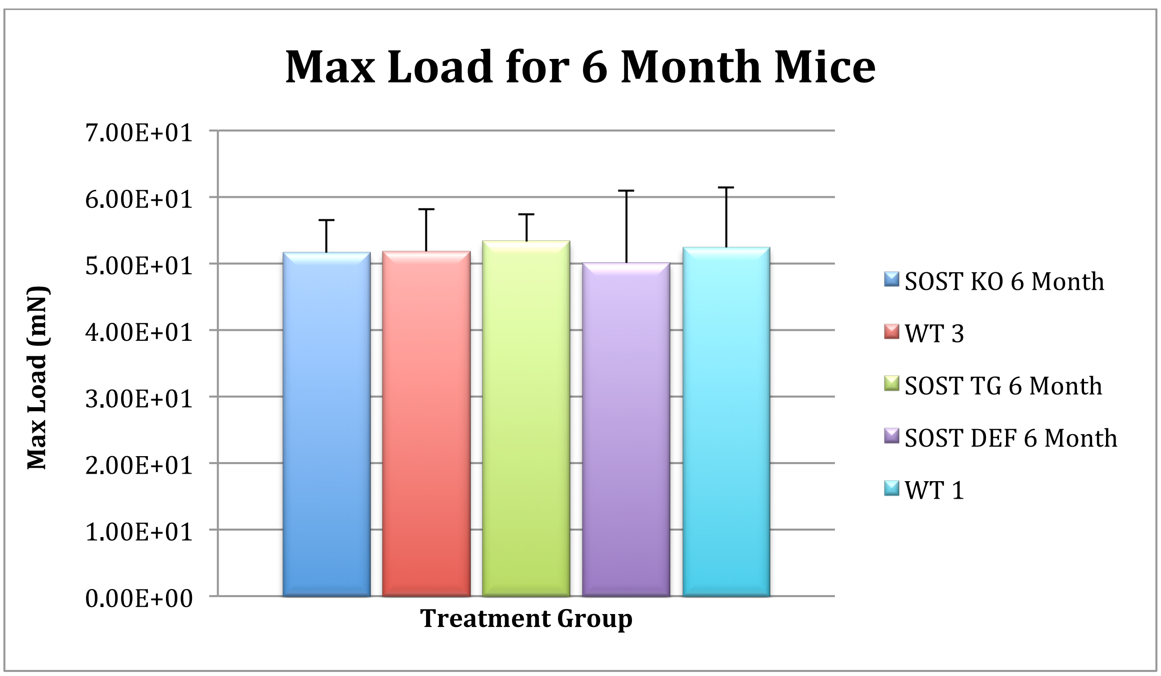


Figure 3.11: Histogram comparison of max load for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

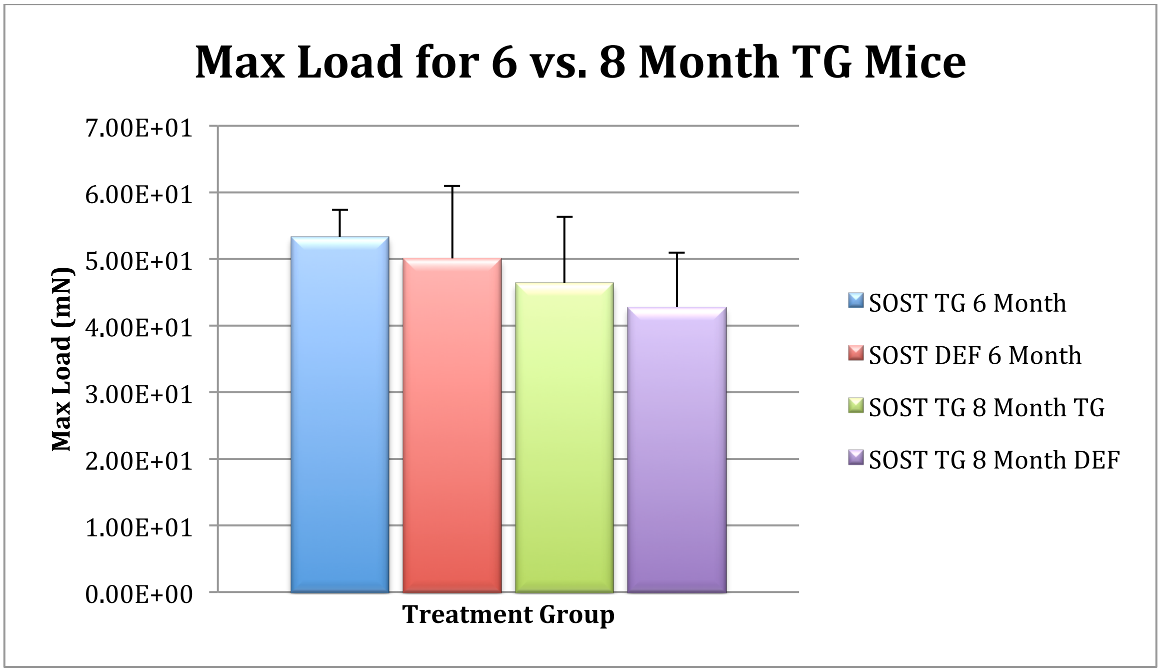


Figure 3.12: Histogram comparison of max load for 6 and 8 month old transgenic mice. Wild type controls were not included in this analysis.

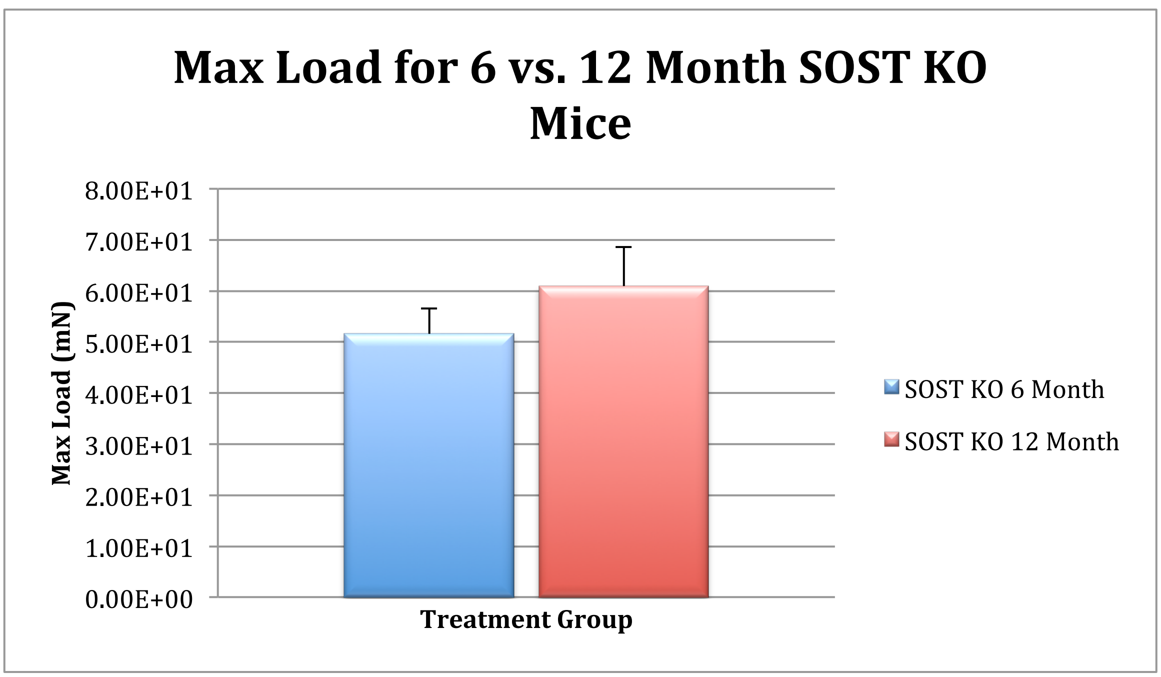


Figure 3.13: Histogram comparison of max load for 6 and 12 month old knockout mice. Wild type controls were not included in this analysis.

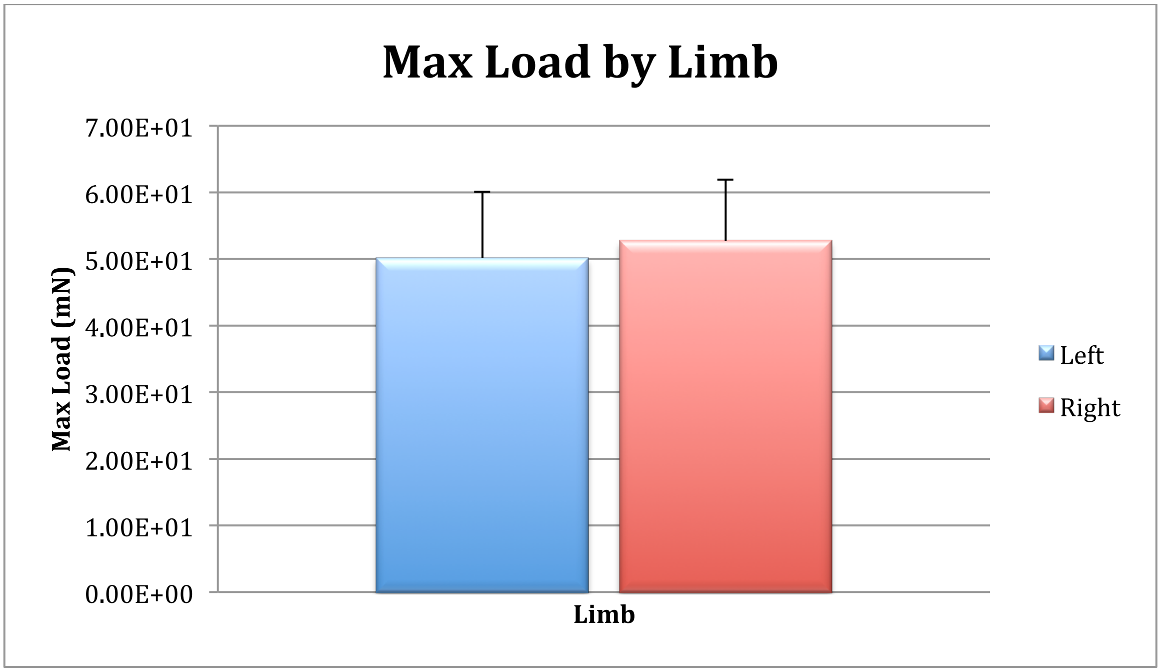


Figure 3.14: Histogram comparison of max load by either left or right limb for all mice. No animals were excluded from this analysis.

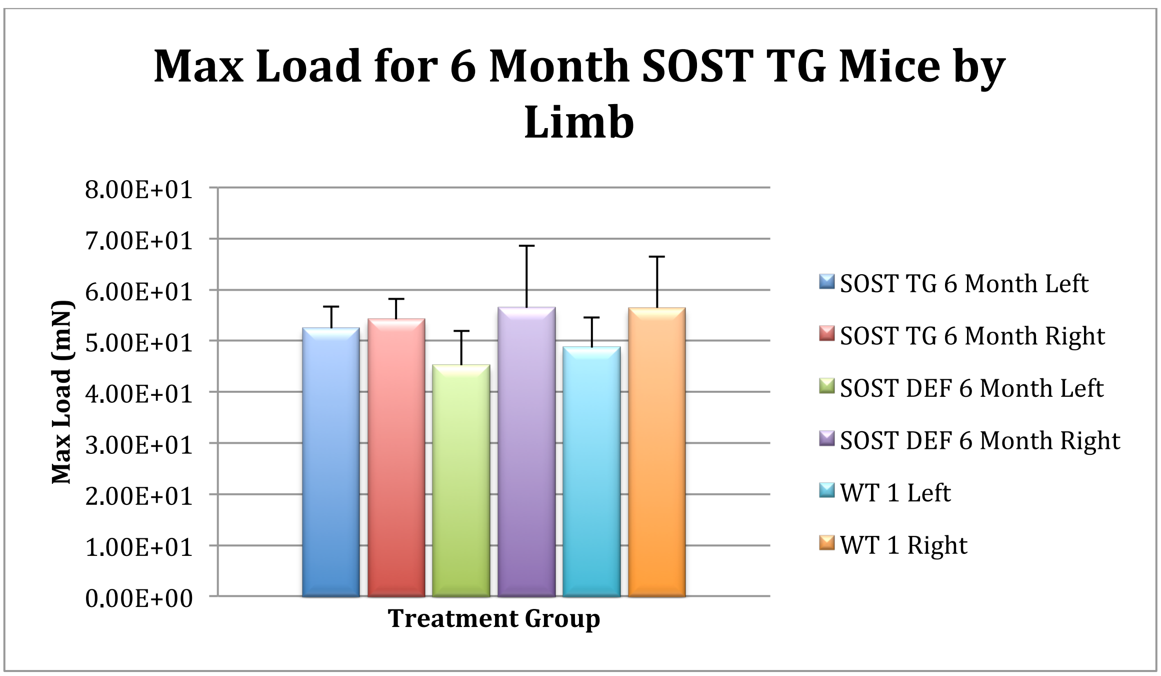


Figure 3.15: Histogram comparison of max load by limb for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

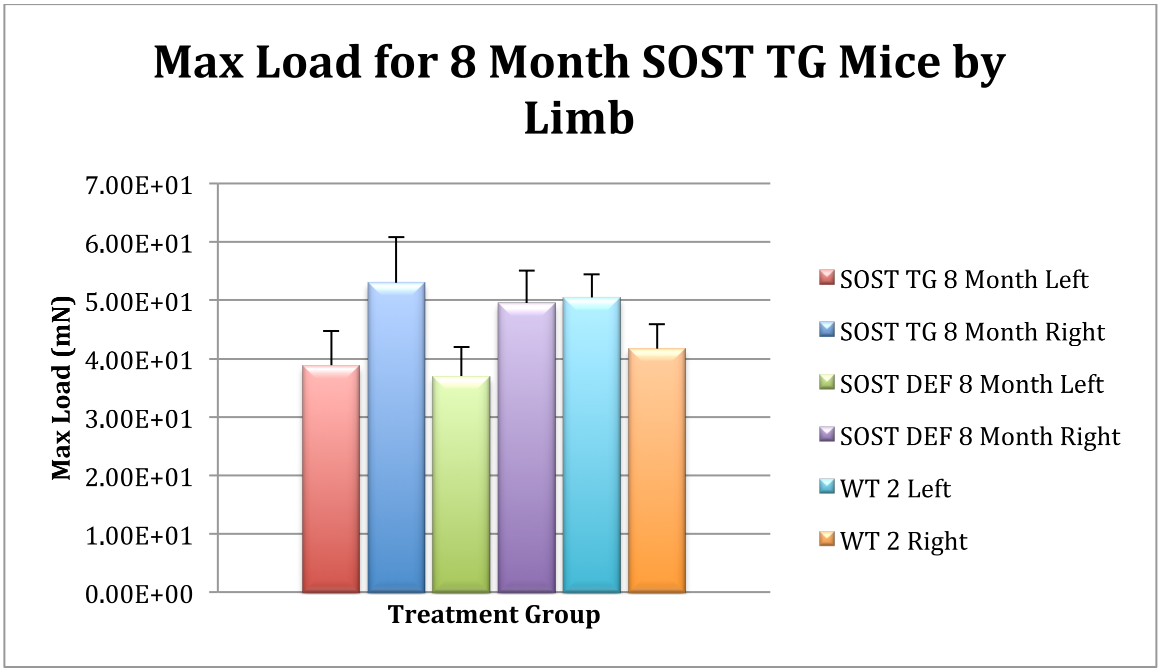


Figure 3.16: Histogram comparison of max load by limb for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis

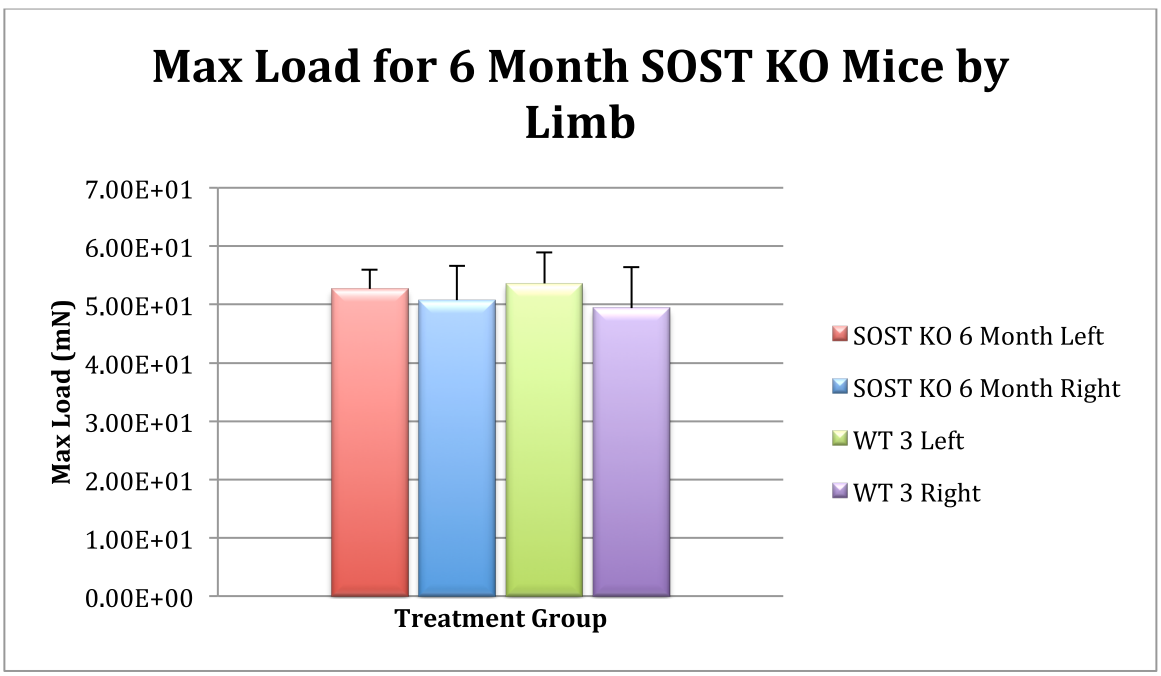


Figure 3.17: Histogram comparison of max load by limb for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis

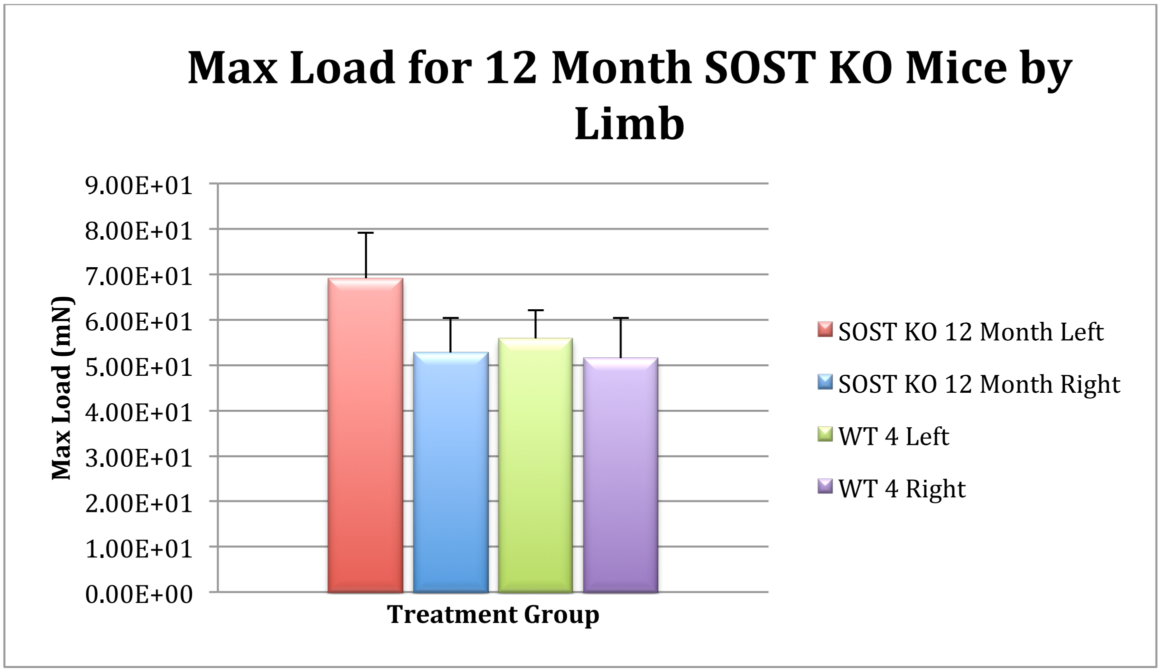


Figure 3.18: Histogram comparison of max load by limb for 12 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis

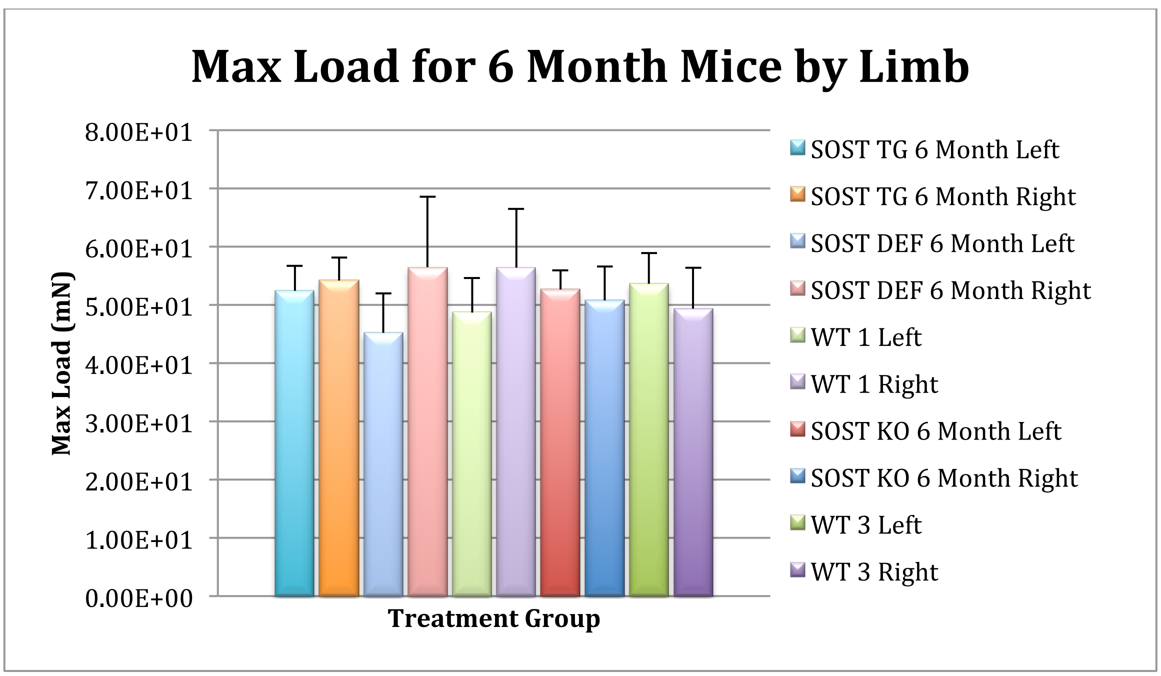


Figure 3.19: Histogram comparison of max load by limb for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

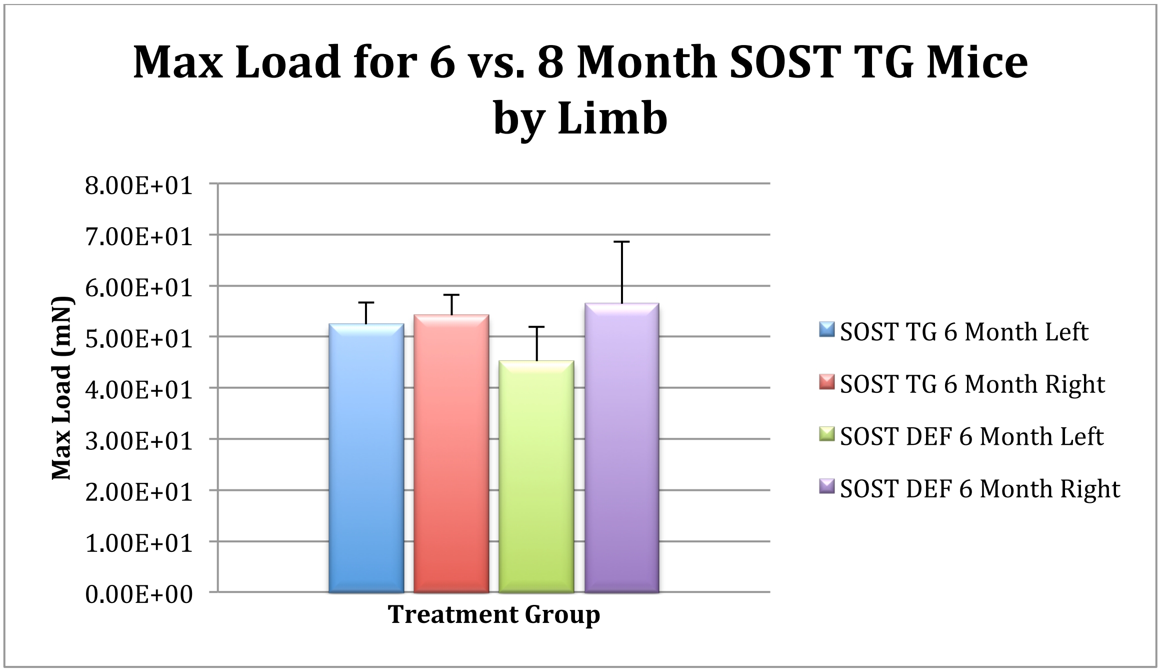


Figure 3.20: Histogram comparison of max load by limb for 6 and 8 month old transgenic mice. Wild type controls were not included in this analysis.

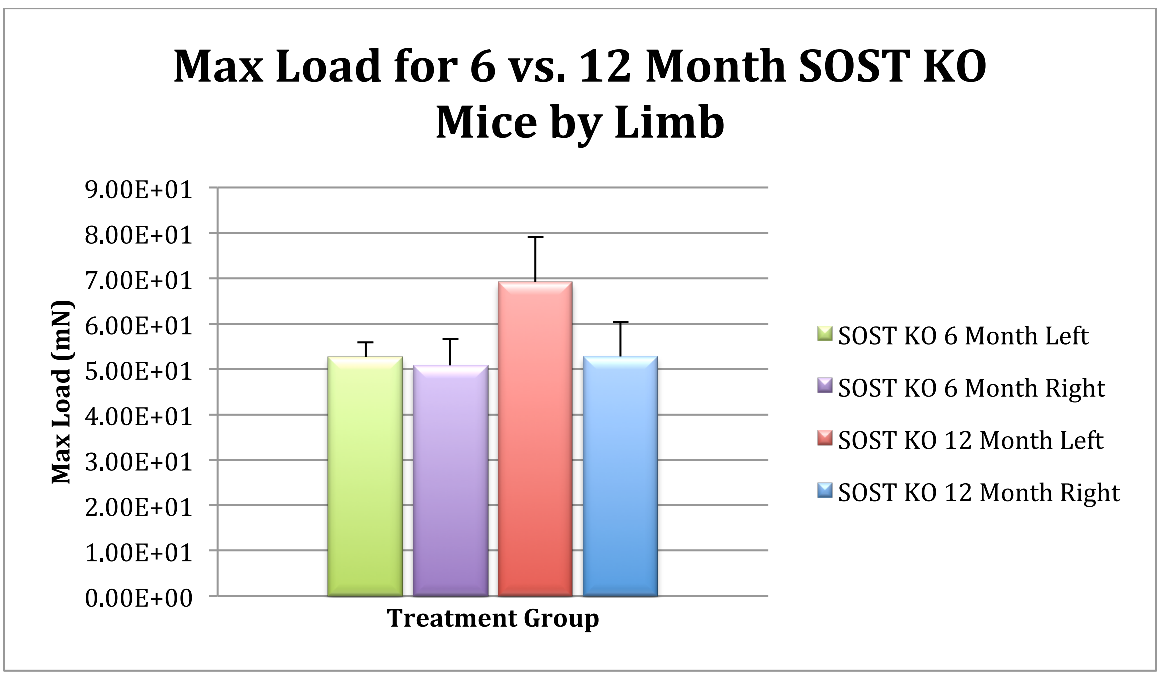


Figure 3.21: Histogram comparison of max load by limb for 6 and 12 month old knockout mice. Wild type controls were not included in this analysis.

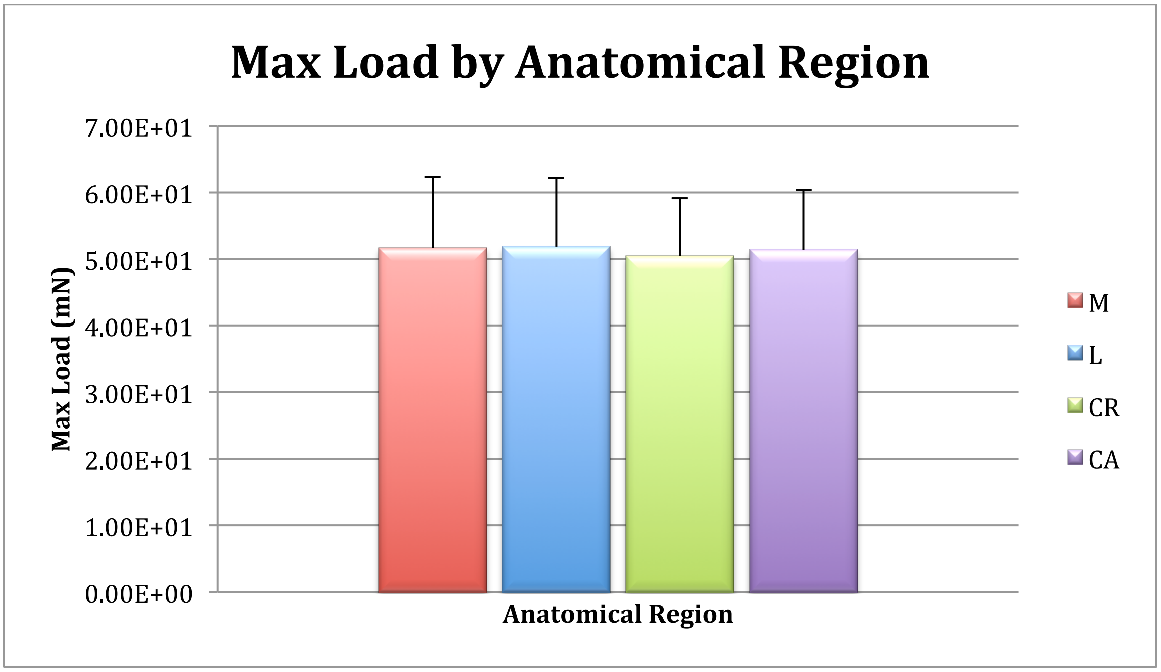


Figure 3.22: Histogram comparison of max load by anatomical region. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis

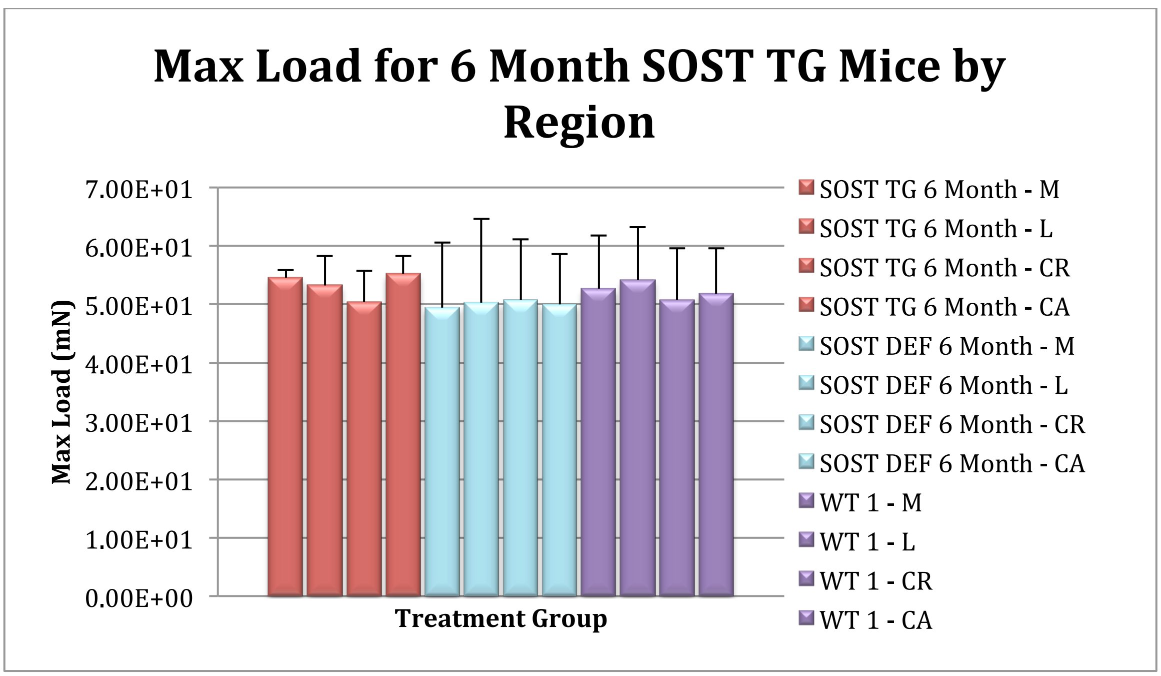


Figure 3.23: Histogram comparison of max load by anatomical region for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

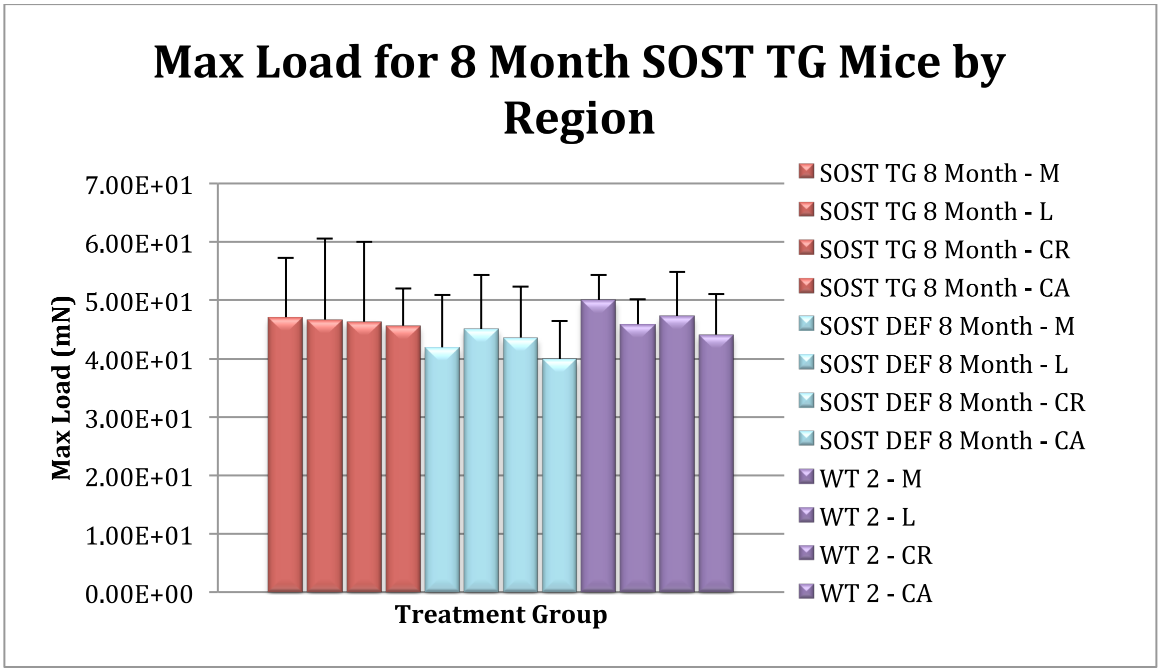


Figure 3.24: Histogram comparison of max load by anatomical region for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

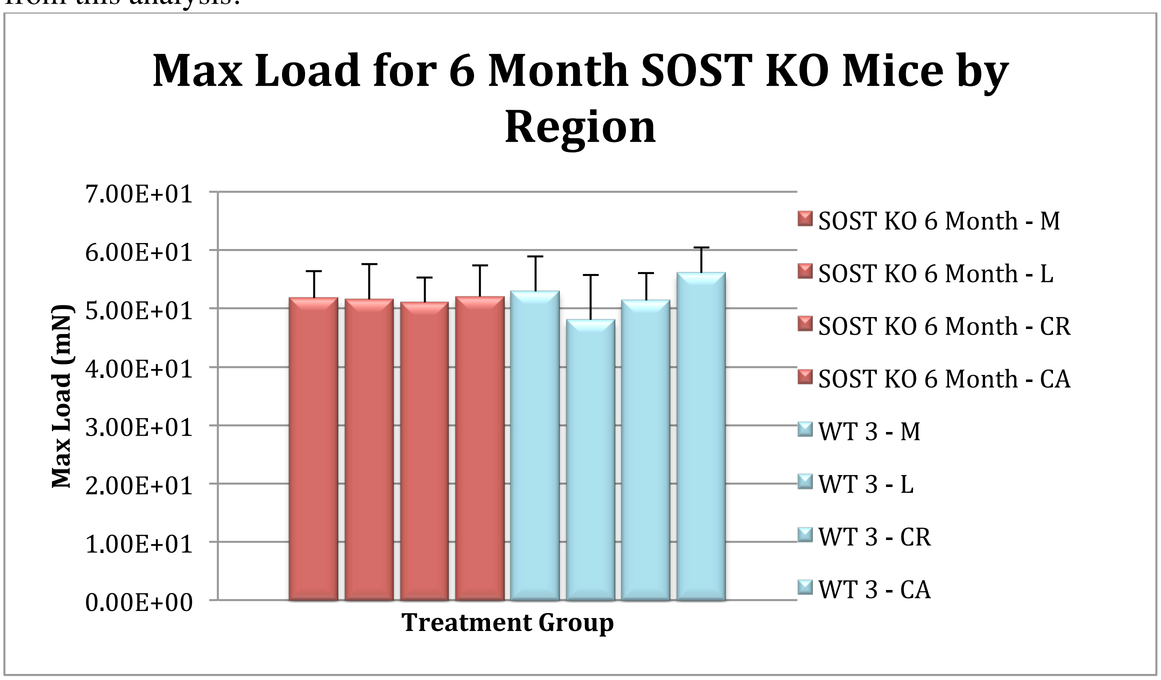


Figure 3.25: Histogram comparison of max load by anatomical region for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

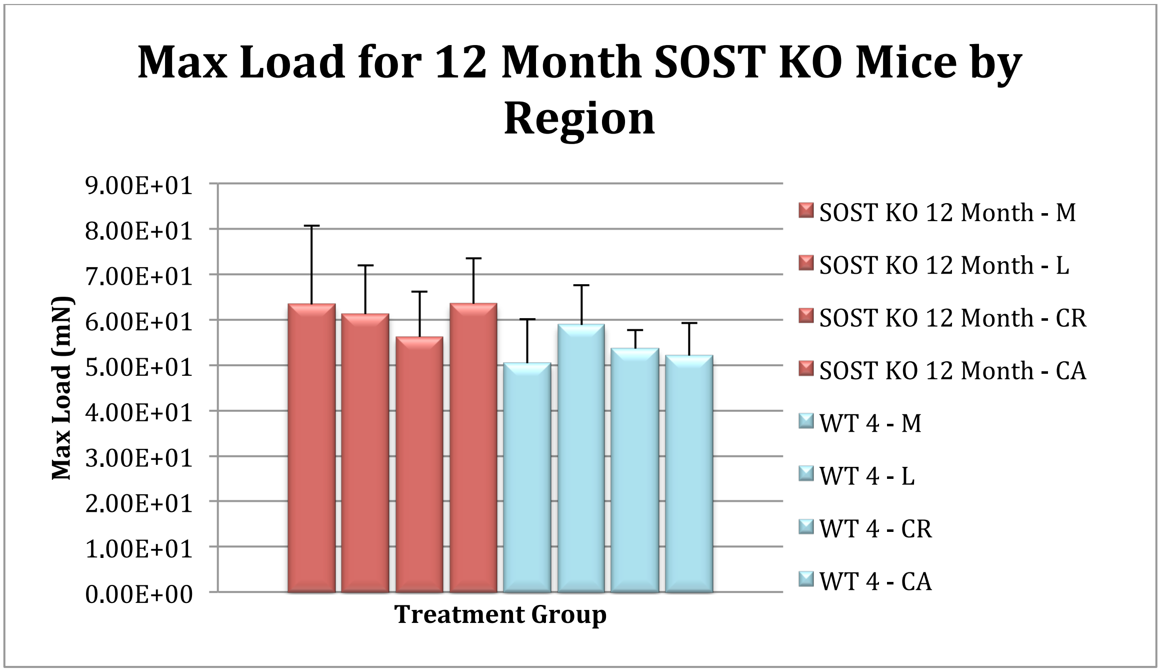


Figure 3.26: Histogram comparison of max load by anatomical region for 12 month old SOST knockout mice. WT 4 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

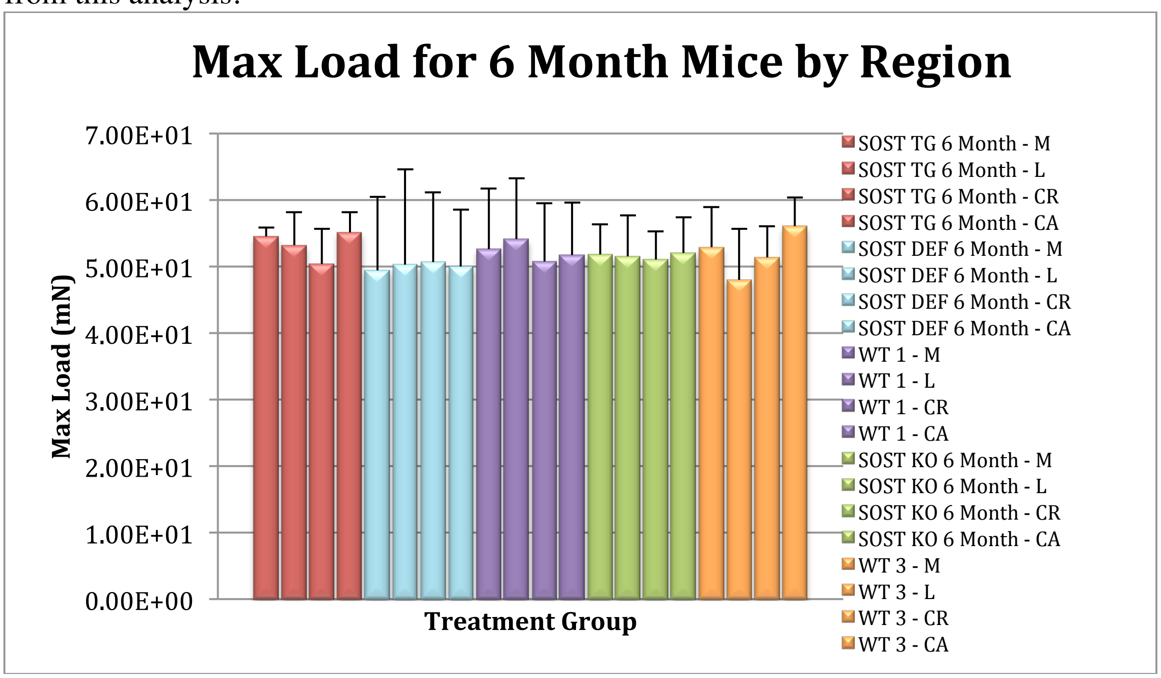


Figure 3.27: Histogram comparison of max load by anatomical region for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

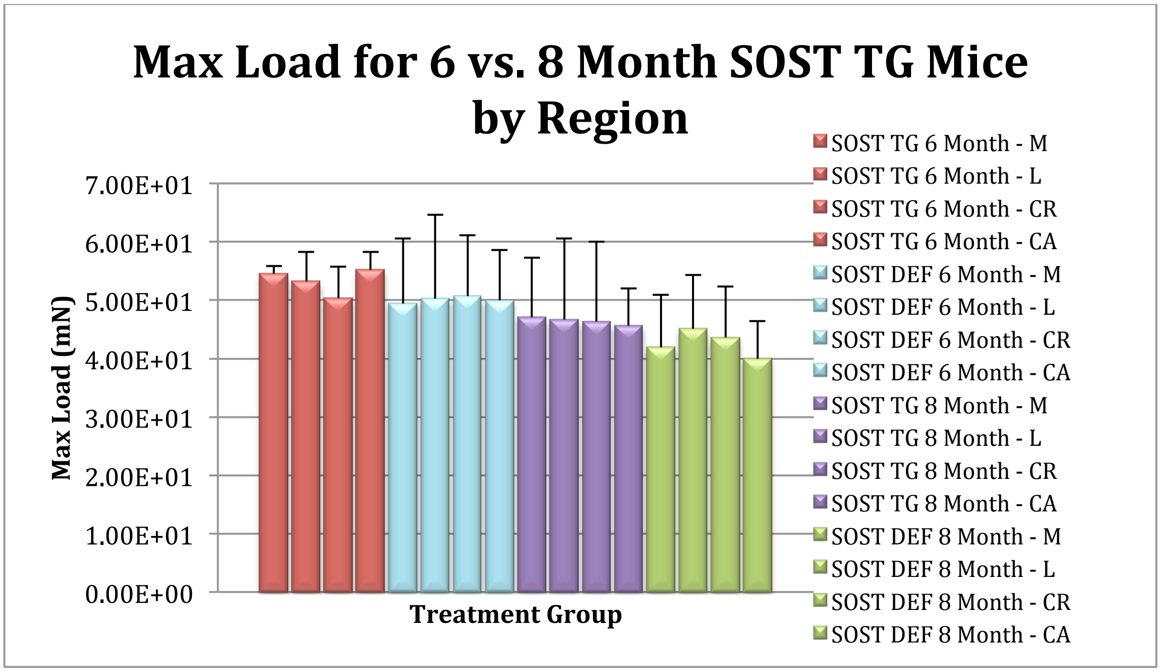


Figure 3.28: Histogram comparison of max load by anatomical region for 6 and 8 month old transgenic mice. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Wild type controls were not included in this analysis.

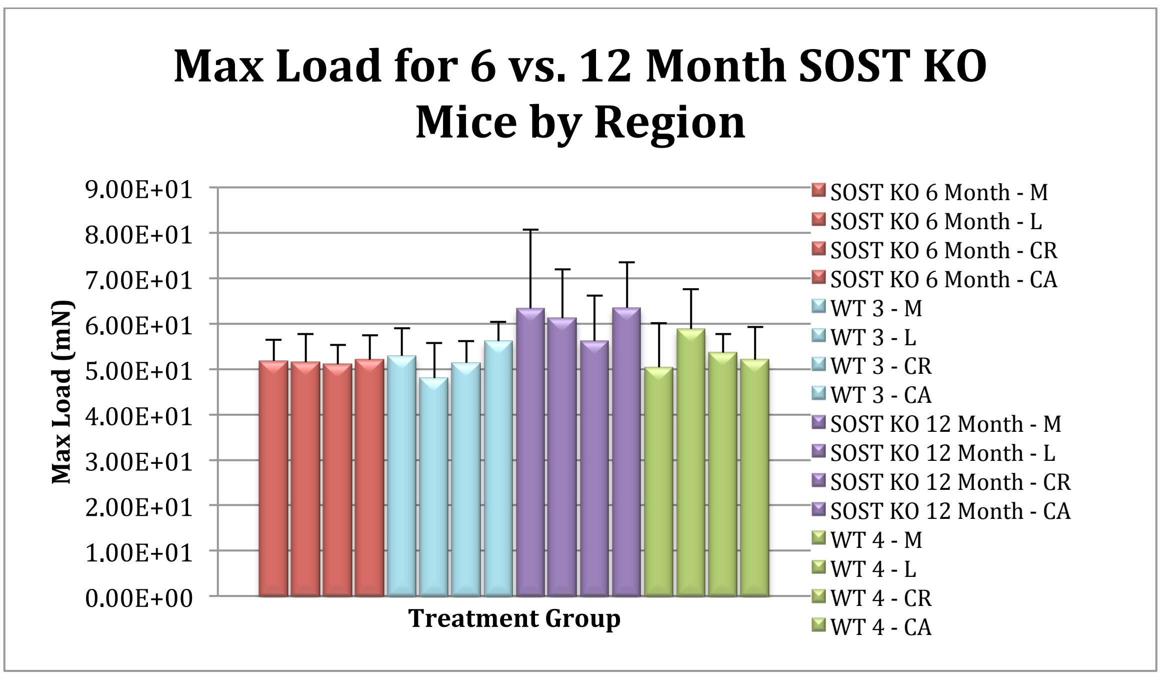


Figure 3.29: Histogram comparison of max load by anatomical region for 6 and 12 month old knockout mice. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Wild type controls were not included in this analysis.

3.2 Hardness

The three way interaction GLM was also run for hardness against treatment group, limb and anatomical region. Initial GLM returned 2 data points surpassing ±2.75 standard residuals which were excluded from subsequent analysis (9294/Right/Lateral, 8602/Right/Caudal). One value was considered to have large leverage over the data, but was included in analysis due to the small sample size of its treatment group. Figure 3.30 shows the associated p-values for the general linear model (GLM) analysis for this data set. Figure 3.31 displays the different treatment groups and their associated means and significantly different groupings, as determined by a Tukey's comparison with a 95% confidence interval.

Analysis of Variance for Hardness (Gpa)_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	13.31455	12.76804	1.41867	25.70	0.000
Limb	1	0.29855	0.44806	0.44806	8.12	0.005
Region	3	0.02191	0.01911	0.00637	0.12	0.951
Genotype+Phenotype*Limb	9	7.73976	7.47065	0.83007	15.04	0.000
Genotype+Phenotype*Region	27	1.28030	1.38367	0.05125	0.93	0.571
Limb*Region	3	0.13861	0.11446	0.03815	0.69	0.558
Genotype+Phenotype*Limb*Region	27	0.85215	0.85215	0.03156	0.57	0.958
Error	263	14.51887	14.51887	0.05520		
Total	342	38.16469				

S = 0.234957   R-Sq = 61.96%   R-Sq(adj) = 50.53%

Figure 3.30: GLM of hardness across treatment groups and associated p-values. Data shown reflects statistical analysis and excludes 2 outlier values.

```
Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype    N  Mean  Grouping
SOST KO 12 Month KO  34   1.6  A
SOST TG 6 Month TG   16   1.6  A
SOST KO 12 Month WT  16   1.6  A
SOST KO 6 Month WT   36   1.5  A
SOST KO 6 Month KO   40   1.5  A
SOST TG 6 Month WT   76   1.4    B
SOST TG 6 Month DEF  59   1.3    B C
SOST TG 8 Month WT   23   1.1      C D
SOST TG 8 Month TG   15   1.1      C D
SOST TG 8 Month DEF  28   1.0        D

Means that do not share a letter are significantly different.
```

Figure 3.31: GLM output of treatment group means and significantly different value groupings for hardness. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

```
Grouping Information Using Tukey Method and 95.0% Confidence

Limb    N  Mean  Grouping
L     183   1.4  A
R     160   1.3    B

Means that do not share a letter are significantly different.
```

Figure 3.32: GLM output of limb means and significantly different value groupings for hardness. Data shown reflects statistical analysis and excludes 2 outlier values.

```
Grouping Information Using Tukey Method and 95.0% Confidence

Region   N  Mean  Grouping
M       87   1.4  A
CA      85   1.4  A
L       86   1.4  A
CR      85   1.3  A

Means that do not share a letter are significantly different.
```

Figure 3.33: GLM output of anatomical region means and significantly different value groupings for hardness. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Data shown reflects statistical analysis and excludes 2 outlier values.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	2.1	A
SOST TG 6 Month TG	R	8	1.6	B
SOST KO 6 Month WT	L	21	1.6	B
SOST KO 12 Month WT	R	8	1.6	B
SOST KO 12 Month WT	L	8	1.6	B C
SOST TG 6 Month TG	L	8	1.6	B C
SOST KO 6 Month KO	L	20	1.5	B C
SOST KO 6 Month KO	R	20	1.5	B C
SOST KO 6 Month WT	R	15	1.5	B C D
SOST TG 6 Month WT	R	36	1.4	B C D E
SOST TG 6 Month DEF	R	25	1.3	B C D E F
SOST TG 6 Month WT	L	40	1.3	B C D E F
SOST TG 6 Month DEF	L	34	1.2	D F G
SOST TG 8 Month WT	L	13	1.2	C D E F G H
SOST KO 12 Month KO	R	17	1.2	D E F G H
SOST TG 8 Month DEF	R	13	1.1	F G H
SOST TG 8 Month TG	L	7	1.1	E F G H
SOST TG 8 Month TG	R	8	1.0	F G H
SOST TG 8 Month WT	R	10	0.9	G H
SOST TG 8 Month DEF	L	15	0.9	H

Means that do not share a letter are significantly different.

Figure 3.34: GLM output of the interaction between treatment group and limb and their associated means and significantly different value groupings for hardness. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	CA	8	1.7	A
SOST KO 12 Month WT	L	4	1.7	A B
SOST KO 12 Month KO	M	8	1.7	A B
SOST TG 6 Month TG	CA	4	1.7	A B C
SOST KO 6 Month WT	CA	8	1.7	A B C
SOST TG 6 Month TG	M	4	1.6	A B C D
SOST KO 12 Month KO	L	9	1.6	A B C D
SOST KO 12 Month WT	CR	4	1.6	A B C D
SOST KO 6 Month KO	CA	10	1.6	A B C D
SOST TG 6 Month TG	L	4	1.6	A B C D E
SOST KO 12 Month WT	CA	4	1.5	A B C D E F
SOST KO 6 Month WT	M	8	1.5	A B C D E F
SOST KO 6 Month WT	CR	10	1.5	A B C D E F
SOST KO 6 Month KO	CR	10	1.5	A B C D E F
SOST TG 6 Month TG	CR	4	1.5	A B C D E F G
SOST KO 6 Month KO	M	10	1.5	A B C D E F G
SOST KO 6 Month KO	L	10	1.5	A B C D E F G
SOST KO 12 Month KO	CR	9	1.5	A B C D E F G
SOST KO 12 Month WT	M	4	1.4	A B C D E F G
SOST KO 6 Month WT	L	10	1.4	A B C D E F G

SOST	TG	6	Month	WT	L	20	1.4	A B C D E F G
SOST	TG	6	Month	WT	M	21	1.4	A B C D E F G
SOST	TG	6	Month	WT	CR	17	1.4	A B C D E F G
SOST	TG	6	Month	DEF	CR	15	1.3	B C D E F G
SOST	TG	6	Month	WT	CA	18	1.3	B C D E F G
SOST	TG	6	Month	DEF	M	15	1.3	B C D E F G
SOST	TG	6	Month	DEF	CA	16	1.3	B C D E F G
SOST	TG	6	Month	DEF	L	13	1.2	C D E F G
SOST	TG	8	Month	WT	M	6	1.1	B C D E F G
SOST	TG	8	Month	WT	CR	4	1.1	B C D E F G
SOST	TG	8	Month	TG	M	4	1.1	B C D E F G
SOST	TG	8	Month	DEF	L	7	1.1	C D E F G
SOST	TG	8	Month	TG	CA	4	1.1	B C D E F G
SOST	TG	8	Month	WT	L	6	1.1	D E F G
SOST	TG	8	Month	DEF	M	7	1.1	E F G
SOST	TG	8	Month	TG	CR	4	1.0	C D E F G
SOST	TG	8	Month	DEF	CR	8	1.0	E F G
SOST	TG	8	Month	TG	L	3	1.0	C D E F G
SOST	TG	8	Month	WT	CA	7	1.0	F G
SOST	TG	8	Month	DEF	CA	6	0.9	G

Means that do not share a letter are significantly different.

Figure 3.35: GLM output of the interaction between treatment group and anatomical region and their associated means and significantly different value groupings for hardness. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Hardness was found to be significantly affected by treatment group (p=0.000) and by limb tested (p=0.005), as well as significant findings were found between the interaction of treatment group and limb tested (p=0.000, Figure 3.43). 6 Month SOST TG mice were found to be significantly greater than its SOST DEF and control littermate (WT 1) counterparts (Figure 3.36). Observations show no significant difference between any treatment groups for the 8 month SOST TG, 6 month SOST KO or 12 month SOST KO mice. Side by side limb differences were observed in the limbs of the 12 month SOST KO mice (Figure 3.47). There were no significant findings amongst the different anatomical regions or its interaction with treatment group or limb.

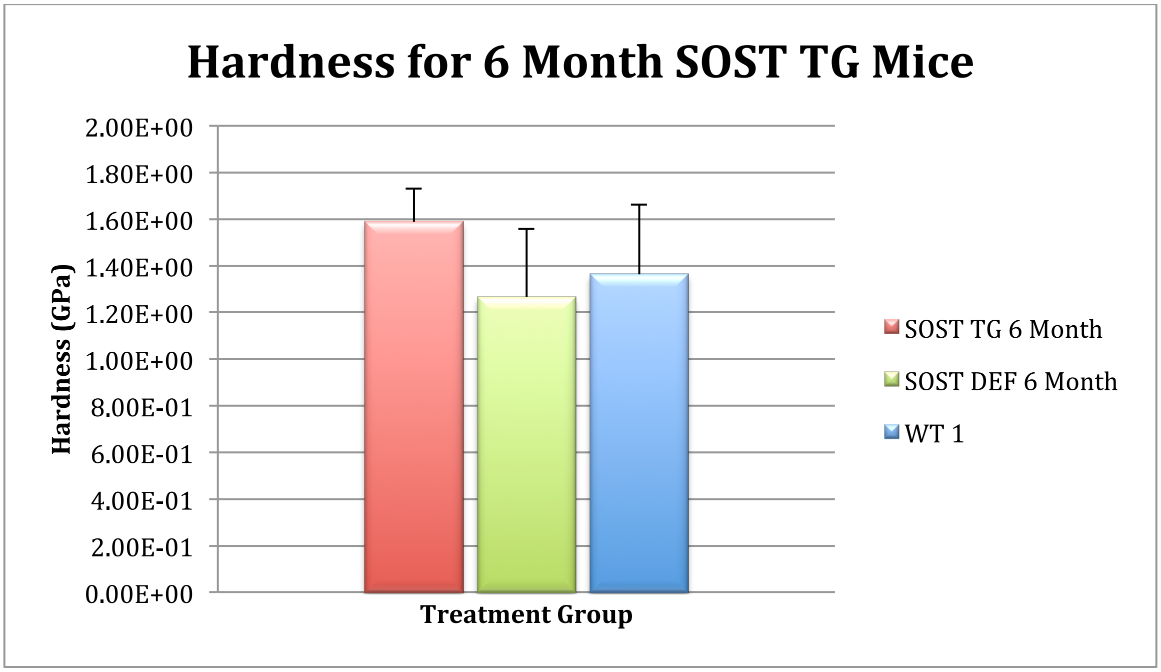


Figure 3.36: Histogram comparison of hardness for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

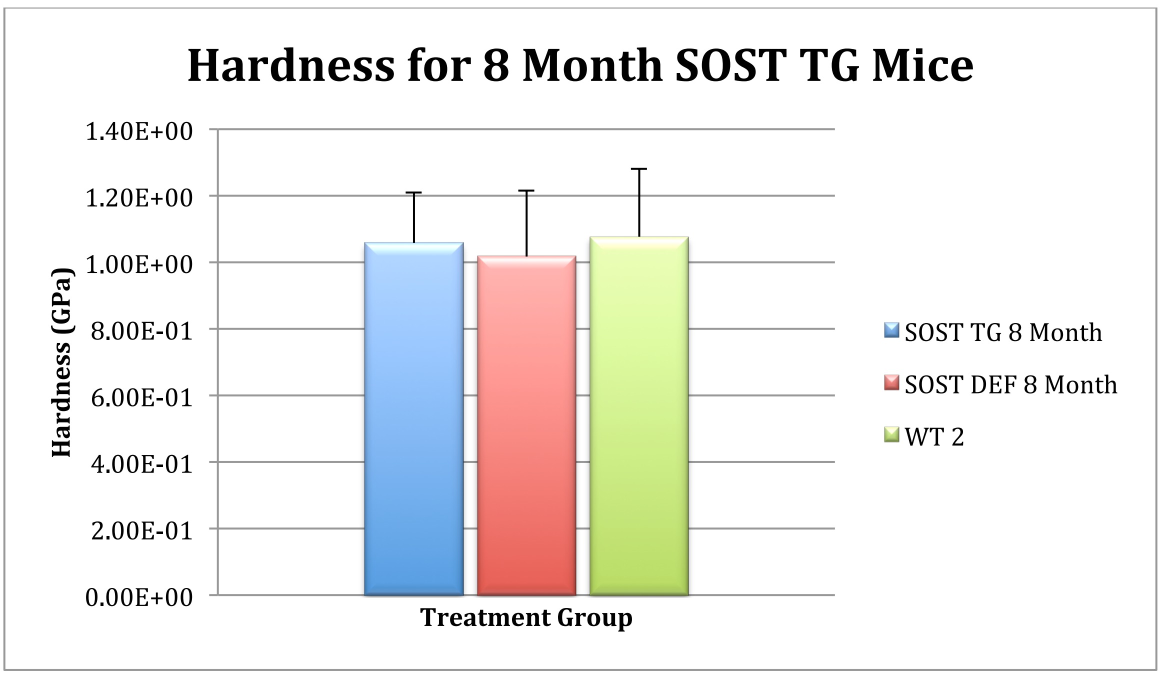


Figure 3.37: Histogram comparison of hardness for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

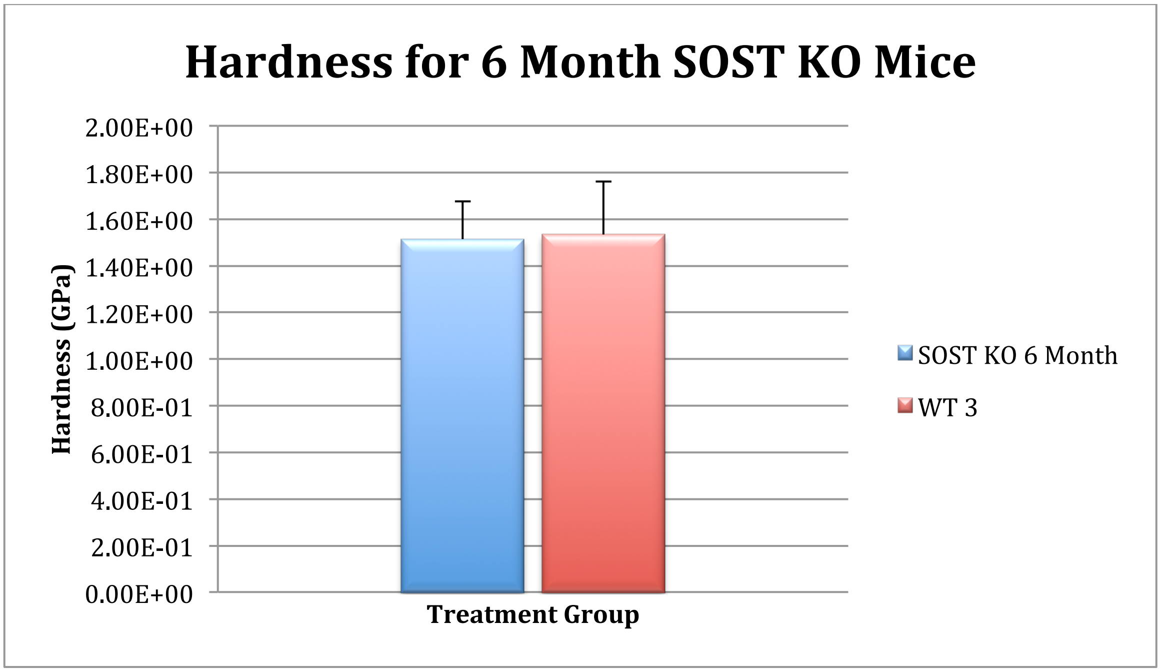


Figure 3.38: Histogram comparison of hardness for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

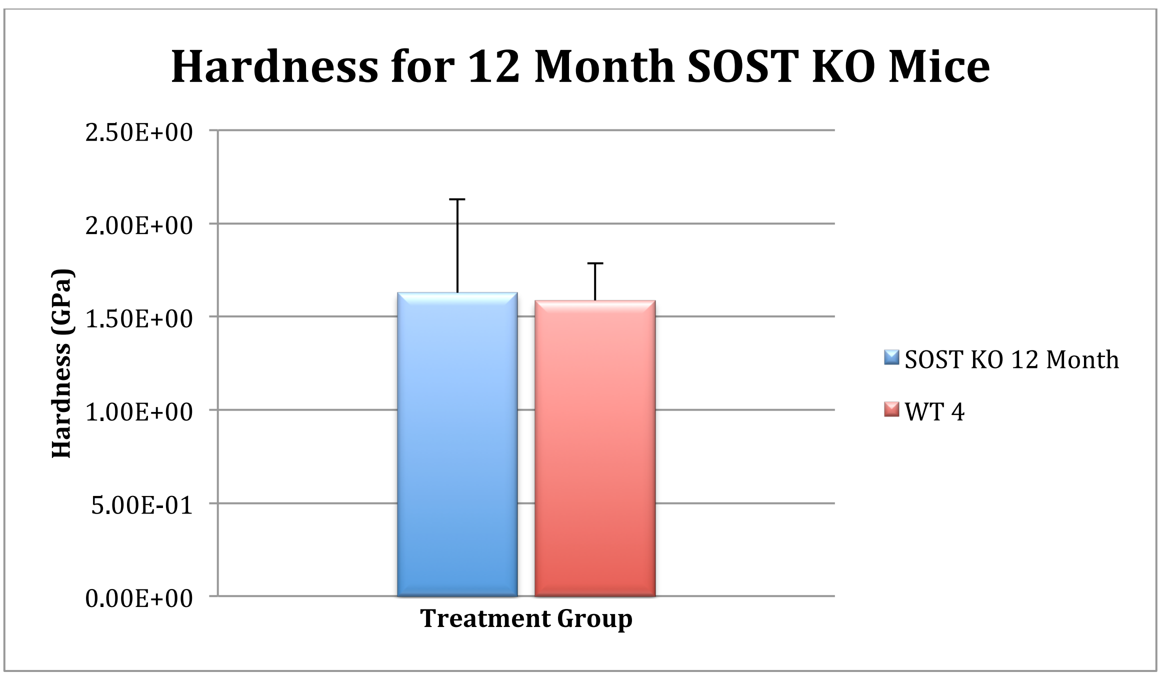


Figure 3.39: Histogram comparison of hardness for 12 month old SOST knockout mice. WT 4 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

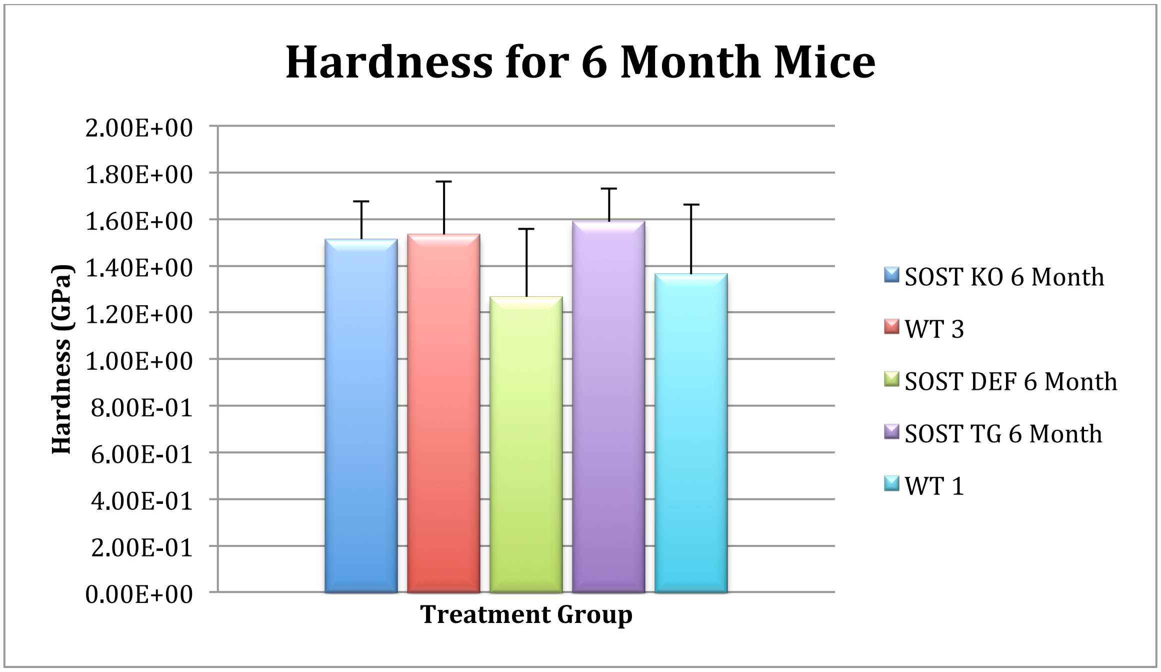


Figure 3.40: Histogram comparison of hardness for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

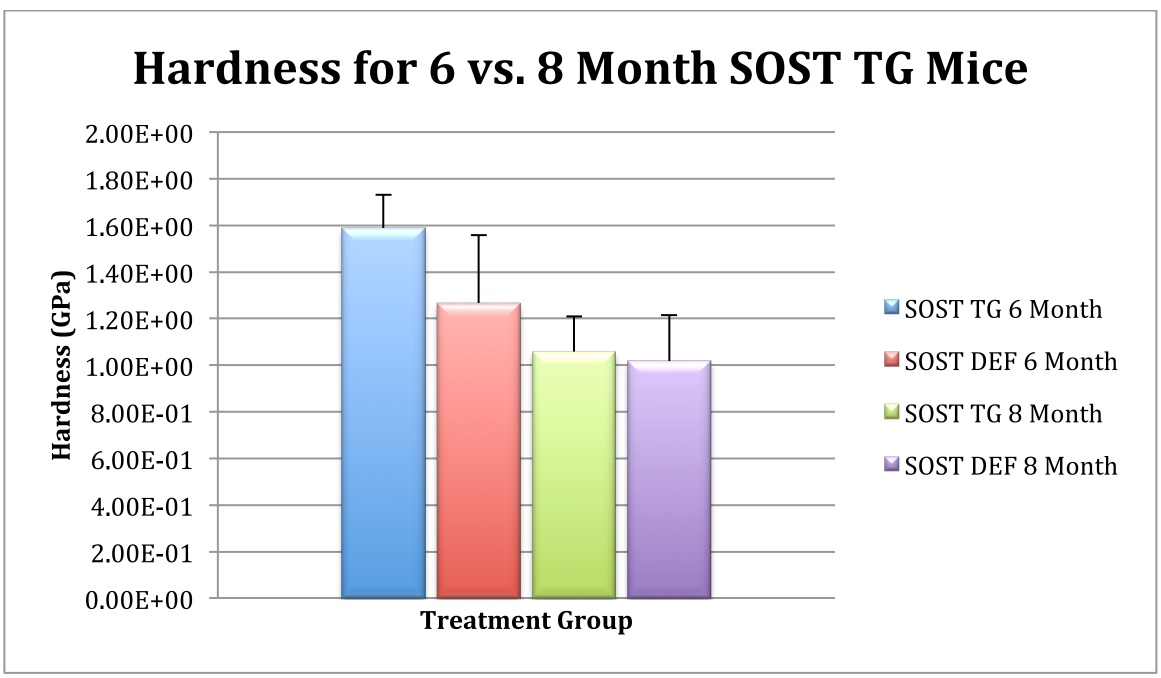


Figure 3.41: Histogram comparison of hardness for 6 and 8 month old transgenic mice. Wild type controls were not included in this analysis.

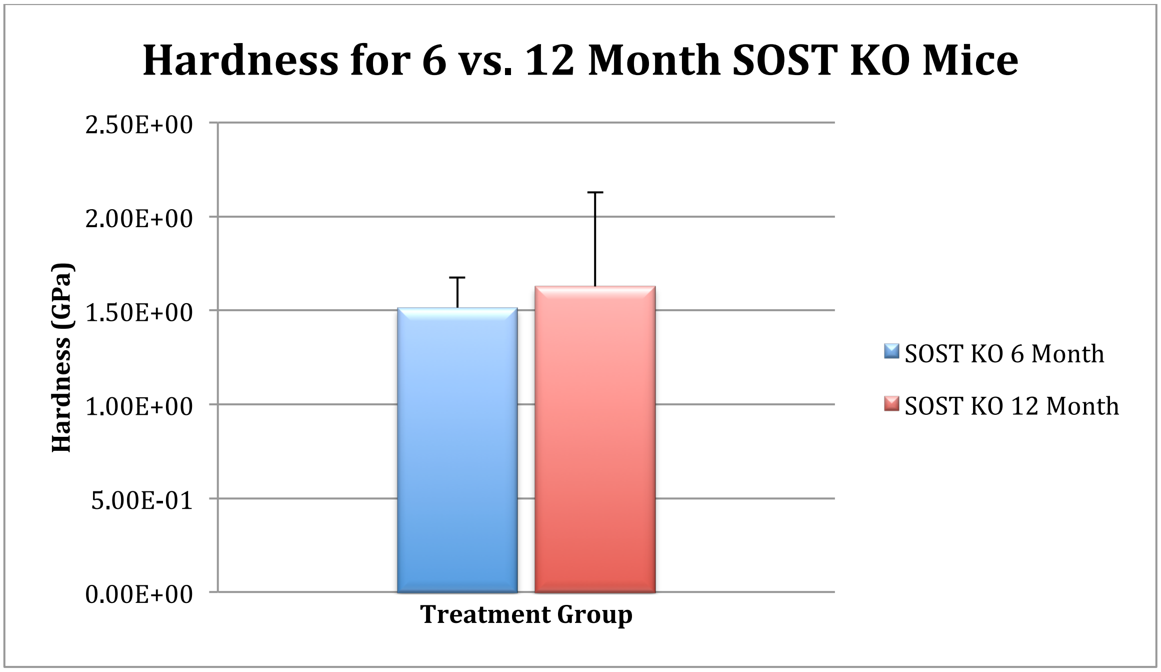


Figure 3.42: Histogram comparison of hardness for 6 and 12 month old knockout mice. Wild type controls were not included in this analysis.

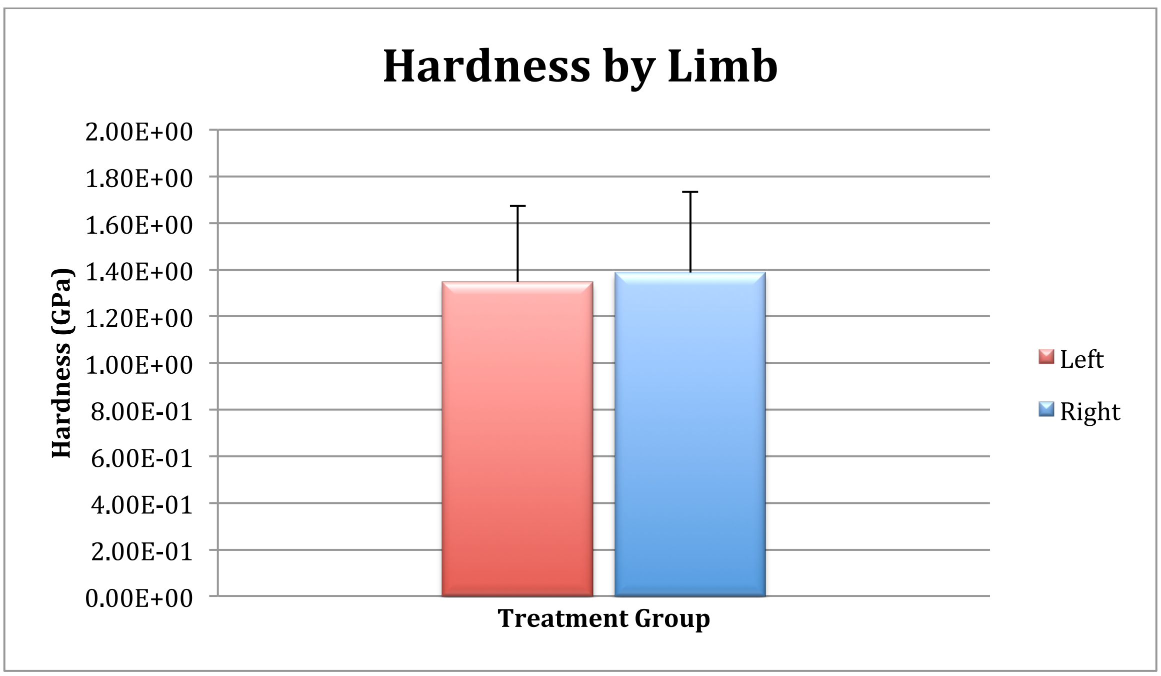


Figure 3.43: Histogram comparison of hardness by either left or right limb for all mice. No animals were excluded from this analysis.

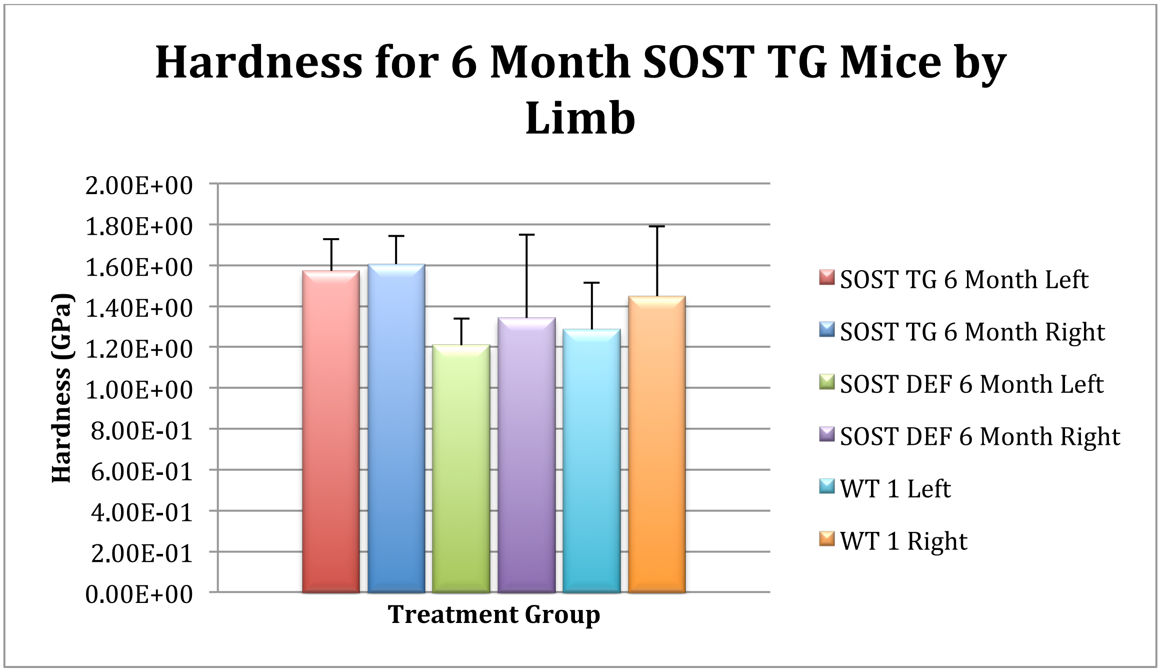


Figure 3.44: Histogram comparison of hardness by limb for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

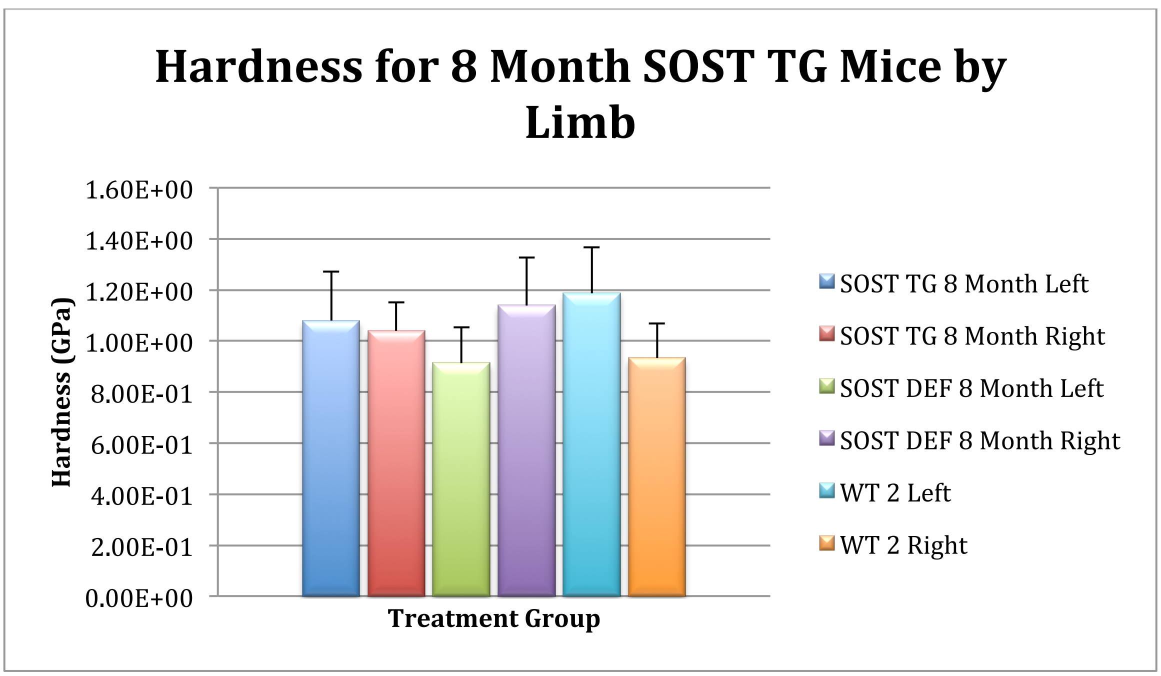


Figure 3.45: Histogram comparison of hardness by limb for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

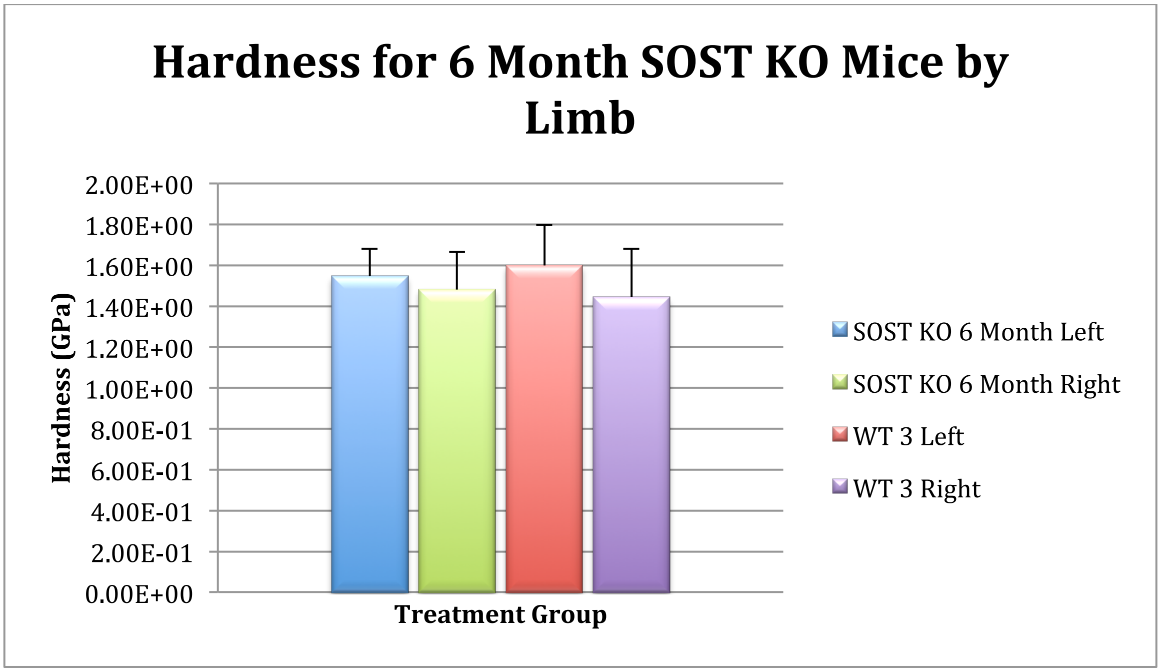


Figure 3.46: Histogram comparison of hardness by limb for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

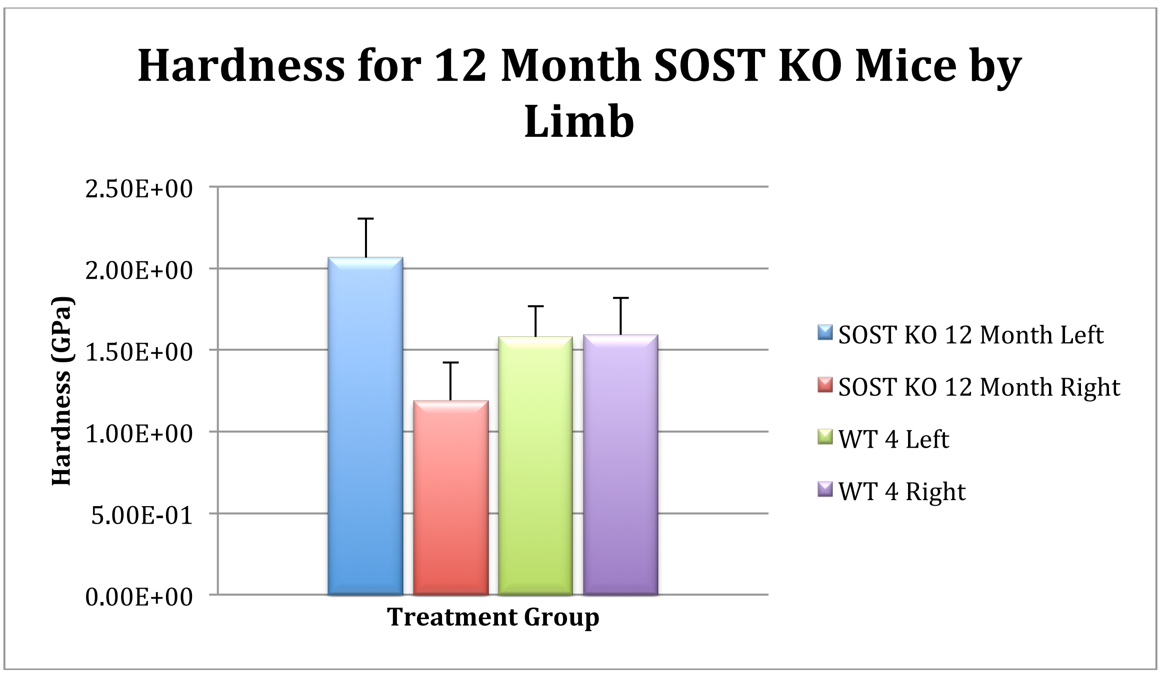


Figure 3.47: Histogram comparison of hardness by limb for 12 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

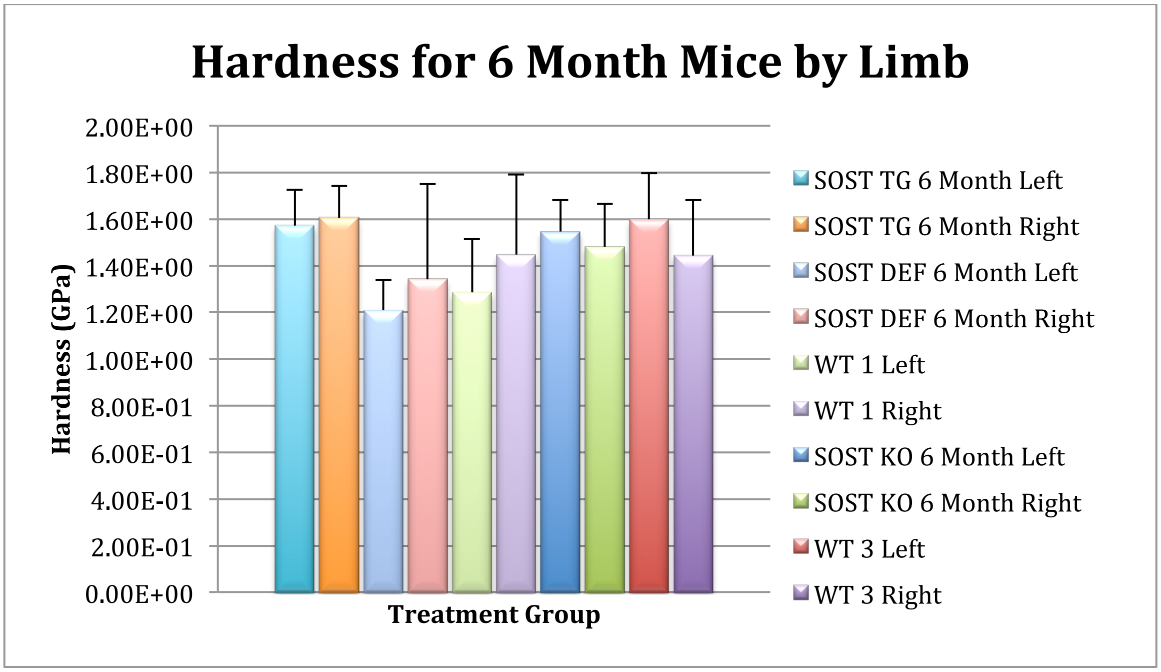


Figure 3.48: Histogram comparison of hardness by limb for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

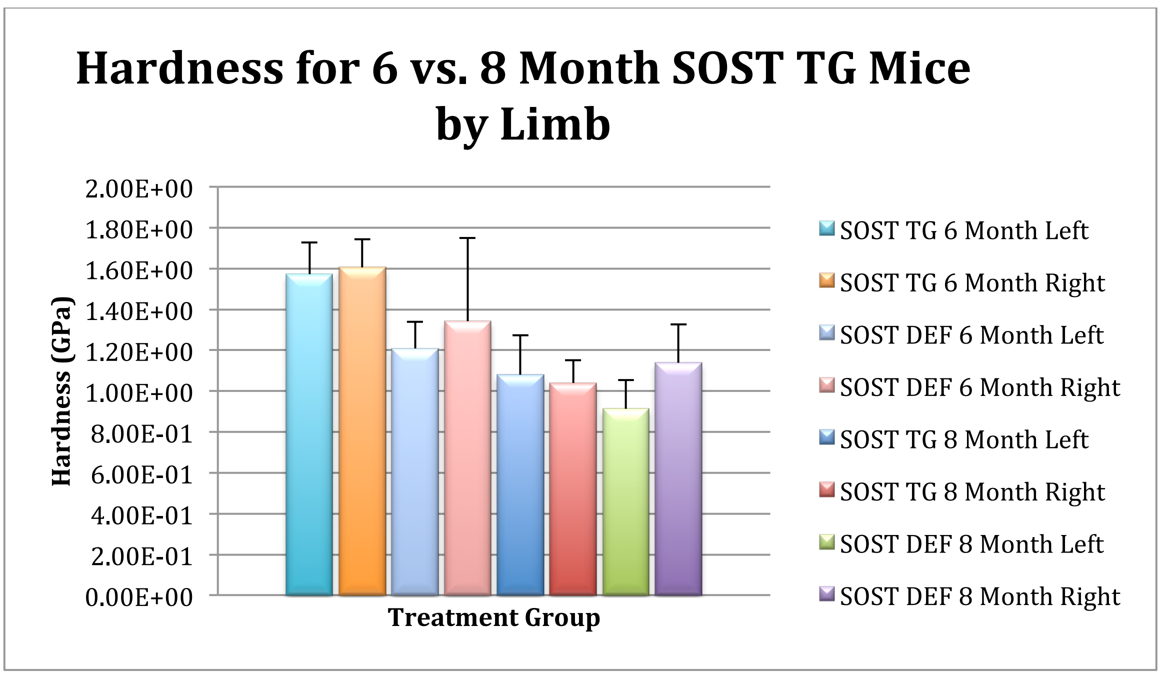


Figure 3.49: Histogram comparison of hardness by limb for 6 and 8 month old transgenic mice. Wild type controls were not included in this analysis.

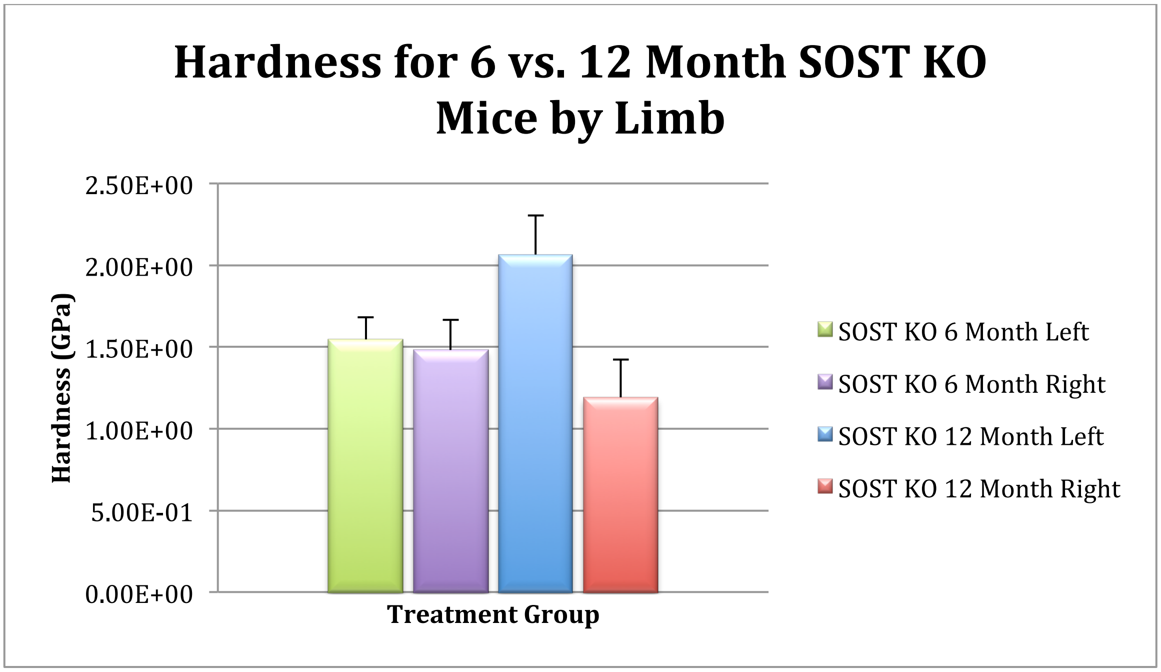


Figure 3.50: Histogram comparison of hardness by limb for 6 and 12 month old knockout mice. Wild type controls were not included in this analysis.

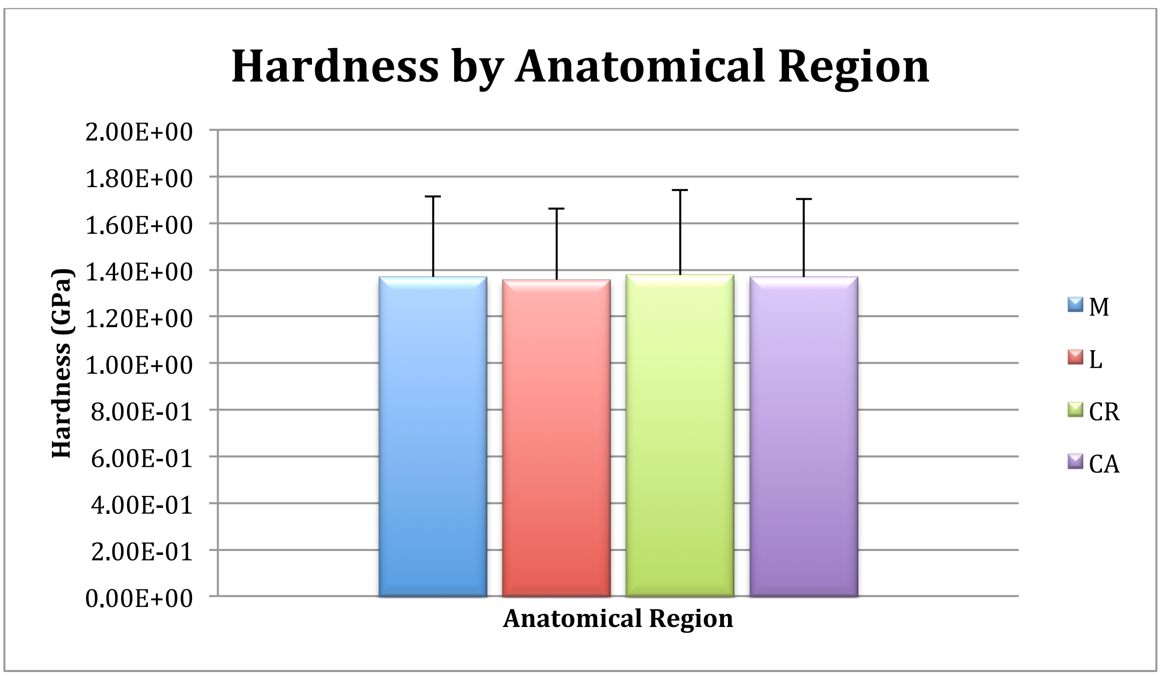


Figure 3.51: Histogram comparison of hardness by anatomical region. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

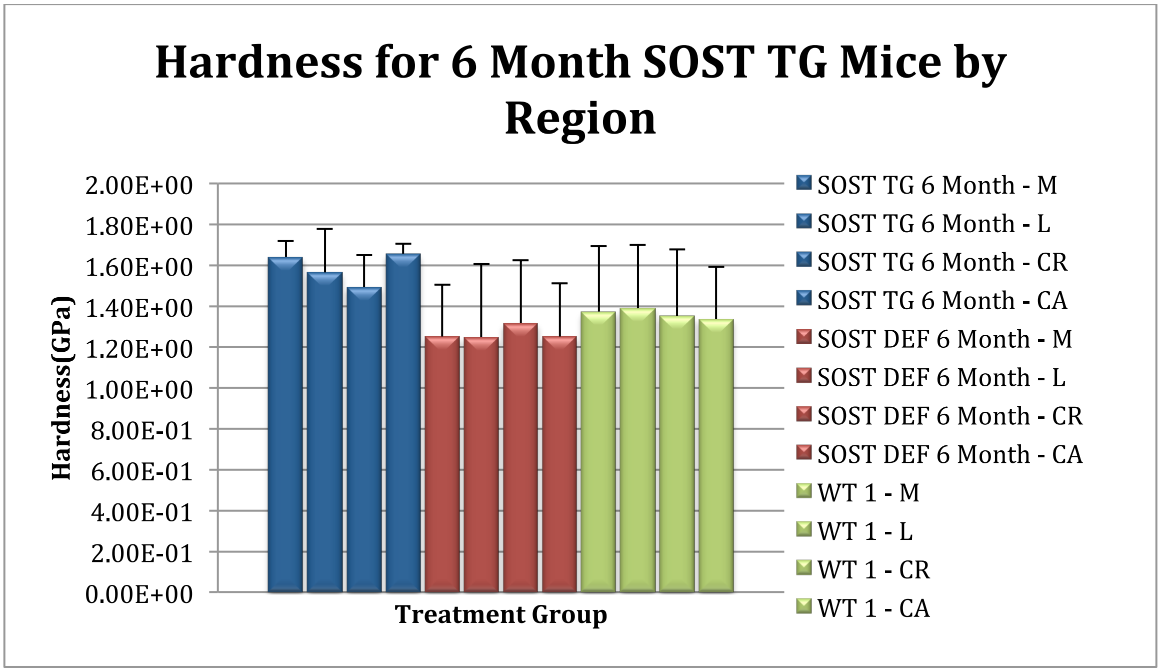


Figure 3.52: Histogram comparison of hardness by anatomical region for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

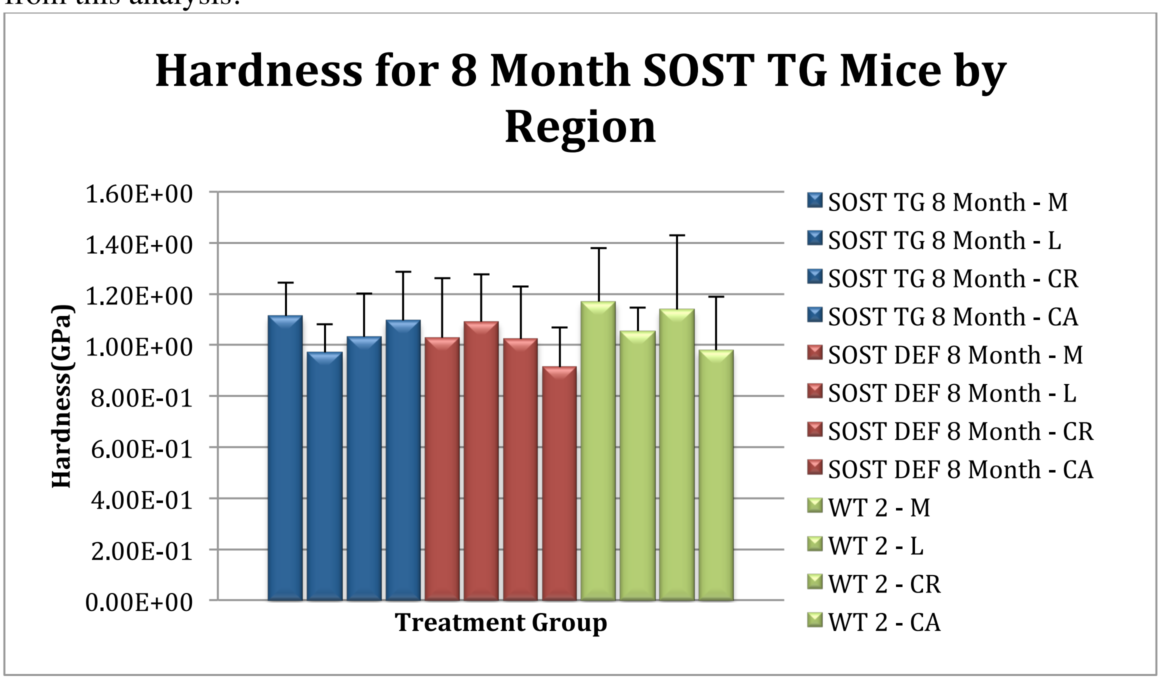


Figure 3.53: Histogram comparison of hardness by anatomical region for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

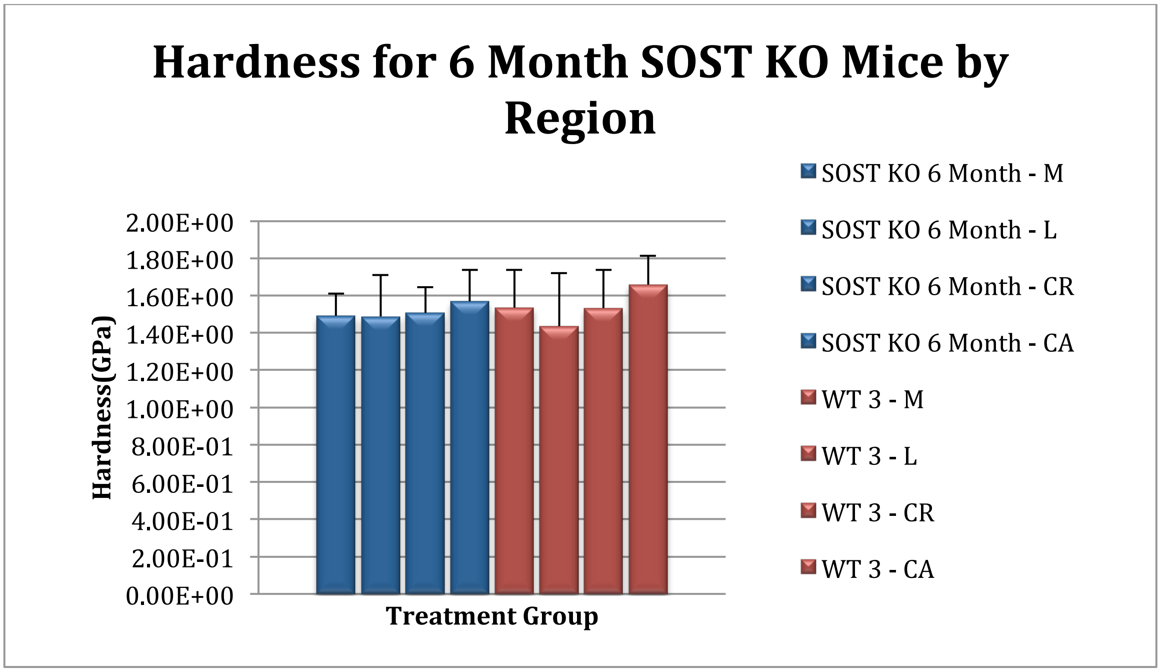


Figure 3.54: Histogram comparison of hardness by anatomical region for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

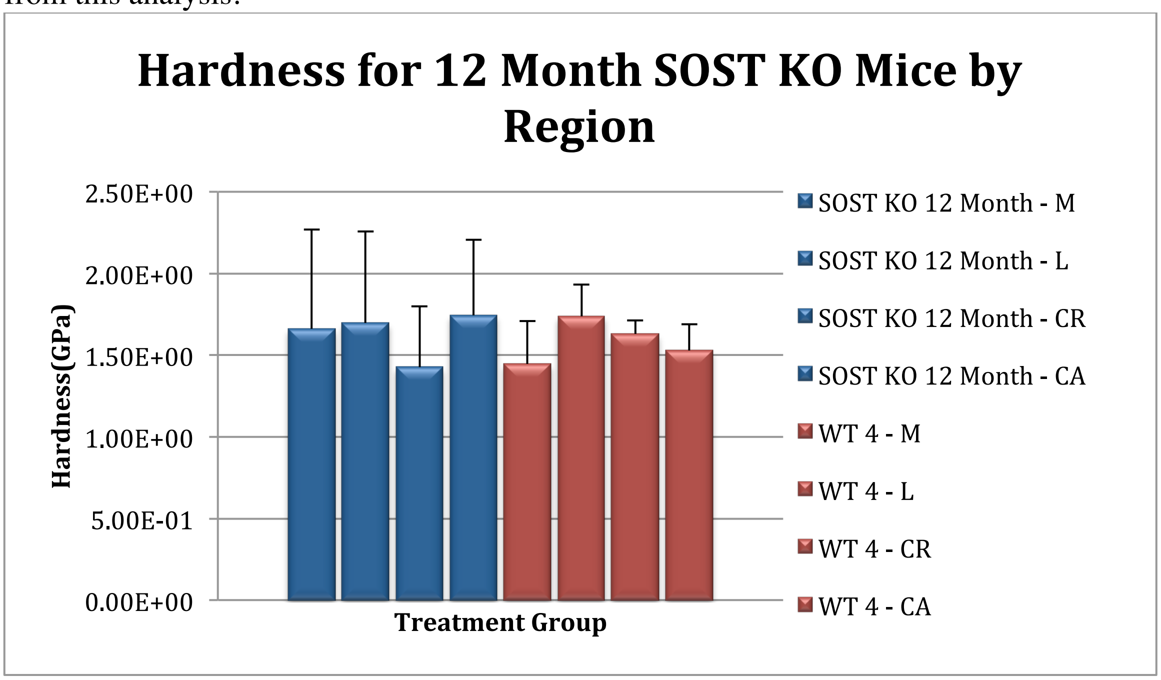


Figure 3.55: Histogram comparison of hardness by anatomical region for 12 month old SOST knockout mice. WT 4 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

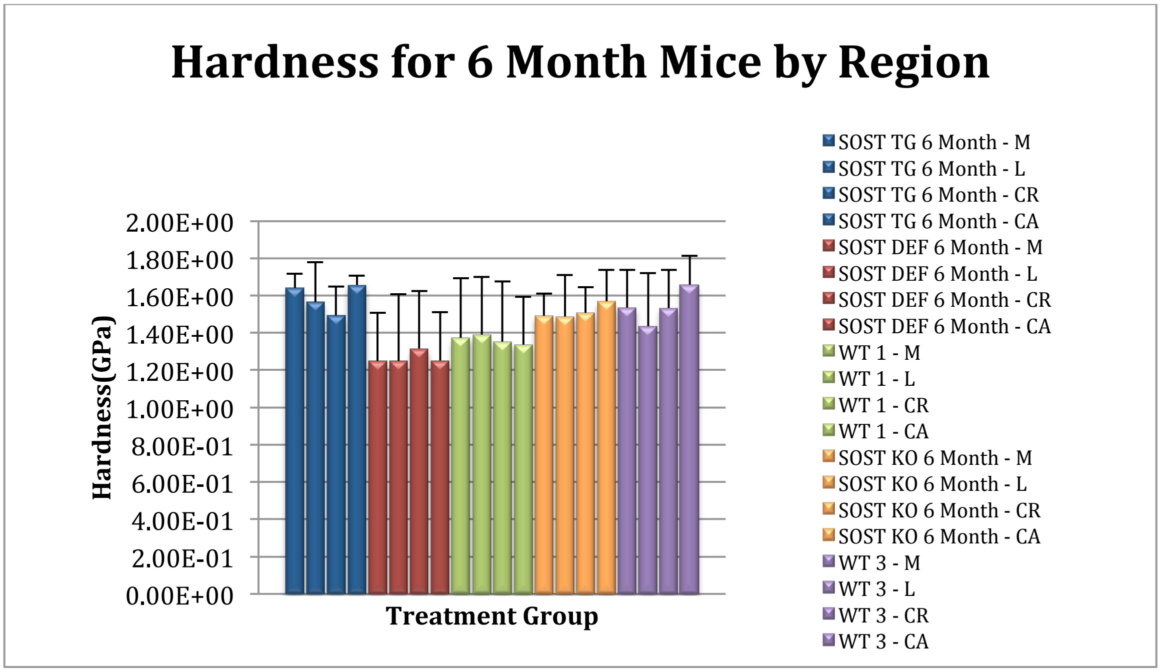


Figure 3.56: Histogram comparison of hardness by anatomical region for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

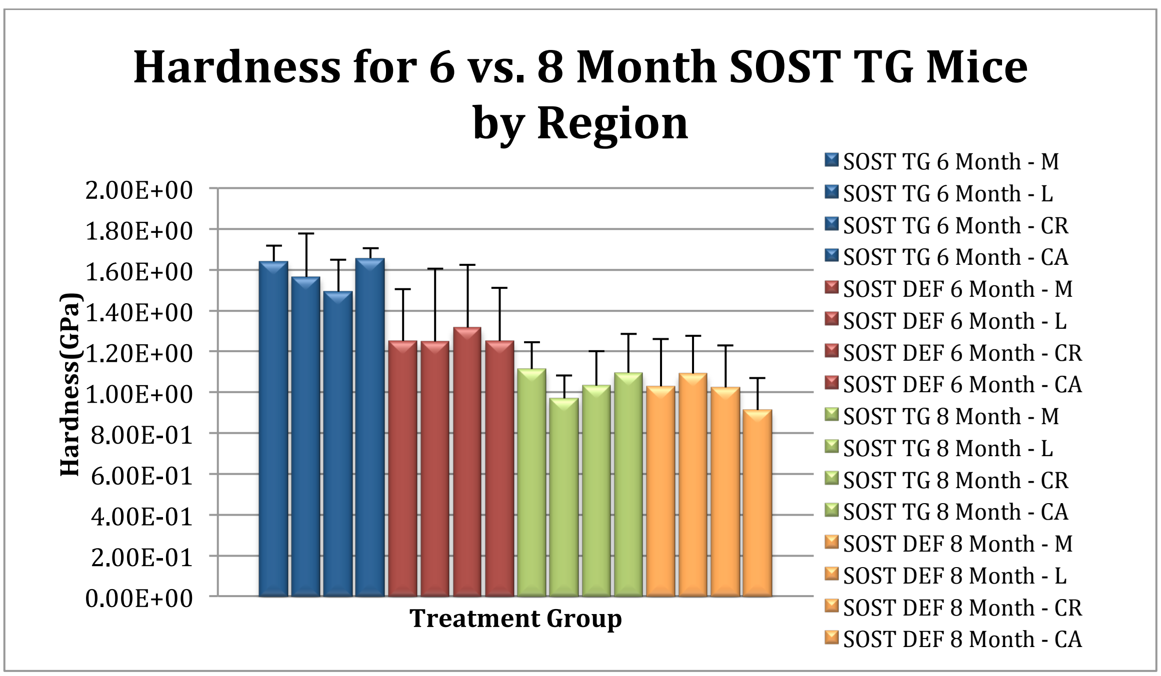


Figure 3.57: Histogram comparison of hardness by anatomical region for 6 and 8 month old transgenic mice. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Wild type controls were not included in this analysis.

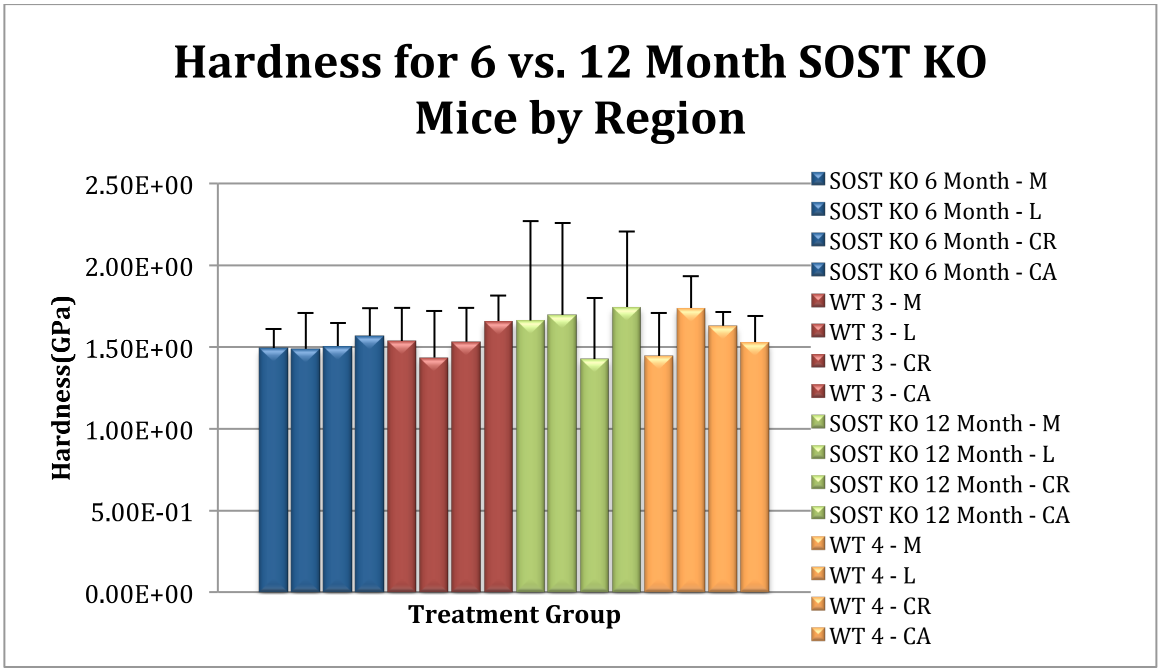


Figure 3.58: Histogram comparison of hardness by anatomical region for 6 and 12 month old knockout mice. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Wild type controls were not included in this analysis.

3.3 Elastic Modulus

The three-way interaction GLM was also run for elastic modulus against treatment group, limb and anatomical region. Initial GLM returned 2 data points surpassing ±2.75 standard residuals which were excluded from subsequent analysis (8603/Right/Medial, 8640/Right/Medial). One value was considered to have large leverage over the data, but was included in analysis due to the small sample size of its treatment group. Figure 3.59 shows the associated p-values for the general linear model (GLM) analysis for this data set. Figure 3.60 displays the different treatment groups and their associated means and significantly different groupings, as determined by a Tukey's comparison with a 95% confidence interval.

Analysis of Variance for E_mod_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	6227.51	5968.48	663.16	46.05	0.000
Limb	1	669.71	219.45	219.45	15.24	0.000
Region	3	46.41	37.69	12.56	0.87	0.456
Genotype+Phenotype*Limb	9	5080.54	4934.56	548.28	38.08	0.000
Genotype+Phenotype*Region	27	403.92	407.07	15.08	1.05	0.406
Limb*Region	3	25.24	38.60	12.87	0.89	0.445
Genotype+Phenotype*Limb*Region	27	442.88	442.88	16.40	1.14	0.295
Error	263	3787.15	3787.15	14.40		
Total	342	16683.35				

Figure 3.59: GLM of elastic modulus across treatment groups and associated p-values. Data shown reflects statistical analysis and excludes 2 outlier values.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	N	Mean	Grouping
SOST KO 12 Month KO	34	43.6	A
SOST TG 6 Month WT	75	34.7	B
SOST TG 6 Month TG	16	33.8	B C
SOST TG 6 Month DEF	60	32.2	C
SOST KO 6 Month KO	40	31.9	C D
SOST KO 12 Month WT	16	31.6	B C D E
SOST KO 6 Month WT	36	31.4	C D E
SOST TG 8 Month WT	23	28.7	D E F
SOST TG 8 Month TG	15	27.5	E F
SOST TG 8 Month DEF	28	27.2	F

Means that do not share a letter are significantly different.

Figure 3.60: GLM output of treatment group means and significantly different value groupings for elastic modulus. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
R	160	33.2	A
L	183	31.3	B

Means that do not share a letter are significantly different.

Figure 3.61: GLM output of limb means and significantly different value groupings for elastic modulus. Data shown reflects statistical analysis and excludes 2 outlier values.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
L	87	32.7	A
M	85	32.6	A
CA	86	32.0	A
CR	85	31.8	A

Means that do not share a letter are significantly different.

Figure 3.62: GLM output of anatomical region means and significantly different value groupings for elastic modulus. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Data shown reflects statistical analysis and excludes 2 outlier values.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	49.7	A
SOST TG 6 Month WT	R	35	38.1	B
SOST TG 6 Month DEF	R	26	37.6	B C
SOST KO 12 Month KO	R	17	37.5	B C D
SOST TG 6 Month TG	R	8	33.9	B C D E
SOST TG 8 Month DEF	R	13	33.8	B C D E
SOST TG 6 Month TG	L	8	33.7	B C D E
SOST TG 8 Month TG	R	8	32.5	C D E
SOST KO 6 Month WT	L	21	32.5	E
SOST KO 6 Month KO	L	20	32.2	E
SOST TG 8 Month WT	L	13	32.1	E
SOST KO 12 Month WT	L	8	31.9	D E F
SOST KO 6 Month KO	R	20	31.5	E F
SOST TG 6 Month WT	L	40	31.4	E F
SOST KO 12 Month WT	R	8	31.3	E F G
SOST KO 6 Month WT	R	15	30.4	E F G
SOST TG 6 Month DEF	L	34	26.8	F G H
SOST TG 8 Month WT	R	10	25.3	G H I
SOST TG 8 Month TG	L	7	22.5	H I
SOST TG 8 Month DEF	L	15	20.6	I

Means that do not share a letter are significantly different.

Figure 3.63: GLM output of the interaction between treatment group and limb and their associated means and significantly different value groupings for elastic modulus. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	9	45.4	A
SOST KO 12 Month KO	CA	8	44.9	A
SOST KO 12 Month KO	M	8	44.5	A
SOST KO 12 Month KO	CR	9	39.6	A B
SOST TG 6 Month WT	M	19	36.1	B C
SOST TG 6 Month TG	M	4	35.2	B C D
SOST TG 6 Month TG	L	4	35.1	B C D
SOST TG 6 Month WT	L	20	34.7	B C D
SOST TG 6 Month WT	CR	17	34.4	B C D

SOST KO 12 Month WT	L	4	34.2	B C D E
SOST TG 6 Month WT	CA	19	33.7	B C D E
SOST TG 6 Month TG	CA	4	33.5	B C D E
SOST KO 6 Month WT	CA	8	32.7	B C D E
SOST KO 6 Month KO	CA	10	32.7	C D E
SOST TG 6 Month DEF	M	15	32.6	C D E
SOST TG 6 Month DEF	CA	16	32.2	C D E
SOST TG 6 Month DEF	CR	15	32.1	C D E
SOST TG 6 Month DEF	L	14	32.0	C D E
SOST KO 6 Month KO	M	10	31.9	C D E
SOST KO 6 Month KO	CR	10	31.8	C D E
SOST KO 6 Month WT	M	8	31.7	C D E
SOST TG 6 Month TG	CR	4	31.5	B C D E
SOST KO 6 Month WT	CR	10	31.4	C D E
SOST KO 6 Month KO	L	10	31.1	C D E
SOST KO 12 Month WT	CR	4	31.1	B C D E
SOST KO 12 Month WT	CA	4	30.8	B C D E
SOST TG 8 Month WT	CR	4	30.5	C D E
SOST KO 12 Month WT	M	4	30.1	C D E
SOST KO 6 Month WT	L	10	30.0	D E
SOST TG 8 Month WT	M	6	29.3	C D E
SOST TG 8 Month DEF	L	7	28.6	D E
SOST TG 8 Month WT	L	6	28.4	D E
SOST TG 8 Month DEF	CR	8	28.0	D E
SOST TG 8 Month TG	M	4	27.7	D E
SOST TG 8 Month TG	CA	4	27.7	D E
SOST TG 8 Month TG	CR	4	27.3	D E
SOST TG 8 Month TG	L	3	27.3	C D E
SOST TG 8 Month DEF	M	7	27.0	D E
SOST TG 8 Month WT	CA	7	26.7	D E
SOST TG 8 Month DEF	CA	6	25.1	E

Means that do not share a letter are significantly different.

Figure 3.64: GLM output of the interaction between treatment group and anatomical region and their associated means and significantly different value groupings for elastic modulus. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Data shown reflects statistical analysis and excludes 2 outlier values. Each wild type was a control littermate for their respective treatment groups. These wild type mice faithfully express murine SOST.

Elastic modulus was found to be significantly affected by treatment group (p=0.000) and by limb tested (p=0.000), as well as significant findings were found between the interaction of treatment group and limb tested (p=0.000). Observations show that the 6 month SOST TG mice to have significantly greater modulus values than the SOST DEF and their control littermate (WT 1) mice (Figure 3.65). The same differences were also observed with the 12 month SOST KO mice having greater modulus values than their control littermate (WT 4) mice (Figure 3.68). No observed differences were seen between

the 8 month SOST TG, SOST DEF and their control littermate (WT 2) mice. Side by side limb differences were observed in the limbs of the 12 month SOST KO mice with the left being greater than the right (Figure 3.76). There were no significant findings amongst the different anatomical regions or its interaction with treatment group or limb.

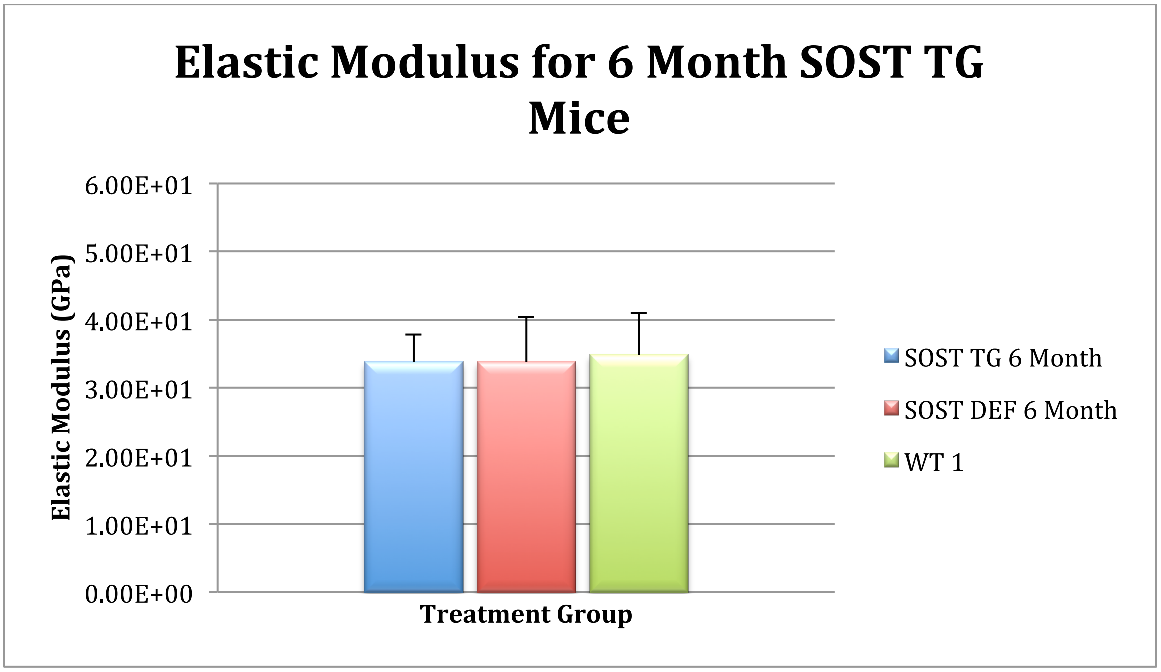


Figure 3.65: Histogram comparison of elastic modulus for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

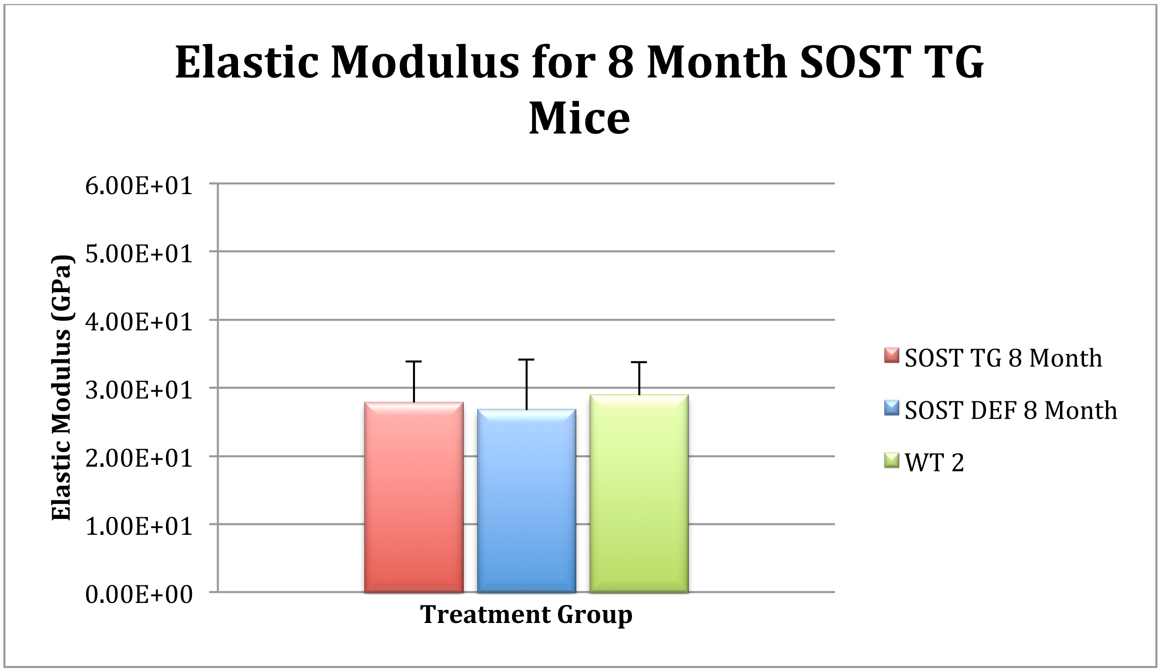


Figure 3.66: Histogram comparison of elastic modulus for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

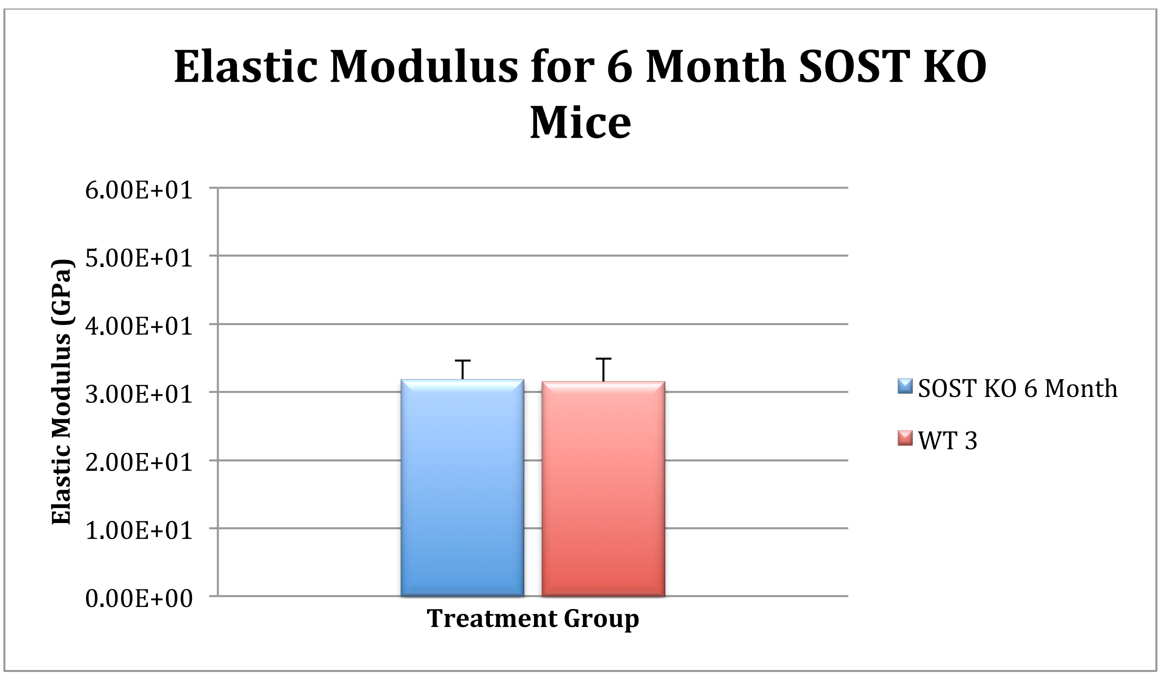


Figure 3.67: Histogram comparison of elastic modulus for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

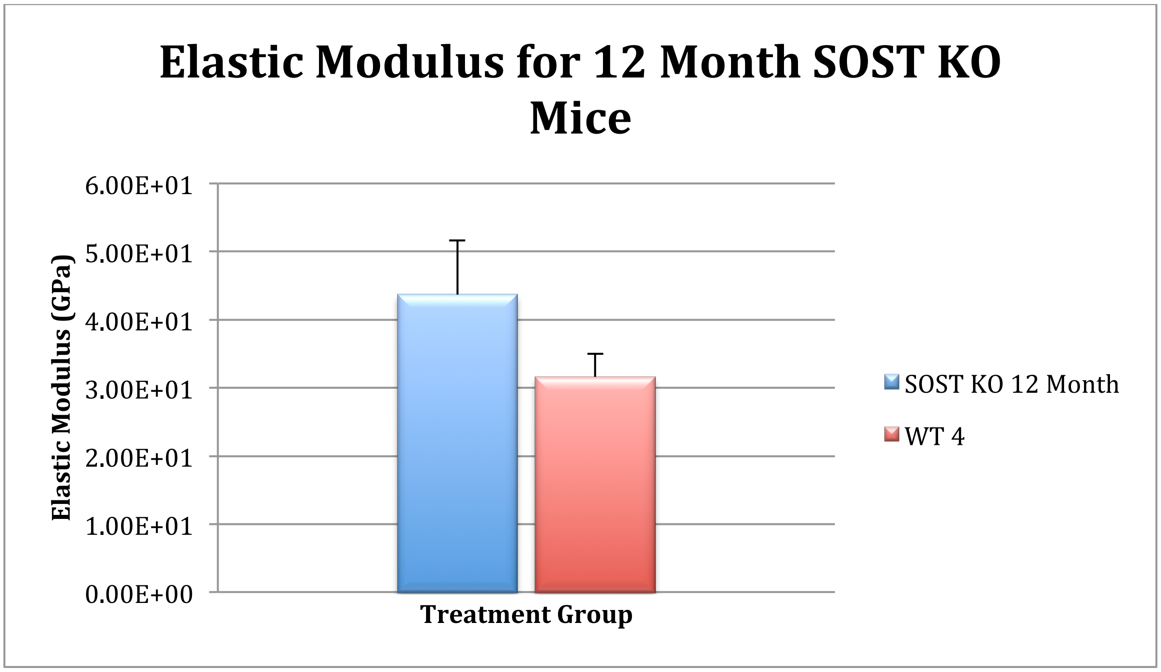


Figure 3.68: Histogram comparison of elastic modulus for 12 month old SOST knockout mice. WT 4 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

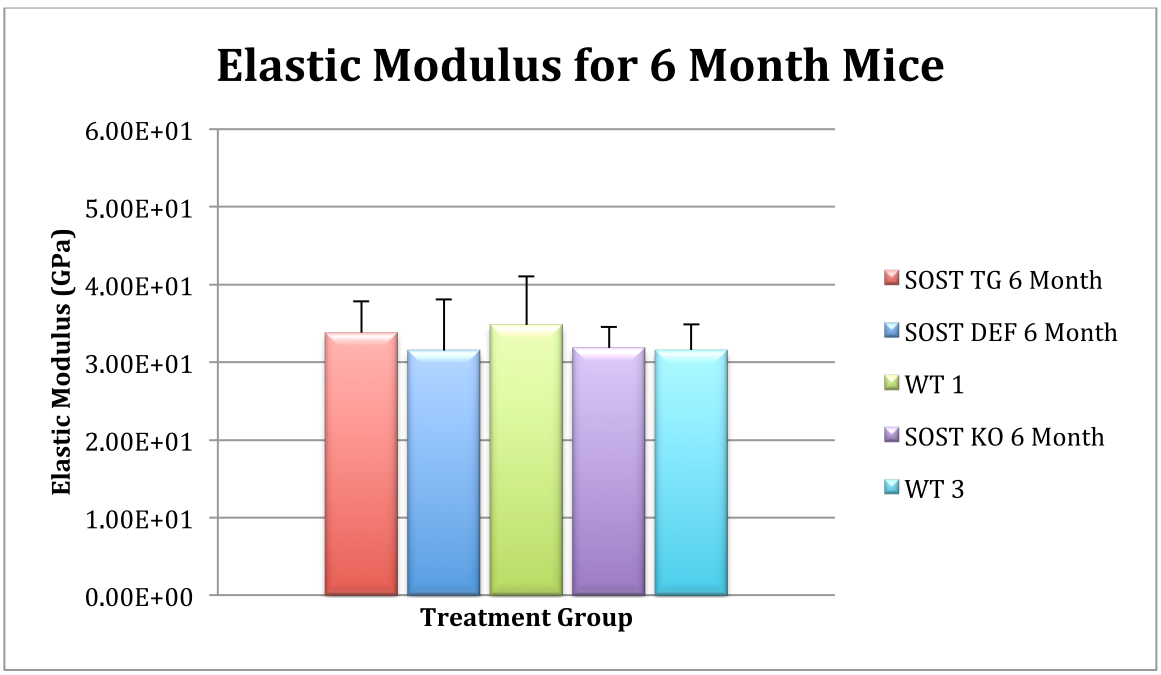


Figure 3.69: Histogram comparison of elastic modulus for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

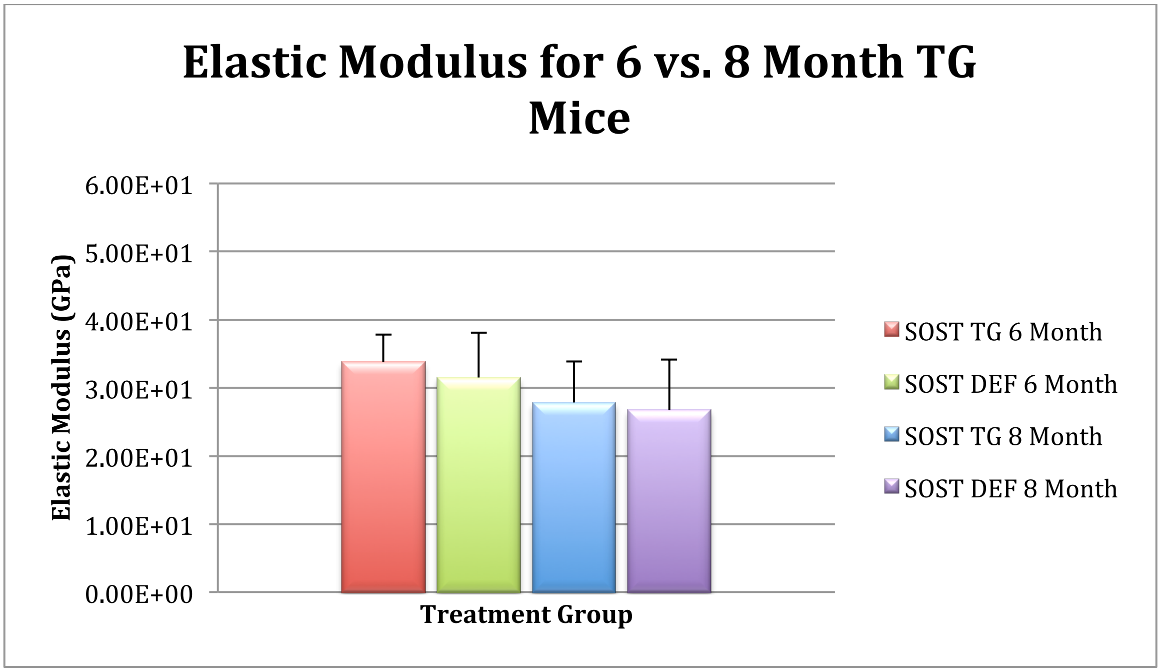


Figure 3.70: Histogram comparison of elastic modulus for 6 and 8 month old transgenic mice. Wild type controls were not included in this analysis.

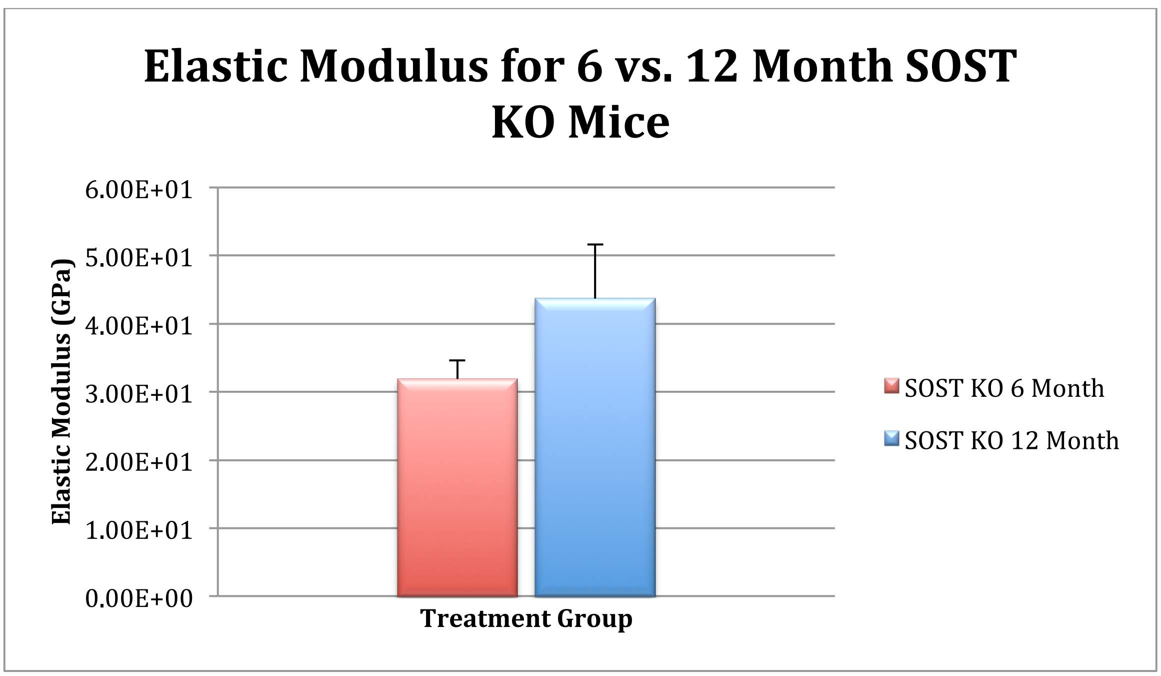


Figure 3.71: Histogram comparison of elastic modulus for 6 and 12 month old knockout mice. Wild type controls were not included in this analysis.

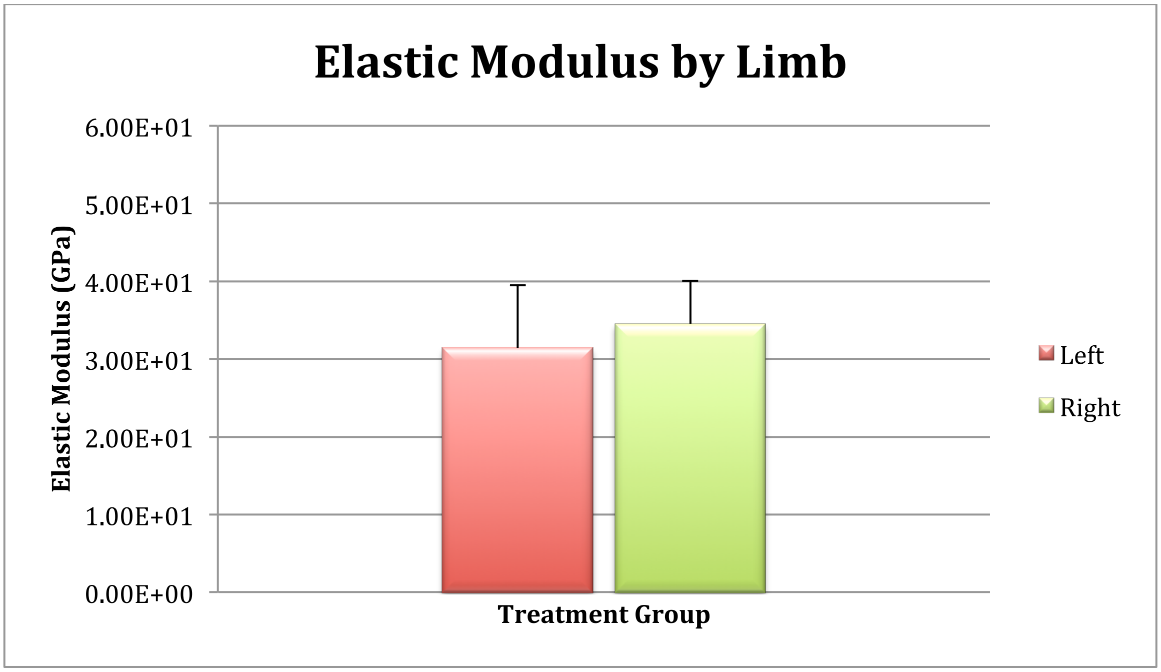


Figure 3.72: Histogram comparison of elastic modulus by either left or right limb for all mice. No animals were excluded from this analysis.

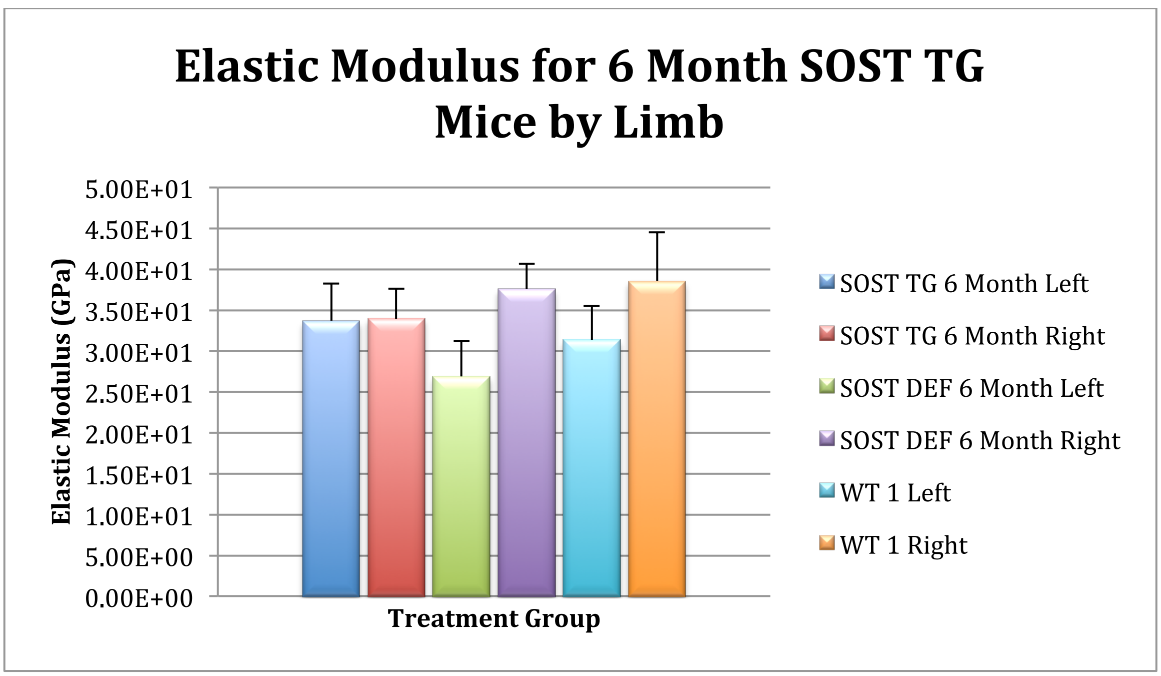


Figure 3.73: Histogram comparison of elastic modulus by limb for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

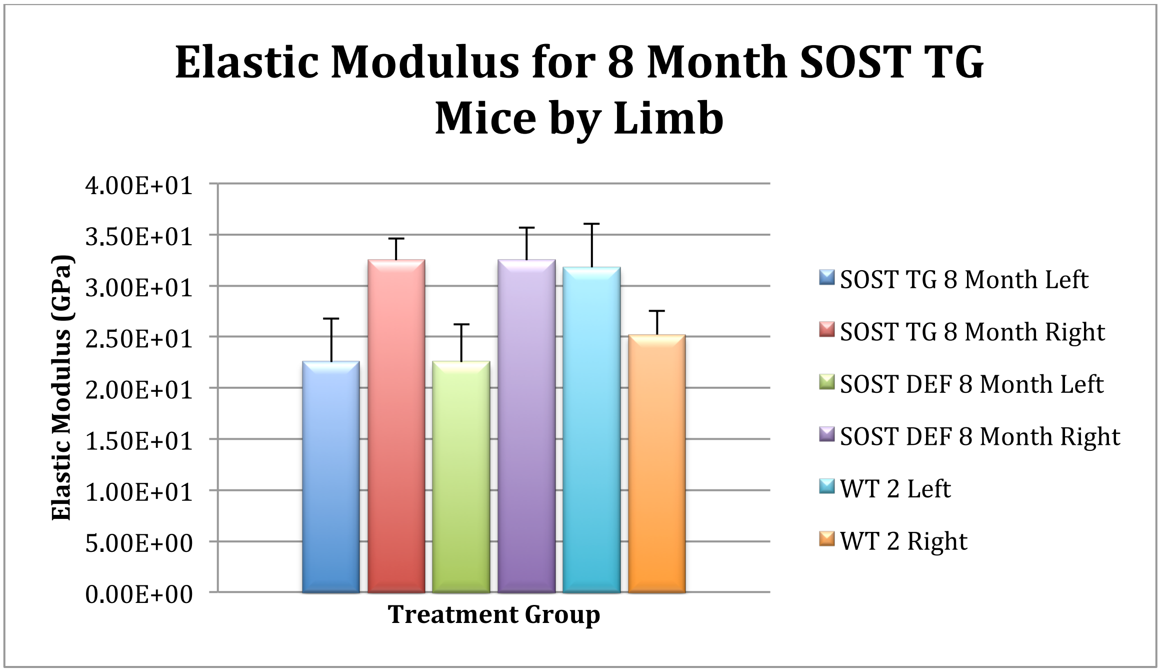


Figure 3.74: Histogram comparison of elastic modulus by limb for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

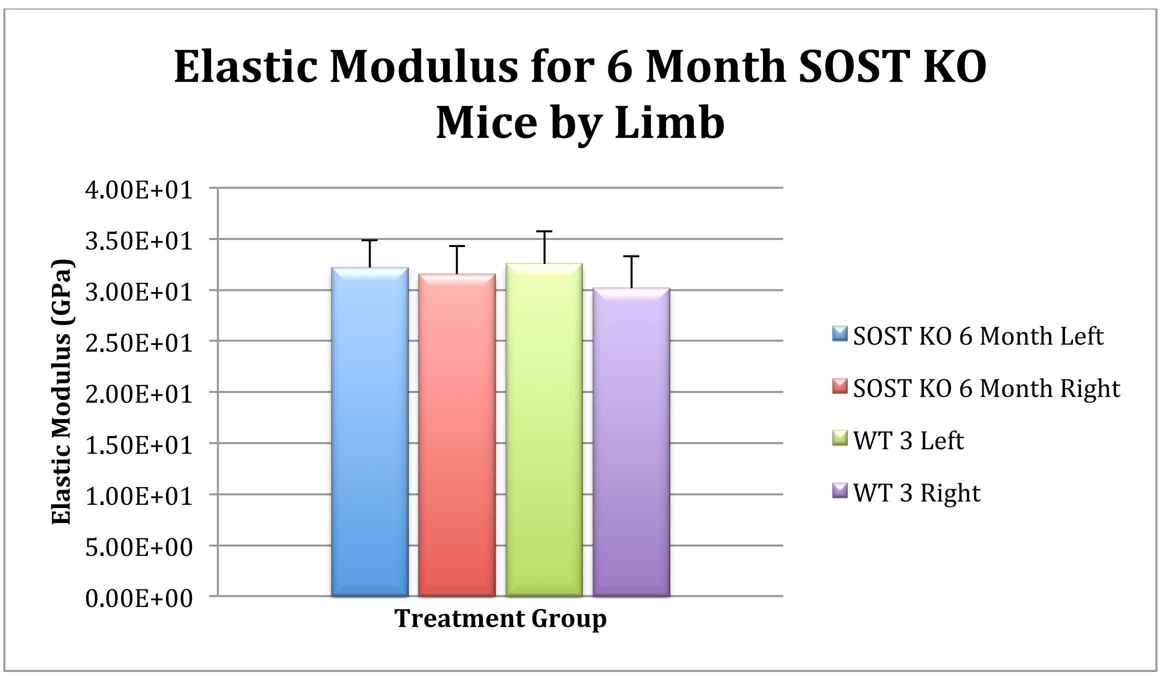


Figure 3.75: Histogram comparison of elastic modulus by limb for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

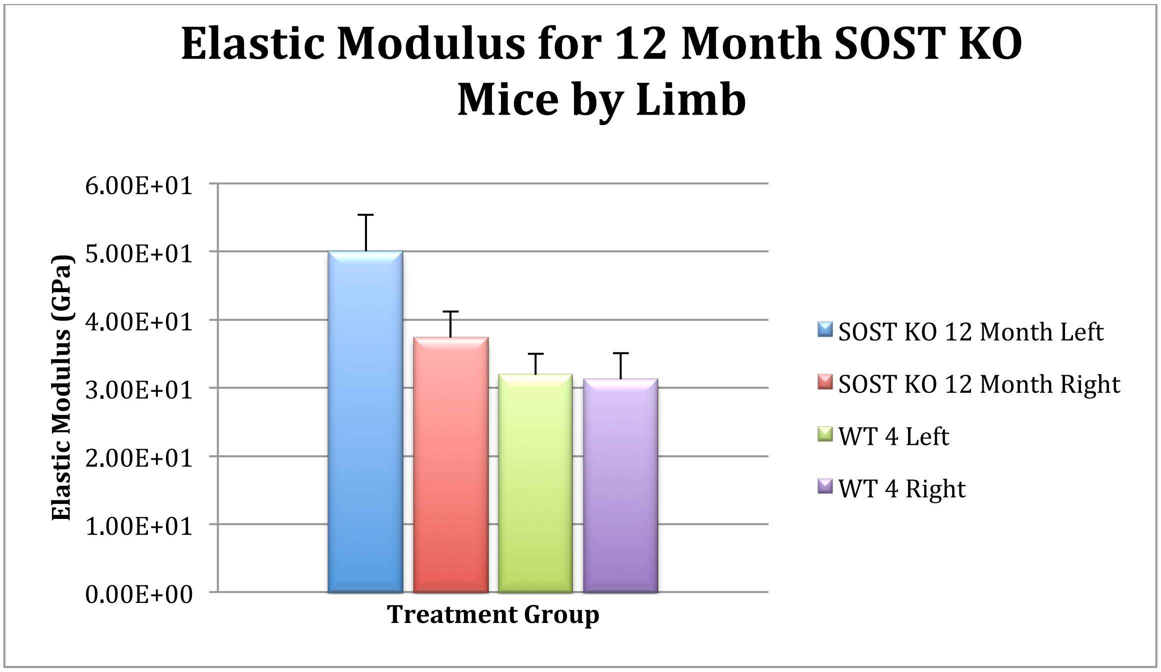


Figure 3.76: Histogram comparison of elastic modulus by limb for 12 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

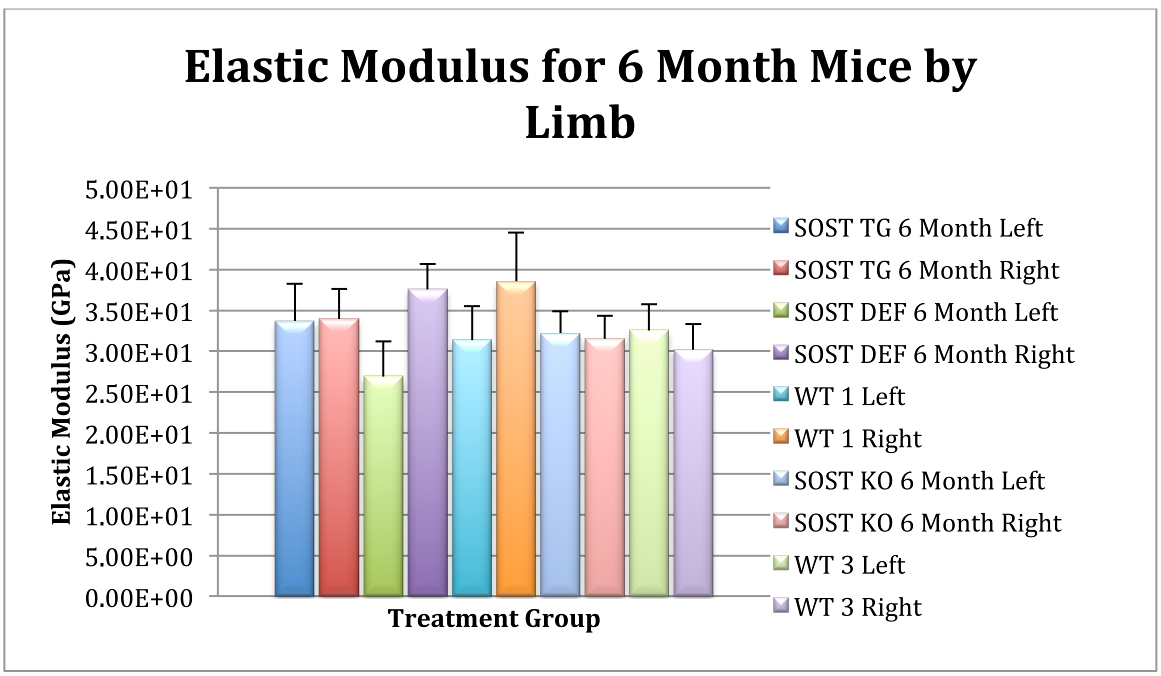


Figure 3.77: Histogram comparison of elastic modulus by limb for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. No animals were excluded from this analysis.

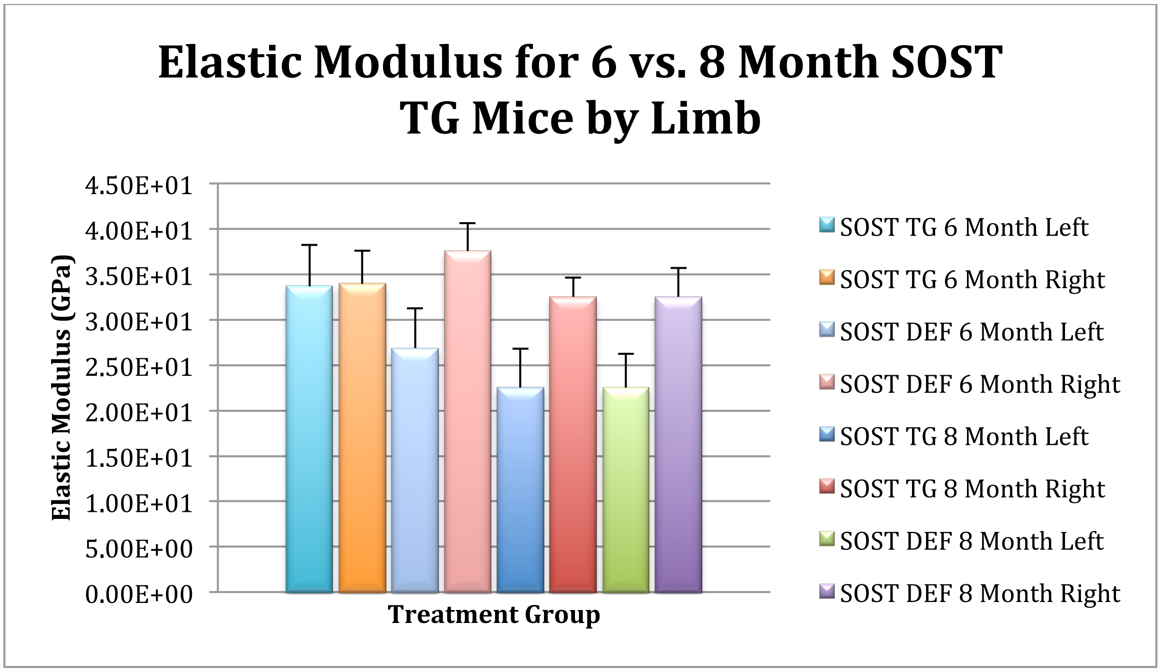


Figure 3.78: Histogram comparison of elastic modulus by limb for 6 and 8 month old transgenic mice. Wild type controls were not included in this analysis.

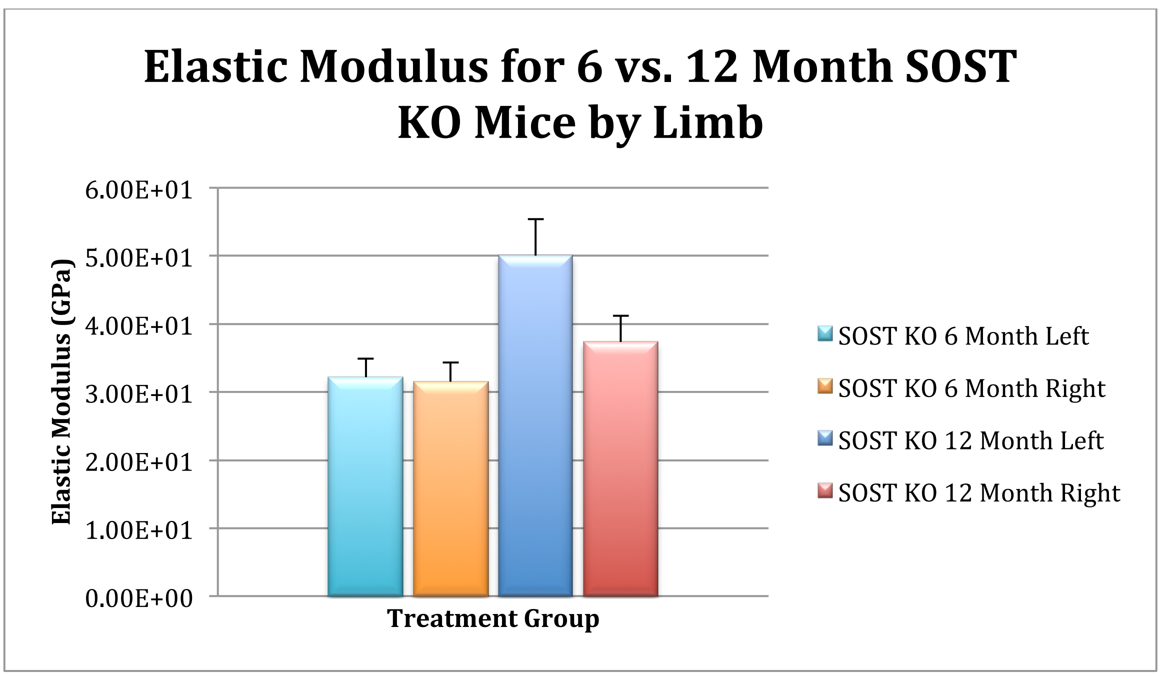


Figure 3.79: Histogram comparison of elastic modulus by limb for 6 and 12 month old knockout mice. Wild type controls were not included in this analysis.

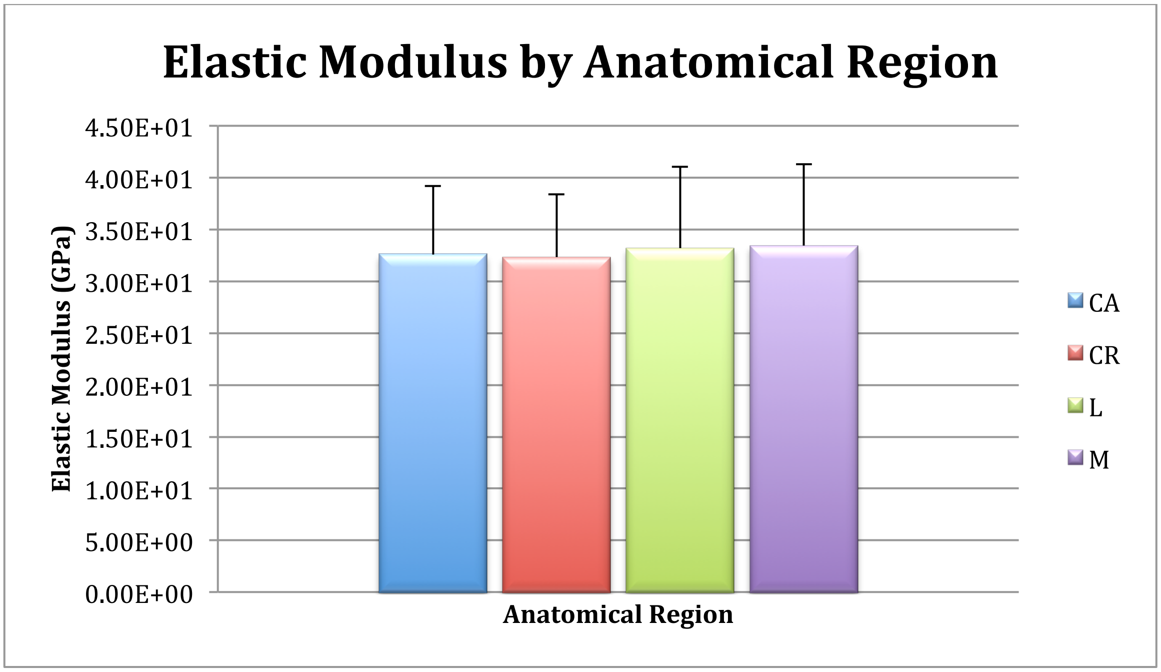


Figure 3.80: Histogram comparison of elastic modulus by anatomical region. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

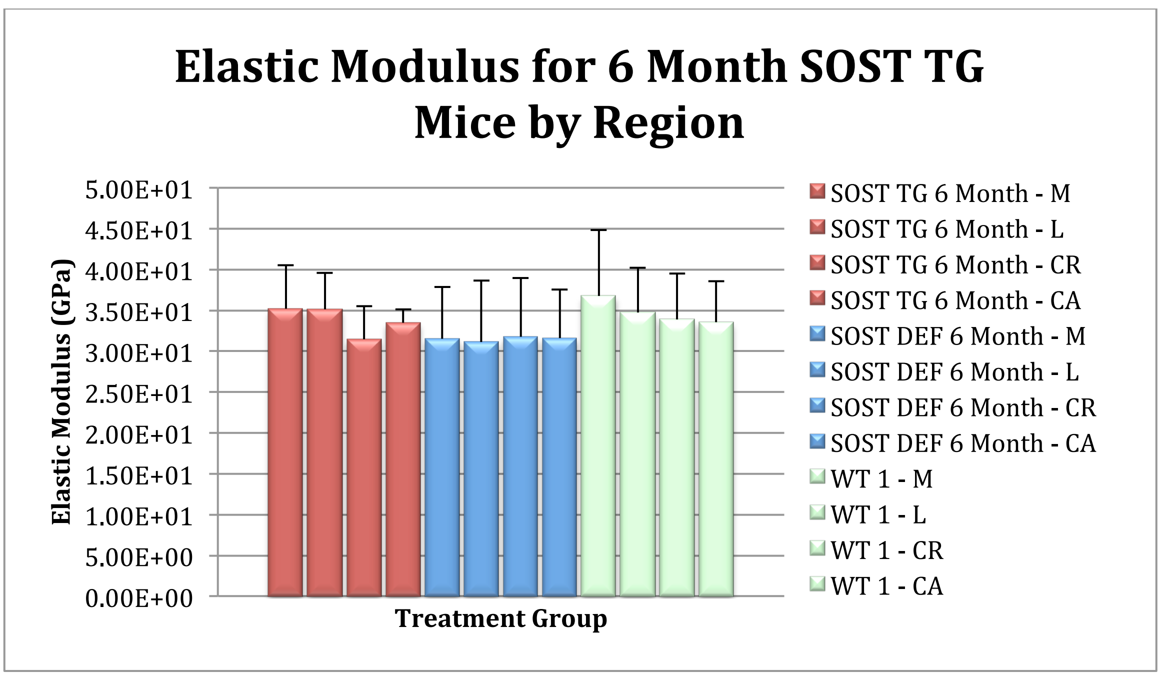


Figure 3.81: Histogram comparison of elastic modulus by anatomical region for 6 month old SOST transgenic mice. WT 1 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

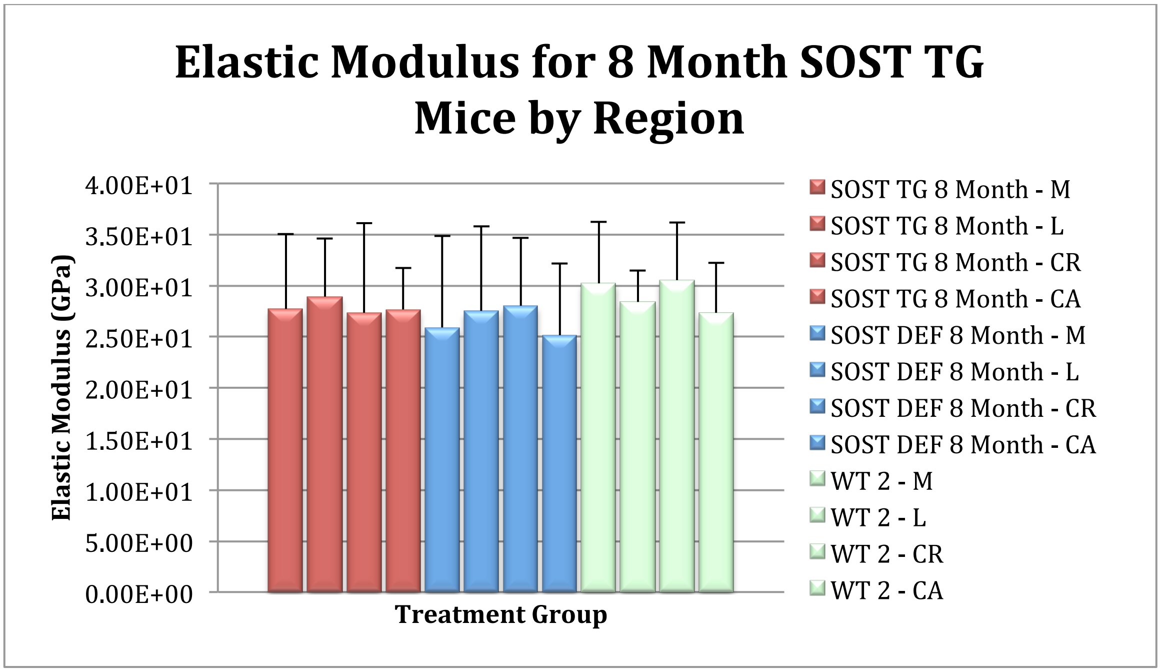


Figure 3.82: Histogram comparison of elastic modulus by anatomical region for 8 month old SOST transgenic mice. WT 2 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

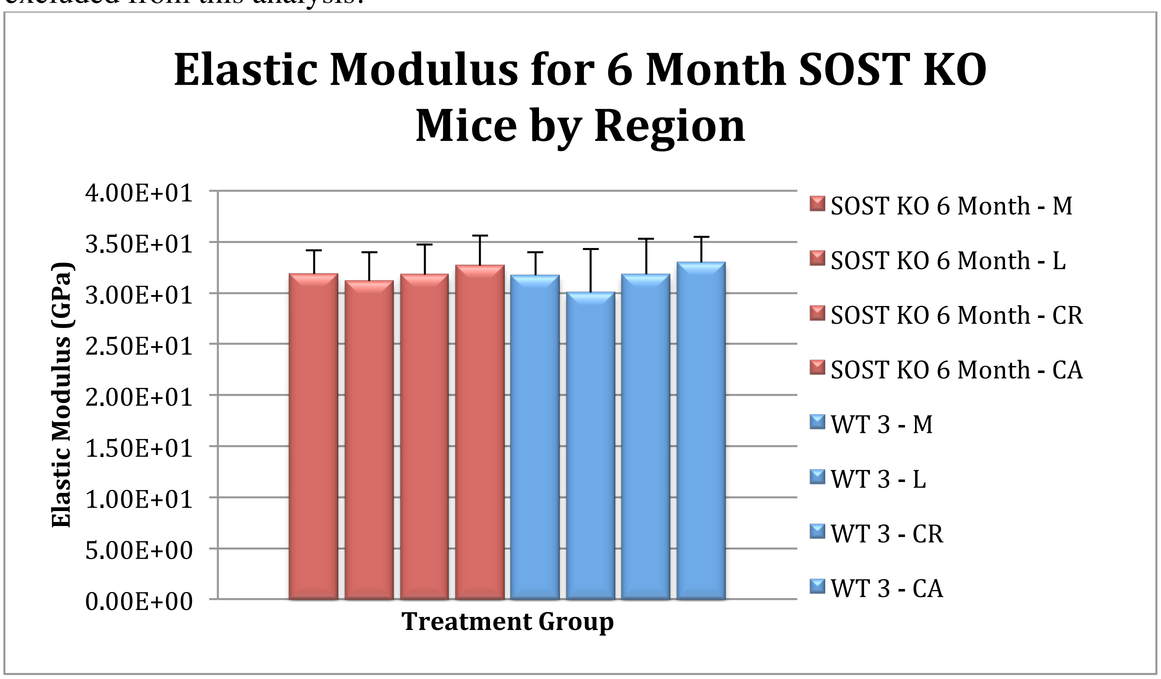


Figure 3.83: Histogram comparison of elastic modulus by anatomical region for 6 month old SOST knockout mice. WT 3 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

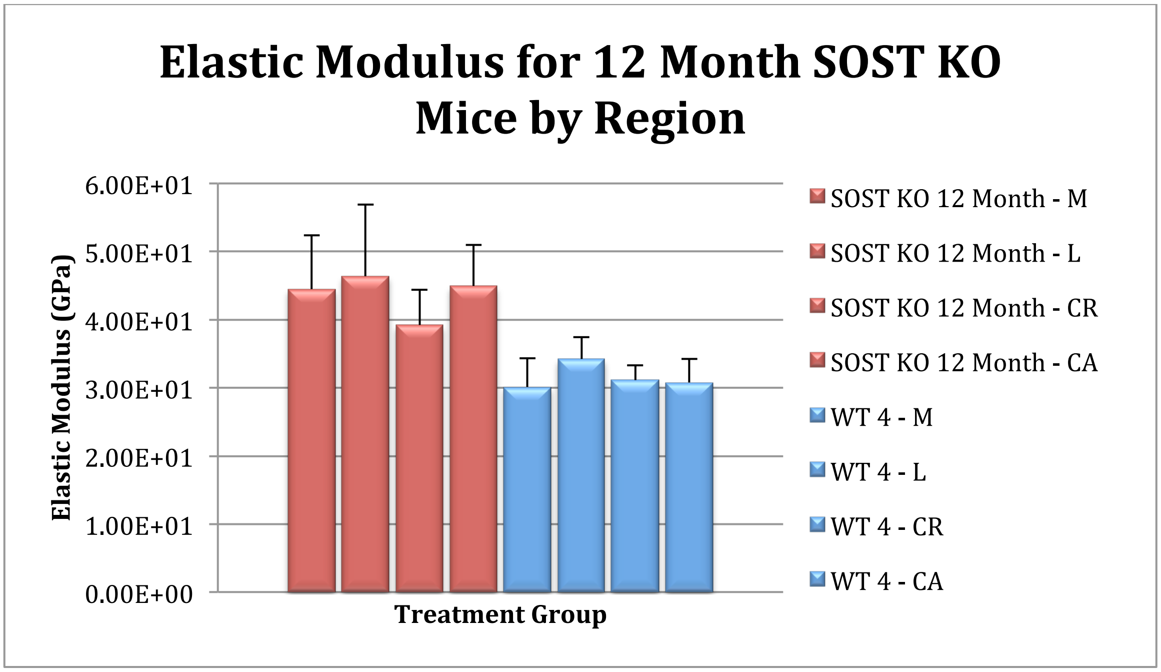


Figure 3.84: Histogram comparison of elastic modulus by anatomical region for 12 month old SOST knockout mice. WT 4 mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

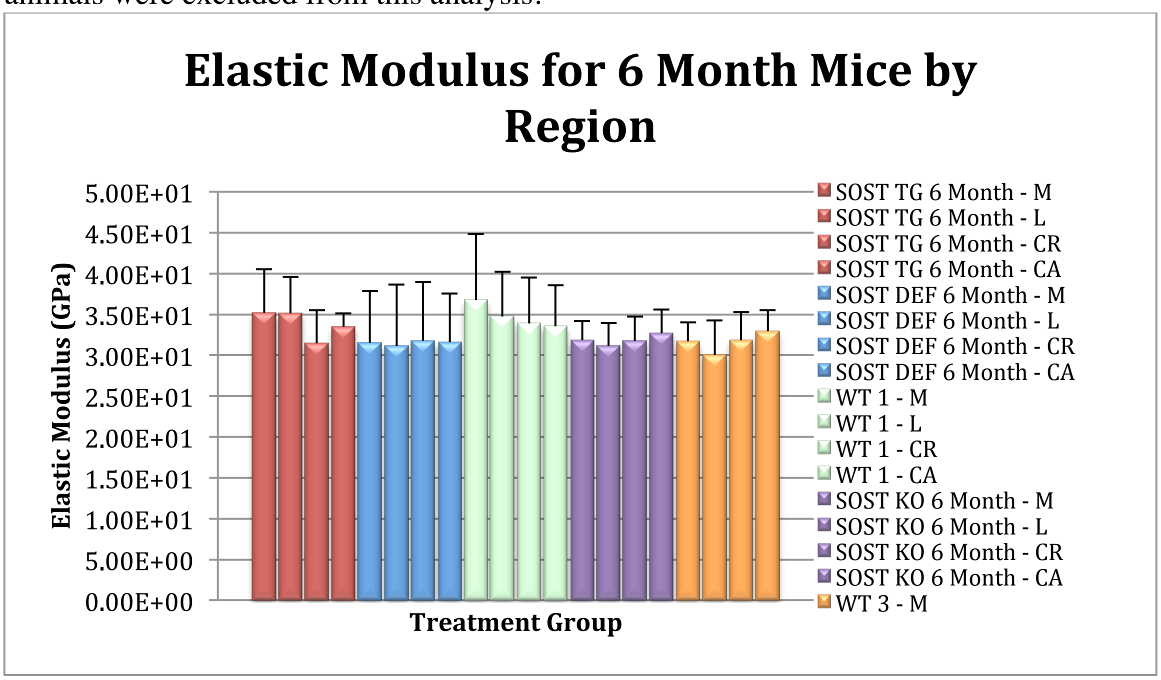


Figure 3.85: Histogram comparison of elastic modulus by anatomical region for all 6 month old SOST transgenic mice. WT 1 (TG) and 3 (KO) mice were control littermates, faithfully expressing murine SOST. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. No animals were excluded from this analysis.

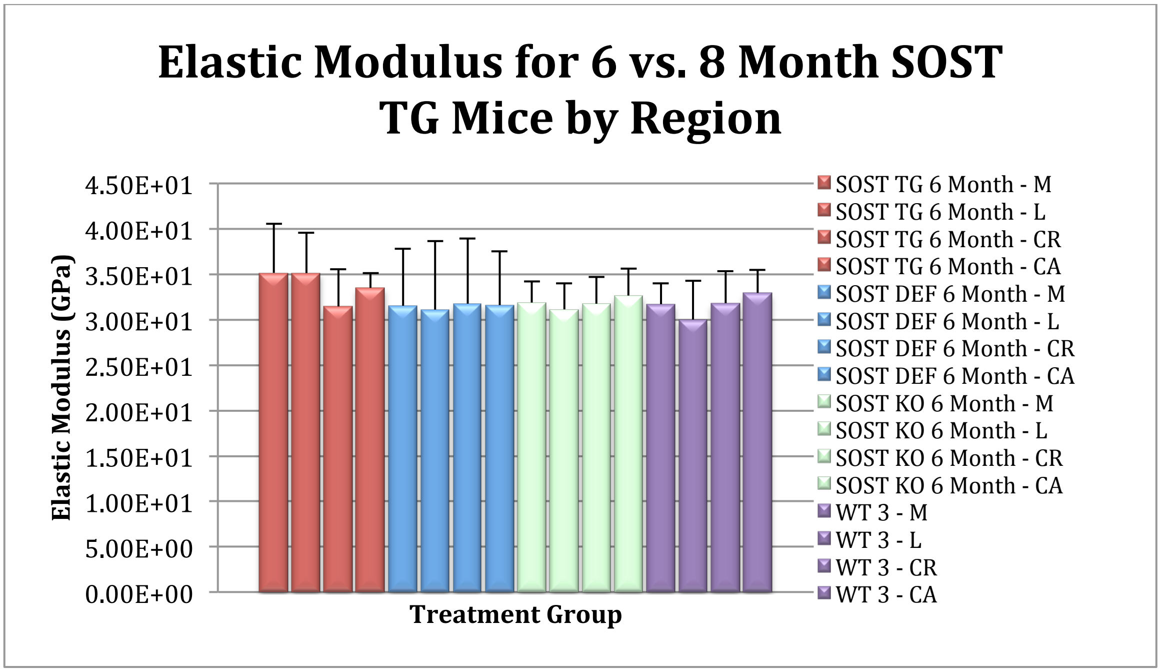


Figure 3.86: Histogram comparison of elastic modulus by anatomical region for 6 and 8 month old transgenic mice. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Wild type controls were not included in this analysis.

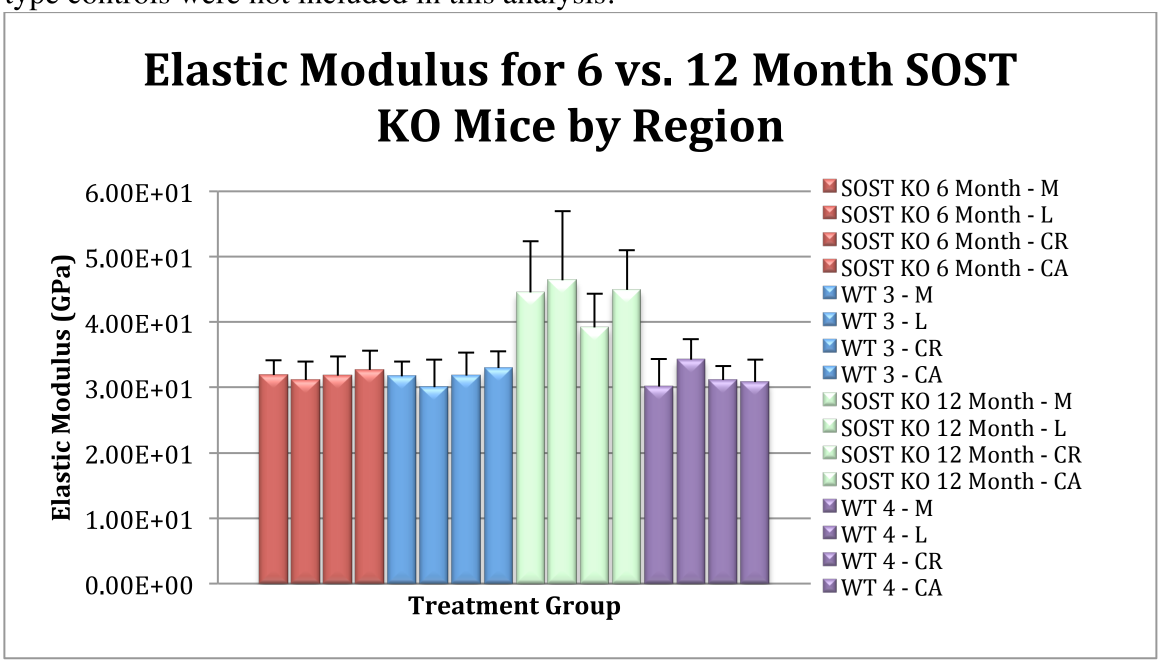


Figure 3.87: Histogram comparison of elastic modulus by anatomical region for 6 and 12 month old knockout mice. M = Medial, L = Lateral, CA = Caudal, CR = Cranial. Wild type controls were not included in this analysis.

IV. DISSCUSSION

Primary goals for this experiment were to determine the mechanical properties of bones expressing various levels of the sclerostin/SOST gene using transgenic mice. Secondary goals included examining any anatomical region differences in testing as well as examining side by side limb differences. Additional goals include determination of the effect of age on the mechanical properties of the tested murine femurs. Femurs were obtained from Lawrence Livermore National Labs and in a prior experiment were broken using 3 point bending techniques. Following 3-point bending, samples were embedded in epoxy and polished for nanoindentation. Each bone was indented in the 4 exposed anatomical regions of the femur. Data collected from the experiments came in the form of maximum load, hardness and elastic modulus values.

It was hypothesized that significant differences would be observed between bones which expressed SOST (TG) and those which had genomes devoid of the region coding for SOST expression (KO). SOST expression negatively regulates bone formation, hence it was it was predicted that the KO mice would have higher values for the tested effects (max load, hardness, elastic modulus) caused by the absence of sclerostin (the protein coded by SOST). In addition, indentations in the medial and lateral regions were predicted to have a greater modulus and hardness values as this axis is the principle direction of loading under normal skeletal conditions. It was also anticipated that there would be no difference between left and right limbs, consistent with previous reports [14, 23].

Results from this experiment were predicted to be useful for further supporting evidence of current bone property knowledge. Observed differences could provide supporting

evidence for continued research into the mechanisms of action of SOST which may lead to the development of gene therapies to combat prevalent bone diseases including osteoporosis.

4.1 Analysis of Results

4.1.1 Max Load

Max load was the first parameter examined. The indentation experiments conducted were depth-controlled experiments, and hence both maximum depth and plastic depth were considered to show less valuable information. Max load was found to be significantly affected by treatment group (p=0.000) as well as both treatment group and limb together (p=0.000). When observing the effect of limb alone, the data suggested low significance (p=0.226). Observations also showed the 12 month SOST KO mice having significantly greater max load values than their control littermate (WT 4) group, which coincides with previous hypothesis that SOST KO mice would have higher mechanical properties, but no differences were observed for the 6 month SOST KO and its control littermate (WT 3). Interesting observations included the absence of a difference in max load between all 6 and 8 month old TG mice (TG, DEF, and WT littermates) along with no difference between 6 and 12 month KO mice and their respective WT littermates. Slight differences were seen in the 8 month SOST DEF compared to its TG and WT mice.

Regional differences were not observed with almost statistically equivalent means for all four anatomical regions (p=0.774) nor any combination of interactions of anatomical region and limb or treatment group. It is important to note again that because the

experiments run were depth-controlled, the data for max load may present to be less valuable because it was somewhat controlled by the depth.

4.1.2 Hardness

Hardness was derived from the resultant depth vs. load curves from the indentation data. Hardness was found to be significantly affected by treatment group (p=0.000) and by limb tested (p=0.005), as well as significant findings were found between the interaction of treatment group and limb tested (p=0.000). 6 Month SOST TG mice were found to be significantly greater in hardness from its SOST DEF and control littermate (WT 1) counterparts. No significant differences were found in hardness between the 12 month SOST KO mice and its control littermates (WT 4) nor any observations between the 6 and 12 month SOST KO mice either. Differences were seen between the 6 and 8 month SOST TG mice.

Side by side limb differences were observed in the limbs of the 12 month SOST KO mice as well as the 6 month SOST DEF limbs. Regional differences were not observed with almost statistically equivalent means for all four anatomical regions (p=0.951) nor any combination of interactions of anatomical region and limb and or treatment group (p=0.958).

4.1.3 Elastic Modulus

Elastic modulus was derived from the slope of the unloading curve and the contact area between the indenter and plastically deformed material at maximum load. Contact areas were determined automatically from the depth vs. load curves. Elastic modulus was

found to be significantly affected by treatment group (p=0.000) and by limb tested (p=0.000), as well as significant findings were found between the interaction of treatment group and limb tested (p=0.000). Observations show significantly higher modulus values in the 6 month SOST TG mice versus its SOST DEF and control littermate (WT 1) mice. Differences were also observed between the 12 month SOST KO and their control littermate (WT 4) mice. No observed differences were seen between the 8 month SOST TG, SOST DEF and their control littermate (WT 2) mice. Side by side limb differences were observed in the limbs of the 12 month SOST KO mice. There were no significant findings amongst the different anatomical regions or its interaction with treatment group or limb. Regional differences were not observed with almost statistically equivalent means for all four anatomical regions (p=0.456), but some low significance was observed between combination of interactions of anatomical region, limb and treatment group (p=0.295).

4.2 Significance of Results

The variation in mechanical properties is important when computing stresses and strains in bone. For example, bone in the region near a metallic implant must be strong enough to withstand the mechanical loads; and at the same time its mechanical attributes must be capable of load transfer from the implant, remodeling, and sustaining itself for long periods of time. In addition, bone that has lost its flexibility and toughness due to osteoporosis, for example, may reflect those changes via altered mechanical properties [32].

Nanoindentation testing indicated that cortical bone from the SOST transgenic mice had significantly higher max load, hardness and elastic modulus. But unlike much research from past studies, a side to side difference was observed amongst the treatment groups. This may be due to the presence of defective limbs. The samples that were considered SOST DEF (defective limb) were not specified as to which limb was considered defective nor the exact process in how these defective mice were determined. Generally this would have been a visual assessment for defective limbs. Neither the 6 nor the 12 month SOST KO treatment groups had defective littermates.

In a study conducted by Silva et al., nanoindentation was used to test SAMP6 transgenic mice (a mouse model for senile osteoporosis), this study used both whole bone bending as well as nanoindentation to determine the mechanical properties of the SAMP6 mice [32]. Their study found average hardness (1.16 GPa) and elastic modulus (31.0 GPa) that coincide with data from this study. Their findings were similar to this nanoindentation experiment and its previous 3-point bending experiment that modulus values for whole bone bending drastically do not compare to those of nanoindentation. In Kai Peterson's 3-point bending experiment from these same mice samples, not only were the modulus values obtained from the whole bone testing smaller in magnitude (roughly between 1~6 GPa across the treatment groups) than that of nanoindentation, but the SOST KO mice were found to have significantly lower modulus values than that of the SOST TG mice. This raises a question of the validity of either whole bone bending and or nanoindentation for determining mechanical properties of bone. Nanoindentation provides a measure of the modulus of the solid phase of bone at the microstructural scale, with no influence of porosity. By contrast, whole-bone bending provides a measure at the continuum scale and

will strongly be influenced by porosity [32]. Conclusions drawn from Silva et al.'s study determined whole bone bending elastic modulus values to be more inaccurate as bones don't fully follow simple beam theory.

Nanoindentation is still a fairly new method of determining bone mechanical properties and past studies have all used different testing parameters. The low-load range test conditions used for the determination of Young's modulus and hardness are similar to those employed by Turner et al. where they selected a depth of 1000 nm, which required a maximum load of approximately 10 mN, a 375 μN/s unloading rate, and 10 s hold time [35]. Hengsberger et al. selected a maximum depth of 900 nm, which required a 10 mN load, followed by a 5 s holding period. Rho et al. tested at a maximum load of 8 mN, with a load/unload rate of 400 μN/s [36]. The difference in Young's modulus and hardness in this range of maximum loads is small. Thus, one might expect only slight variation in bone properties within this nanoindentation scale [34-36]. Nanoindentation has proven to be a good source of determination of mechanical properties of bone at the microstructure scale [37].

Findings from this nanoindentation experiment and past studies provide evidence that increased BMD and overall strength is seen in SOST KO mice that have the deletion of the gene on the 17q12 chromosome [12]. For patients afflicted by bone disorders such as Osteoporosis, a potential gene therapy can some day be developed to strategically control the expression of the SOST gene, fighting the regulation of bone growth. Gene therapy for bone regeneration has applications in veterinary, as well as human, medicine and the

regulatory route is less burdensome. Success in veterinary applications would facilitate their adoption into human clinical use [38].

4.3 Challenges/Limitations

Some limitations were found in the experiments that can be improved upon for future experiments. Firstly, and importantly, there were small and unequal sample sizes for the different treatment groups, including varying numbers of male and female mice in each group. These inequalities may have resulted in non-significant differences between some of the treatment groups.

Other challenges arose in the methods of sample polishing and testing. When grinding down the bones, depending on the nature of the break, some bones needed to be ground down a great amount to expose an entire cross section for nanoindentation testing. This grinding on some bones greatly surpassed the middle shaft region of the bone and came much closer to the proximal (or distal) end of the bone where the bone is primarily trabecular, changing much of the bones structure as well as its mechanical properties.

Regional testing was also difficult to determine at times. In the experiments, the anatomical bone regions were generalized by a wider range, instead of in more precise and exact locations. Using the microscope within the cabinet at 40x made it very difficult to determine where indentations are being placed in regards to the whole bone. The indenter wasn't always assumed to hit the exact spot the crosshairs were pointed at but was generally close to the point of specification.

4.4 Future Work

Once nanoindentation had actually begun, it became apparent that many steps in the methods process could have been different in order to make the nanoindentation process and data collection more efficient.

4.4.1 Embedding Process Improvements

One such thing is during embedding, making sure the bones don't float up and out of the tubes while the epoxy is setting can prove to be a very difficult task, especially when watching over multiple sample holders of epoxy at a time. If only a small later of epoxy was poured in, enough to cover half of the tubes, leaving the top half remaining out of the surface of the epoxy, not only would the bones not float up and out of the tubes, but the bones could all also very easily be oriented in the same direction so that they all faced the same direction. This would make the identifications of the anatomical regions much easier once polished down. Then after the epoxy has set a little, more epoxy can be mixed and poured over the rest of the samples to cover them completely.

4.4.2 Indentation Efficiency

The nanoindentation process is very long, roughly 12-15 minutes per indent. While only 1 sample can be loaded into the nanoindenter at a time, up to 100 schedules can be set, with only 1 schedule being 9 indents in one anatomical region. The actual programming of the indentation locations does not take long, if more than 1 set of bones were embedded per sample, not only could more schedules be set at a time, but there is less room for polishing errors. Even if two sets were put in each epoxy puck, that would cut

the sample number from 20 to 10 and would allow for much more efficient experimentation data collection and timing. As well as timing, depth calibrations were conducted every 5 samples tested, while re-calibrations always resulted the same, testing more samples under a single calibration could potentially reduce any calibration discrepancies.

4.4.3 SOST Ingrowth Regional Testing

The SOST KO bones when polished to the 0.05-micron level revealed many bone structures grown inwards where the marrow cavity usually resides. Future works can use nanoindentation to test different regions within these structures to test the micro-mechanical properties of these structures.

4.5 Conclusions

The goal of this study was to identify any significant differences in mechanical properties of bones from mice that do and do not express the SOST gene. Results from this study were meant to help advance the understanding of how SOST/sclerostin works and if it can be used as a target for gene therapies to battle bone disorders. The findings of this study coincide with data from other studies and show that mice hybridized by Lawrence Livermore National Lab have greater mechanical properties in comparison to their wild type littermate controls and transgenic mice. Further analysis should be done on these bones in regions of higher ingrowth in order to generate an accurate picture of the overall impact of SOST on bone formation. With continued studies and relevant data showing a definitive increase in quality of bone resulting from the removal of the SOST gene

compared to that of normal, healthy bone, the use of gene therapies to combat bone diseases such as osteoporosis may be fruitful for the medical world and has the capability to help the millions affected by bone diseases/disorders.

REFERENCES

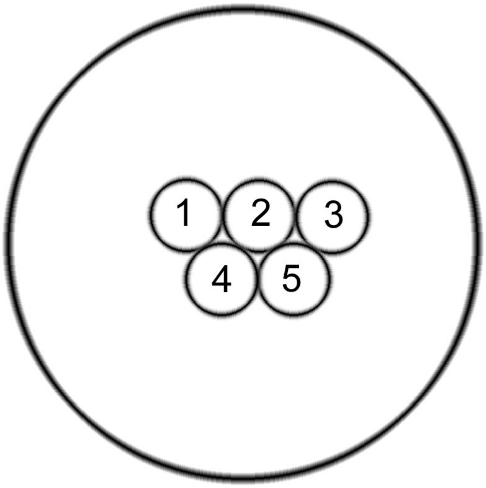
1. Taichman, R. S. "Blood and Bone: Two Tissues Whose Fates Are Intertwined to Create the Hematopoietic Stem-cell Niche." *Blood* 105.7 (2005): 2631-639.
2. Frost, Harold M. "Bone's Mechanostat: A 2003 Update." *The Anatomical Record* 275A.2 (2003): 1081-101
3. Clarke, B. "Normal Bone Anatomy and Physiology." *Clinical Journal of the American Society of Nephrology* 3.Supplement 3 (2008): S131-139.
4. Martin, R. Bruce, David B. Burr, , et al. Sharkey. *Skeletal Tissue Mechanics*. New York: Springer, 1998. Print.
5. Wang, X. *Fundamental Biomechanics in Bone Tissue Engineering*. [San Rafael, Calif.]: Morgan & Claypool, 2010.
6. Goff, M. G., and C. R. Sylfield. "Three-dimensional Characterization of Resorption Cavity Size and Location in Human Vertebral Trabecular Bone." *NIH Public Access* (2012): 28-37.
7. Kanis, J. "Diagnosis of Osteoporosis and Assessment of Fracture Risk." *Journal of Bone and Mineral Research* 9.8 (1994): 1929-936.
8. Kanis, J. A., D. Black, , et al. "A New Approach to the Development of Assessment Guidelines for Osteoporosis." *Osteoporosis International* 13.7 (2002): 527-36.
9. Bonewald, L. F. "Mechanosensation and Transduction in Osteocytes." *Bonekey Osteovision* **3**(2006): 7-15
10. Jacobs, P. "Van Buchem Disease." *Postgraduate Medical Journal* 53.622 (1977): 497-506.
11. Van Bezooijen, R.L., A.L. Bronckers, R.A. Gortzak, , et al. "Sclerostin in Mineralized Matrices and Van Buchem Disease." *Journal of Dental Research* 88.6 (2009): 569-74.
12. Balemans, W., Vandenende, J., et al. "Localization of the Gene for Sclerosteosis to the Van Buchem Disease–Gene Region on Chromosome 17q12–q21." *The American Journal of Human Genetics* 64.6 (1999): 1661-669.
13. Staehling-Hampton, K., Proll,S., et al. "A 52-kb Deletion in TheSOST-MEOX1 Intergenic Region on 17q12-q21 Is Associated with Van Buchem Disease in the Dutch Population." *American Journal of Medical Genetics* 110.2 (2002): 144-52.
14. Loots, G. G. "Genomic Deletion of a Long-range Bone Enhancer Misregulates Sclerostin in Van Buchem Disease." *Genome Research* 15.7 (2005): 928-35.
15. Vanhul, W., and W. Balemans. "Van Buchem Disease (Hyperostosis Corticalis Generalisata) Maps to Chromosome 17q12-q21." *The American Journal of Human Genetics* 62.2 (1998): 391-99.
16. Balemans, W., and W. Van Hul. "Human Genetics of SOST." *J Musculoskelet Neuronal Interact* 6.4 (2006): 355-56.
17. Balemans, Wendy, and Martin Ebeling. "Increased Bone Density in Sclerosteosis

Is Due to the Deficiency of a Novel Secreted Protein (SOST)." *Human Molecular Genetics* 10.5 (2001): 537-43.
18. Van Bezooijen, R. L. "Sclerostin Is an Osteocyte-expressed Negative Regulator of Bone Formation, But Not a Classical BMP Antagonist." *Journal of Experimental Medicine* 199.6 (2004): 805-14
19. Löwik, C.W.G.M., and R.L. Van Bezooijen. "Wnt Signaling Is Involved in the Inhibitory Action of Sclerostin on BMP-stimulated Bone Formation." *J Musculoskelet Neuronal Interact* 6.4 (2006): 357.
20. Muschler, G.F., Raut, V.P., et al. "The Design and Use of Animal Models for Translational Research in Bone Tissue Engineering and Regenerative Medicine." *Tissue Engineering Part B: Reviews* 16.1 (2010): 123-45.
21. Marturano, Joseph E., Benjamin C. Cleveland, Melissa A. Byrne, and Shannon L. O'Connell. "An Improved Murine Femur Fracture Device for Bone Healing Studies." *Journal of Biomechanics* 41.6 (2008): 1222-228.
22. Boskey, A. L., Wright, T.M., et al. "Guidelines for Describing Mouse Skeletal Phenotype." *Journal of Orthopaedic Research* 21.1 (2003): 1-5.
23. Li, X., Ominsky, M.S.m et al. "Targeted Deletion of the Sclerostin Gene in Mice Results in Increased Bone Formation and Bone Strength." *Journal of Bone and Mineral Research* 23.6 (2008): 860-69.
24. Winkler, D. G. "Osteocyte Control of Bone Formation via Sclerostin, a Novel BMP Antagonist." *The EMBO Journal* 22.23 (2003): 6267-276
25. Currey, John. "Measurement of the Mechanical Properties of Bone: A Recent History." *Clinical Orthopaedics and Related Research®* 467.8 (2009): 1948-954.
26. Rho, J., Kuhn-Spearing, L., et al. "Mechanical Properties and the Hierarchical Structure of Bone." *Medical Engineering & Physics* 20.2 (1998): 92-102.
27. Leppänen, O., Sievänen, H., et al. "Three-Point Bending of Rat Femur in the Mediolateral Direction: Introduction and Validation of a Novel Biomechanical Testing Protocol." *Journal of Bone and Mineral Research* 21.8 (2006): 1231-237.
28. Malecki, E. A., and Buhl, K. M. et al. "Bone Structural and Mechanical Properties Are Affected by Hypotransferrinemia But Not by Iron Deficiency in Mice." *Journal of Bone and Mineral Research* 15.2 (2000): 271-77.
29. Peterson, Kainoa J. "MECHANICAL PROPERTIES OF BONE DUE TO SOST EXPRESSION: A 3-POINT BENDING ASSESSMENT OF MURINE FEMURS." Thesis. California Polytechnic University, San Luis Obispo, 2012.
30. Rho, JY, and P. Zioupos. "Microstructural Elasticity and Regional Heterogeneity in Human Femoral Bone of Various Ages Examined by Nano-indentation." *Journal of Biomechanics* 35 (2002): 189-98.
31. *TheNanoTest User Manual*. 2004. User Manual for Micro Materials Ltd The NanoTest System. Wrexham Technology Park, Wrexham, UK.
32. Silva, M. "Nanoindentation and Whole-bone Bending Estimates of Material Properties in Bones from the Senescence Accelerated Mouse SAMP6." *Journal of Biomechanics* 37.11 (2004): 1639-646

33. Hoffler, C.E., and Guo, X. E,, et al. "An Application of Nanoindentation Technique to Measure Bone Tissue Lamellae Properties." *Journal of Biomechanical Engineering* 127.7 (2005): 1046. Print.
34. Zhang, J., Niebur, G.L., et al.. "Mechanical Property Determination of Bone through Nano- and Micro-indentation Testing and Finite Element Simulation." *Journal of Biomechanics* 41.2 (2008): 267-75
35. Turner, CH, Rho, J., et al. "The Elastic Properties of Trabecular and Cortical Bone Tissues Are Similar: Results from Two Microscopic Measurement Techniques." *J Biomech* 32.4 (1999): 437-41
36. Hengsberger, S., J. Enstroem, et al. "How Is the Indentation Modulus of Bone Tissue Related to Its Macroscopic Elastic Response? A Validation Study." *Journal of Biomechanics* 36.10 (2003): 1503-509.
37. Němeček, Jiří. *Nanoindentation in Materials Science*. Rijeka: InTech, 2012.
38. Evans, Christopher. "Gene Therapy for the Regeneration of Bone." *Injury* 42.6 (2011): 599-604.

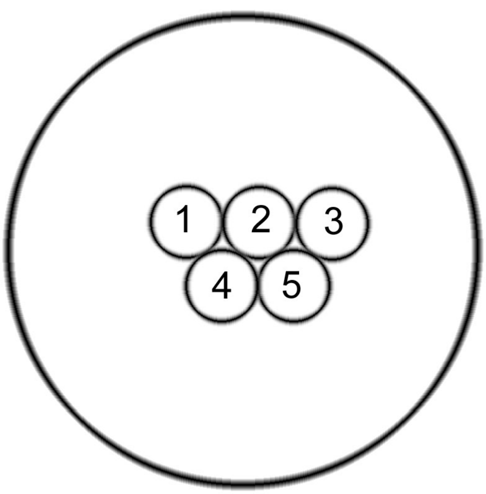
Appendix A: Bone Sample Configurations and Treatment Groups

Puck 1



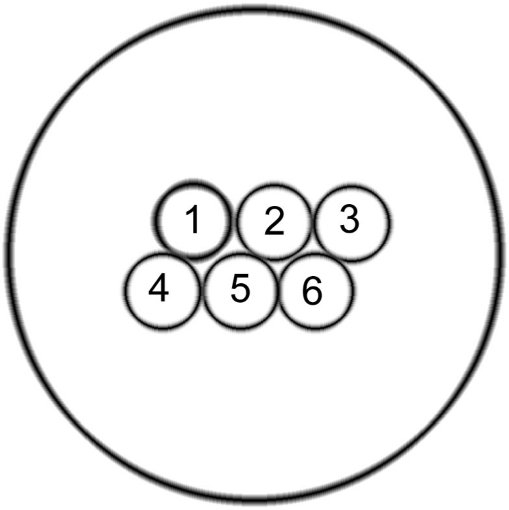
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	9200	SOST KO 6 Month KO	Full	Left	M
2	9203	SOST KO 6 Month KO	Full	Left	M
3	9201	SOST KO 6 Month KO	Full	Left	M
4	9202	SOST KO 6 Month KO	Full	Left	M
5	9198	SOST KO 6 Month KO	Full	Left	M

Puck 2



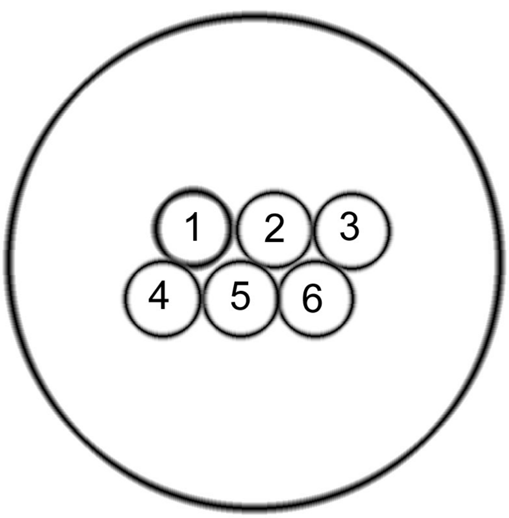
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	9200	SOST KO 6 Month KO	Full	Right	M
2	9203	SOST KO 6 Month KO	Full	Right	M
3	9201	SOST KO 6 Month KO	Full	Right	M
4	9202	SOST KO 6 Month KO	Full	Right	M
5	9198	SOST KO 6 Month KO	Full	Right	M

Puck 3



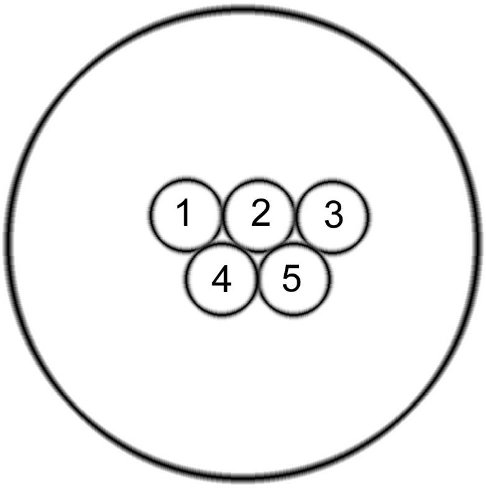
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	9209	SOST KO 6 Month WT	Partial - CR	Left	M
2	9208	SOST KO 6 Month WT	Full	Left	M
3	9240	SOST KO 6 Month WT	Full	Left	M
4	9239	SOST KO 6 Month WT	Full	Left	M
5	9207	SOST KO 6 Month WT	Full	Left	M
6	9238	SOST KO 6 Month WT	Full	Left	M

Puck 4



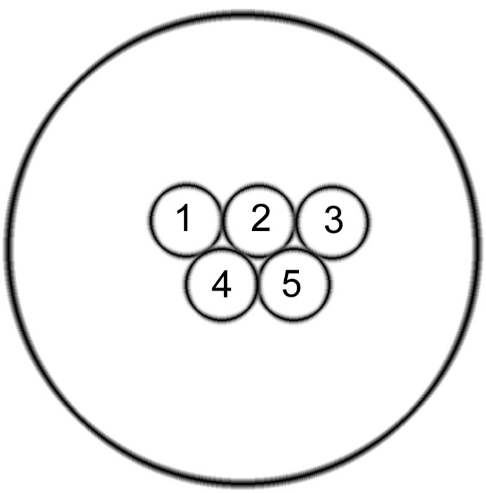
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	9209	SOST KO 6 Month WT	Partial - L	Right	M
2	9208	SOST KO 6 Month WT	Partial - CR	Right	M
3	9240	SOST KO 6 Month WT	Full	Right	M
4	9239	SOST KO 6 Month WT	Full	Right	M
5	9207	SOST KO 6 Month WT	Partial - L	Right	M
6	9238	SOST KO 6 Month WT	Full	Right	M

Puck 5



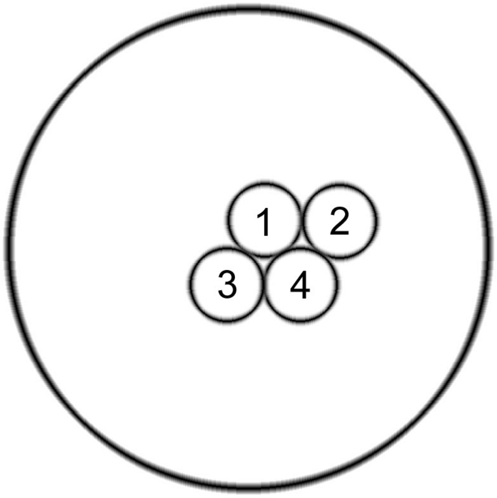
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	7594	SOST KO 12 Month KO	Partial - L	Left	M
2	7544	SOST KO 12 Month KO	Full	Left	M
3	7593	SOST KO 12 Month KO	Full	Left	M
4	7565	SOST KO 12 Month KO	Full	Left	M
5	7592	SOST KO 12 Month KO	Full	Left	M

Puck 6



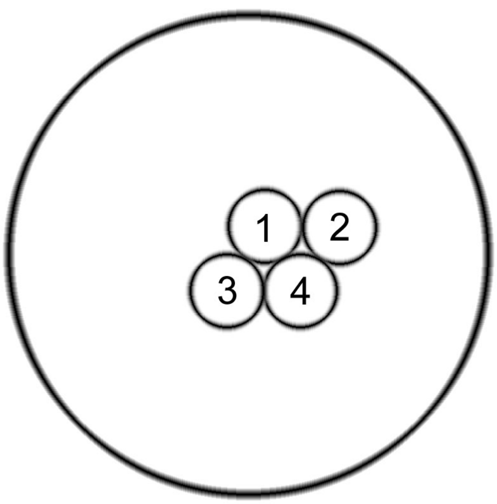
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	7594	SOST KO 12 Month KO	Partial - CR	Right	M
2	7544	SOST KO 12 Month KO	Full	Right	M
3	7593	SOST KO 12 Month KO	Full	Right	M
4	7565	SOST KO 12 Month KO	Full	Right	M
5	7592	SOST KO 12 Month KO	Full	Right	M

Puck 7



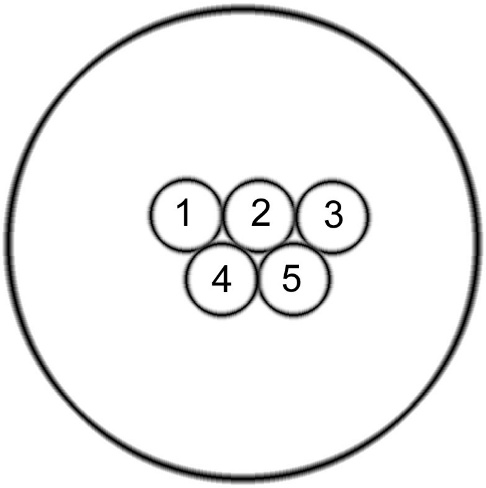
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	9296	SOST TG 6 Month TG	Full	Left	F
2	7564	SOST KO 12 Month WT	Full	Left	M
3	9293	SOST TG 6 Month TG	Full	Left	F
4	7595	SOST KO 12 Month WT	Full	Left	M

Puck 8



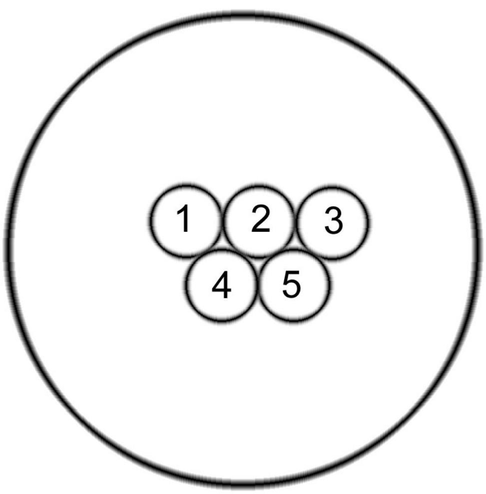
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	9296	SOST TG 6 Month TG	Full	Right	F
2	7564	SOST KO 12 Month WT	Full	Right	M
3	9293	SOST TG 6 Month TG	Full	Right	F
4	7595	SOST KO 12 Month WT	Full	Right	M

Puck 9



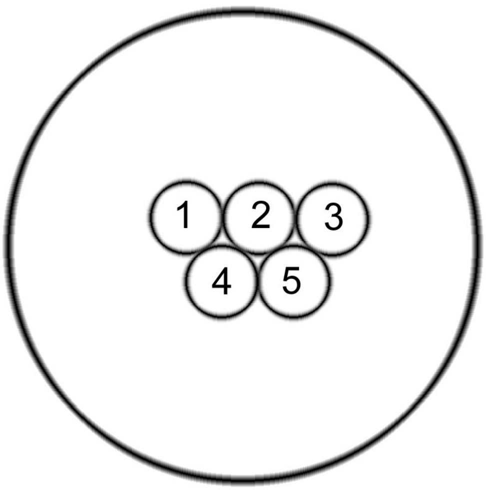
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	8601	SOST TG 6 Month DEF	Full	Left	M
2	8642	SOST TG 6 Month DEF	Full	Left	M
3	8658	SOST TG 6 Month DEF	Full	Left	M
4	8587	SOST TG 6 Month DEF	Full	Left	M
5	8641	SOST TG 6 Month DEF	Full	Left	M

Puck 10



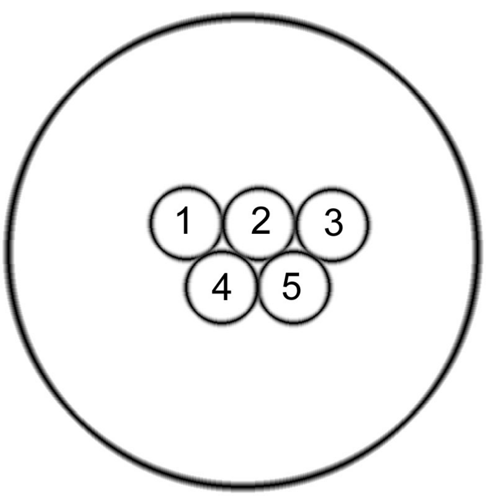
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	8601	SOST TG 6 Month DEF	Full	Right	M
2	8642	SOST TG 6 Month DEF	Full	Right	M
3	8658	SOST TG 6 Month DEF	Full	Right	M
4	8587	SOST TG 6 Month DEF	Full	Right	M
5	8641	SOST TG 6 Month DEF	Partial - CA	Right	M

Puck 11



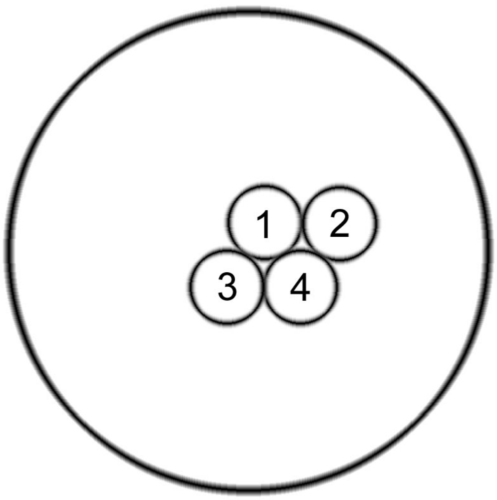
Ring	Number	Genotype+Phenotype	Exposer	Limb	Se
1	8640	SOST TG 6 Month WT	Full	Left	M
2	8600	SOST TG 6 Month WT	Full	Left	M
3	8657	SOST TG 6 Month WT	Full	Left	M
4	8599	SOST TG 6 Month WT	Partial - CR,M	Left	M
5	8656	SOST TG 6 Month WT	Full	Left	M

Puck 12



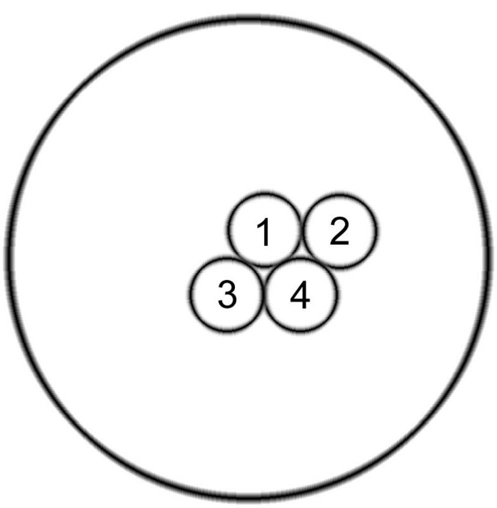
Ring	Number	Genotype+Phenotype	Exposer	Limb	Se
1	8640	SOST TG 6 Month WT	Full	Right	M
2	8600	SOST TG 6 Month WT	Full	Right	M
3	8657	SOST TG 6 Month WT	Full	Right	M
4	8599	SOST TG 6 Month WT	Full	Right	M
5	8656	SOST TG 6 Month WT	Partial - CA,M,L	Right	M

Puck 13



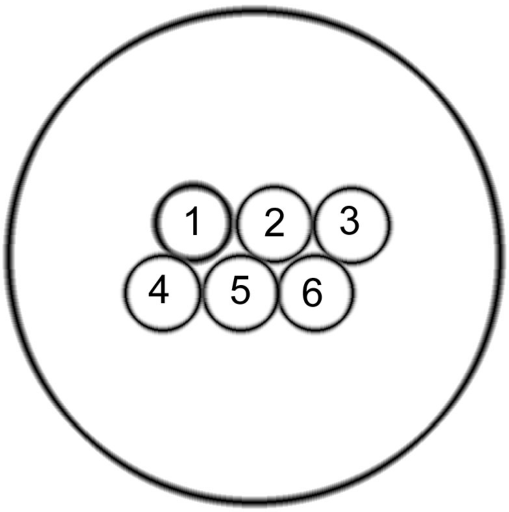
Ring	Number	Genotype+Phenotype	Exposer	Limb	Se
1	9294	SOST TG 6 Month DEF	Full	Left	F
2	9528	SOST TG 6 Month DEF	Full	Left	F
3	9530	SOST TG 6 Month DEF	Partial - CA, M	Left	F
4	9295	SOST TG 6 Month DEF	Full	Left	F

Puck 14



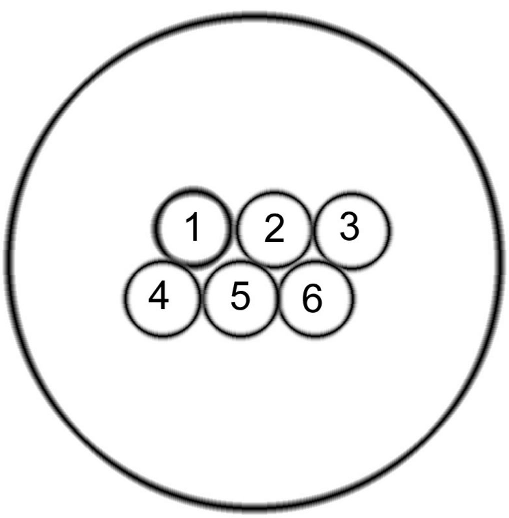
Ring	Number	Genotype+Phenotype	Exposer	Limb	Se
1	9294	SOST TG 6 Month DEF	Full	Right	F
2	9528	SOST TG 6 Month DEF	Full	Right	F
3	9530	SOST TG 6 Month DEF	NONE	Right	F
4	9295	SOST TG 6 Month DEF	Partial - CR	Right	F

Puck 15



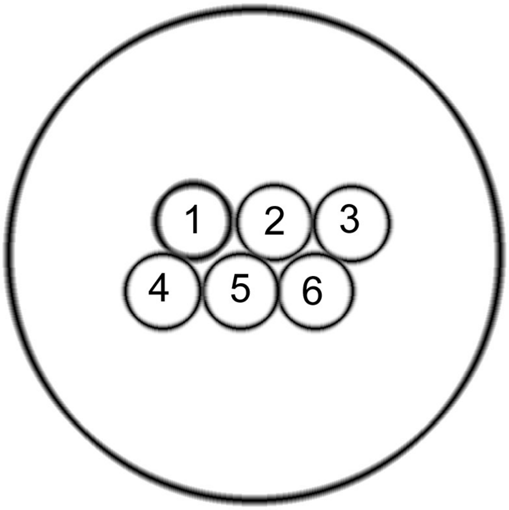
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	8603	SOST TG 6 Month WT	Full	Left	F
2	8643	SOST TG 6 Month WT	Full	Left	F
3	8602	SOST TG 6 Month WT	Full	Left	F
4	9529	SOST TG 6 Month WT	Full	Left	F
5	9526	SOST TG 6 Month WT	Full	Left	F
6	9527	SOST TG 6 Month WT	Partial - CA, L	Left	F

Puck 16



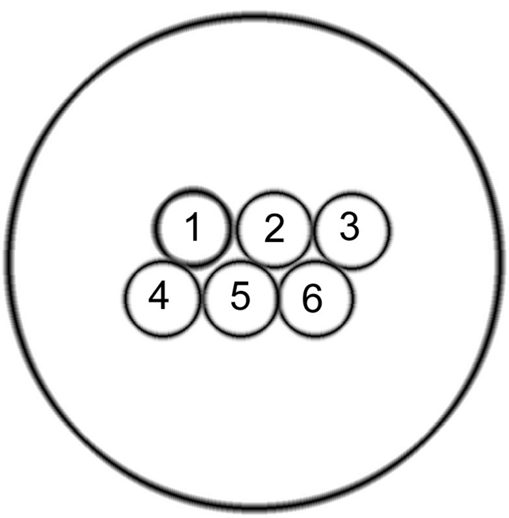
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	8603	SOST TG 6 Month WT	Full	Right	F
2	8643	SOST TG 6 Month WT	Full	Right	F
3	8602	SOST TG 6 Month WT	Full	Right	F
4	9529	SOST TG 6 Month WT	Full	Right	F
5	9526	SOST TG 6 Month WT	Full	Right	F
6	9527	SOST TG 6 Month WT	Full	Right	F

Puck 17



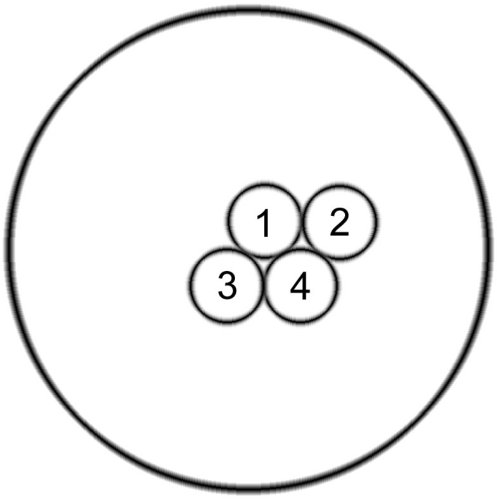
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	5599	SOST TG 8 Month TG	Full	Left	F
2	5592	SOST TG 8 Month TG	Full	Left	F
3	5588	SOST TG 8 Month DEF	Full	Left	F
4	5589	SOST TG 8 Month DEF	Full	Left	F
5	8007	SOST TG 8 Month DEF	Full	Left	F
6	8008	SOST TG 8 Month DEF	Full	Left	F

Puck 18



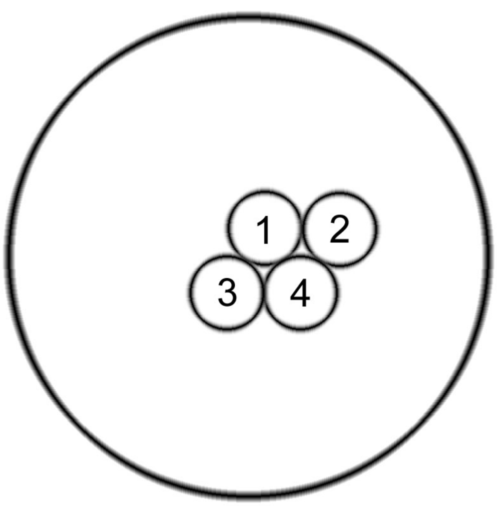
Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	5599	SOST TG 8 Month TG	Full	Right	F
2	5592	SOST TG 8 Month TG	Full	Right	F
3	5588	SOST TG 8 Month DEF	Full	Right	F
4	5589	SOST TG 8 Month DEF	Full	Right	F
5	8007	SOST TG 8 Month DEF	Partial - CR	Right	F
6	8008	SOST TG 8 Month DEF	Full	Right	F

Puck 19



Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	5590	SOST TG 8 Month WT	Full	Left	F
2	5598	SOST TG 8 Month WT	Partial – CA, M	Left	F
3	5593	SOST TG 8 Month WT	Full	Left	F
4	5597	SOST TG 8 Month WT	Full	Left	F

Puck 20



Ring	Number	Genotype+Phenotype	Exposer	Limb	Sex
1	5590	SOST TG 8 Month WT	Full	Right	F
2	5598	SOST TG 8 Month WT	Partial – CA,	Right	F
3	5593	SOST TG 8 Month WT	Full	Right	F
4	5597	SOST TG 8 Month WT	NONE	Right	F

Appendix B: Untestable Bone Regions

Bone	Limb	Region	Reason For No Data
9209	L	CA	No Exposure
9209	L	L	No Exposure
9209	L	M	No Exposure
9209	R	CA	No Exposure
9209	R	CR	No Exposure
9209	R	M	No Exposure
9208	R	CA	No Exposure
9208	R	L	No Exposure
9208	R	M	No Exposure
9207	R	CA	No Exposure
9207	R	CR	No Exposure
9207	R	M	No Exposure
7594	L	CA	No Exposure
7594	L	CR	No Exposure
7594	L	M	No Exposure
7594	R	CA	No Exposure
7594	R	L	No Exposure
7594	R	M	No Exposure
8641	R	CR	No Exposure
8641	R	L	No Exposure
8641	R	M	No Exposure
8599	L	CA	No Exposure
8599	L	L	No Exposure
8656	R	CR	No Exposure
9530	L	CR	No Exposure
9530	L	L	No Exposure
9530	R	ALL	No Exposure
9295	R	CA	No Exposure
9295	R	L	No Exposure
9295	R	M	No Exposure
9527	L	CR	No Exposure
9527	L	M	No Exposure
8603	R	CR	Shift during nanotest. Failed 1 attempted retest.
8643	R	CA	Shift during nanotest. Failed 1 attempted retest.
8643	R	CR	Shift during nanotest. Failed 1 attempted retest.
8643	R	L	Shift during nanotest. Failed 1 attempted retest.
9529	R	CA	Shift during nanotest. Failed 1 attempted retest.
9529	R	CR	Shift during nanotest. Failed 1 attempted retest.
5592	L	L	Shift during nanotest. Failed 1 attempted retest.

5589	L	CA	Shift during nanotest. Failed 1 attempted retest.
8807	R	CA	No Exposure
8807	R	L	No Exposure
8807	R	M	No Exposure
5598	L	CR	No Exposure
5593	L	CR	Shift during nanotest. Failed 1 attempted retest.
5593	L	L	Shift during nanotest. Failed 1 attempted retest.
5598	R	CR	No Exposure
5598	R	L	No Exposure
5597	R	ALL	No Exposure

Appendix C: Raw Indentation Data

Genotype+Phenotype	Limb	Sex	Number	Region	Max Depth (nm)	Plastic Depth (nm)	Max Load (mN)	Hardness (Gpa)	E_mod (Gpa)
SOST KO 12 Month KO	L	M	7594	CA	-	-	-	-	-
SOST KO 12 Month KO	L	M	7594	CR	-	-	-	-	-
SOST KO 12 Month KO	L	M	7594	M	-	-	-	-	-
SOST KO 12 Month KO	R	M	7594	CA	-	-	-	-	-
SOST KO 12 Month KO	R	M	7594	L	-	-	-	-	-
SOST KO 12 Month KO	R	M	7594	M	-	-	-	-	-
SOST KO 12 Month KO	R	M	7594	CR	1521.2374	1381.5409	47.9124	1.3352	36.9835
SOST KO 12 Month KO	L	M	7594	L	1289.0970	1148.9691	55.3917	2.1696	50.2705
SOST KO 12 Month KO	R	M	7593	M	1920.0210	1797.3796	57.8596	0.9778	39.8958
SOST KO 12 Month KO	R	M	7593	CR	1710.0295	1590.1895	46.9267	0.9996	37.0222
SOST KO 12 Month KO	R	M	7593	CA	1700.2311	1566.4371	65.2324	1.4314	47.2195
SOST KO 12 Month KO	L	M	7593	M	1673.0359	1506.7911	88.8294	2.0840	52.6310
SOST KO 12 Month KO	L	M	7593	CR	1643.5768	1485.1223	66.8369	1.6119	42.1165
SOST KO 12 Month KO	R	M	7593	L	1637.1093	1507.7071	52.7483	1.2374	40.2669
SOST KO 12 Month KO	L	M	7593	L	1622.4466	1462.0802	81.1935	2.0168	51.3523
SOST KO 12 Month KO	L	M	7593	CA	1529.7970	1349.8942	81.3623	2.3498	49.4071
SOST KO 12 Month KO	R	M	7592	L	1720.0003	1584.6376	46.4265	1.0009	32.8417
SOST KO 12 Month KO	R	M	7592	CA	1624.6958	1491.6861	50.4612	1.2058	37.7881
SOST KO 12 Month KO	R	M	7592	M	1619.6995	1497.2943	38.4254	0.9136	31.3910
SOST KO 12 Month KO	R	M	7592	CR	1615.1813	1470.7158	53.2388	1.3257	37.5659
SOST KO 12 Month KO	L	M	7592	CA	1590.4290	1437.4390	71.8653	1.8566	48.5268
SOST KO 12 Month KO	L	M	7592	L	1493.1327	1344.3358	70.8483	2.0605	52.2126
SOST KO 12 Month KO	L	M	7592	CR	1432.5717	1285.5744	60.5854	1.9237	47.1307
SOST KO 12 Month KO	L	M	7592	M	1342.1315	1206.0987	56.7652	2.0319	50.7286
SOST KO 12 Month KO	R	M	7565	L	1826.2787	1666.2617	62.1547	1.2102	34.8170
SOST KO 12 Month KO	R	M	7565	CR	1761.9411	1643.1301	39.5837	0.7932	30.2348
SOST KO 12 Month KO	R	M	7565	M	1738.9833	1622.4132	49.8328	1.0180	39.4857
SOST KO 12 Month KO	R	M	7565	CA	1697.4792	1556.3927	54.2224	1.2350	37.9728
SOST KO 12 Month KO	L	M	7565	M	1443.0054	1279.6260	76.2928	2.4619	53.8734
SOST KO 12 Month KO	L	M	7565	CR	1430.9071	1285.4879	58.0576	1.8484	45.6892
SOST KO 12 Month KO	L	M	7565	L	1358.8795	1239.2949	63.5671	2.1581	63.6079
SOST KO 12 Month KO	L	M	7565	CA	1300.5361	1158.3479	59.0435	2.2897	52.9804
SOST KO 12 Month KO	R	M	7544	L	1802.4642	1671.3567	52.8847	1.0321	36.9211
SOST KO 12 Month KO	R	M	7544	CR	1778.8189	1612.8524	63.9092	1.3339	36.3247
SOST KO 12 Month KO	L	M	7544	CR	1662.1334	1486.0982	68.8792	1.6583	39.5797
SOST KO 12 Month KO	L	M	7544	M	1617.4141	1438.4572	82.4755	2.1118	47.5502

SOST KO 12 Month KO	R	M	7544	CA	1600.9383	1442.3523	58.5867	1.4964	38.0013
SOST KO 12 Month KO	R	M	7544	M	1491.9463	1334.2640	56.3750	1.6859	40.3325
SOST KO 12 Month KO	L	M	7544	CA	1460.9790	1300.7407	67.2900	2.0862	47.5317
SOST KO 12 Month KO	L	M	7544	L	1340.3616	1194.0448	65.7580	2.3897	55.1501
SOST KO 12 Month WT	L	M	7595	M	1585.5138	1409.9647	46.1374	1.2268	27.5675
SOST KO 12 Month WT	L	M	7595	L	1569.3243	1374.2035	56.1937	1.5693	30.9315
SOST KO 12 Month WT	L	M	7595	CR	1561.3208	1362.9243	57.1619	1.6233	31.2002
SOST KO 12 Month WT	L	M	7595	CA	1553.4874	1365.4067	53.1272	1.4998	30.5159
SOST KO 12 Month WT	R	M	7595	M	1455.1704	1282.2731	38.7884	1.2321	25.7056
SOST KO 12 Month WT	R	M	7595	L	1426.2948	1254.0195	47.3919	1.5701	32.1904
SOST KO 12 Month WT	R	M	7595	CR	1406.1121	1232.3120	49.7856	1.7137	34.1602
SOST KO 12 Month WT	R	M	7595	CA	1404.6698	1235.8695	45.2209	1.5405	31.7677
SOST KO 12 Month WT	R	M	7564	CA	1573.7087	1376.8018	48.2035	1.3404	26.2371
SOST KO 12 Month WT	L	M	7564	M	1567.8464	1382.5334	56.9730	1.5757	32.8093
SOST KO 12 Month WT	L	M	7564	CA	1562.3400	1370.1761	61.7936	1.7344	34.6435
SOST KO 12 Month WT	R	M	7564	CR	1559.9223	1345.5404	57.2656	1.6617	29.2650
SOST KO 12 Month WT	L	M	7564	L	1544.1539	1354.0547	65.6335	1.8822	37.6075
SOST KO 12 Month WT	R	M	7564	L	1540.6477	1340.4595	65.9255	1.9302	36.2171
SOST KO 12 Month WT	R	M	7564	M	1529.4357	1338.7627	59.7287	1.7510	34.4733
SOST KO 12 Month WT	L	M	7564	CR	1501.8306	1314.6756	50.0176	1.5181	29.9327
SOST KO 6 Month KO	L	M	9203	M	1559.1559	1369.8757	52.8708	1.4870	30.1040
SOST KO 6 Month KO	R	M	9203	CA	1553.3658	1362.8393	51.1895	1.4557	29.0865
SOST KO 6 Month KO	R	M	9203	M	1538.6143	1356.7591	50.9564	1.4611	30.4753
SOST KO 6 Month KO	L	M	9203	CR	1536.6753	1367.5220	51.8572	1.4674	33.2403
SOST KO 6 Month KO	R	M	9203	L	1534.3337	1361.9240	50.8008	1.4473	31.9566
SOST KO 6 Month KO	R	M	9203	CR	1528.3383	1341.7121	60.7799	1.7750	35.7974
SOST KO 6 Month KO	L	M	9203	CA	1513.9083	1337.5805	46.8785	1.3791	29.3326
SOST KO 6 Month KO	L	M	9203	L	1477.1490	1299.5148	47.6075	1.4963	30.4496
SOST KO 6 Month KO	L	M	9202	CA	-	-	-	1.6941	34.6583
SOST KO 6 Month KO	L	M	9202	CR	-	-	-	1.5786	33.1670
SOST KO 6 Month KO	L	M	9202	L	-	-	-	1.2899	29.5375
SOST KO 6 Month KO	L	M	9202	M	-	-	-	1.3919	31.3908
SOST KO 6 Month KO	R	M	9202	M	1542.2175	1359.1866	52.4341	1.5041	31.1401
SOST KO 6 Month KO	R	M	9202	CA	1515.4784	1332.7021	56.5091	1.6733	34.1787
SOST KO 6 Month KO	R	M	9202	CR	1510.3830	1327.4865	46.7962	1.3974	28.4180
SOST KO 6 Month KO	R	M	9202	L	1490.4715	1304.1764	59.4332	1.8316	36.0124
SOST KO 6 Month KO	R	M	9201	CR	1563.8952	1394.7564	50.0413	1.3602	31.3248
SOST KO 6 Month KO	R	M	9201	L	1558.8384	1391.2606	45.7022	1.2479	28.9902
SOST KO 6 Month KO	L	M	9201	M	1525.7745	1352.0027	56.1063	1.6157	35.2022
SOST KO 6 Month KO	L	M	9201	L	1512.0660	1340.3934	47.9930	1.4127	30.7117

SOST KO 6 Month KO	R	M	9201	CA	1509.0634	1340.4054	43.0773	1.2629	28.1211
SOST KO 6 Month KO	L	M	9201	CR	1490.8159	1311.4560	49.6316	1.5164	31.0957
SOST KO 6 Month KO	R	M	9201	M	1478.1614	1309.7864	40.6543	1.2759	27.5879
SOST KO 6 Month KO	L	M	9201	CA	1446.4577	1273.1816	55.7519	1.8000	37.1845
SOST KO 6 Month KO	L	M	9200	CR	1565.6103	1364.2560	50.3881	1.4271	27.1190
SOST KO 6 Month KO	R	M	9200	L	1557.7710	1390.2751	42.9086	1.1736	27.1954
SOST KO 6 Month KO	L	M	9200	L	1555.6378	1356.0005	53.2141	1.5232	29.0453
SOST KO 6 Month KO	L	M	9200	M	1551.3022	1367.5811	52.8621	1.4877	31.0183
SOST KO 6 Month KO	L	M	9200	CA	1543.3553	1356.8881	55.7336	1.5922	32.4859
SOST KO 6 Month KO	R	M	9200	M	1538.9065	1372.2468	51.5325	1.4843	33.8642
SOST KO 6 Month KO	R	M	9200	CR	1527.7783	1361.8012	48.8700	1.3901	31.9473
SOST KO 6 Month KO	R	M	9200	CA	1440.5771	1278.2541	46.3838	1.4835	32.8442
SOST KO 6 Month KO	R	M	9198	M	1532.6418	1362.3526	52.2764	1.4831	33.2222
SOST KO 6 Month KO	R	M	9198	CA	1528.0567	1340.4317	57.1522	1.6732	33.5065
SOST KO 6 Month KO	R	M	9198	L	1523.3789	1334.4597	60.6258	1.7872	35.4627
SOST KO 6 Month KO	L	M	9198	L	1517.5001	1325.0577	55.4478	1.6667	32.0824
SOST KO 6 Month KO	L	M	9198	M	1505.0958	1320.4498	56.4810	1.7252	34.5703
SOST KO 6 Month KO	L	M	9198	CA	1501.7725	1325.2579	55.7563	1.6739	35.1365
SOST KO 6 Month KO	R	M	9198	CR	1481.4615	1300.7796	46.8764	1.4501	29.3341
SOST KO 6 Month KO	L	M	9198	CR	1464.7915	1294.1568	54.0354	1.6981	36.1841
SOST KO 6 Month WT	L	M	9240	CA	1586.7906	1391.2493	52.4086	1.4399	28.5085
SOST KO 6 Month WT	R	M	9240	L	1567.9555	1391.9072	44.6790	1.2189	26.9555
SOST KO 6 Month WT	R	M	9240	CR	1565.4851	1388.8462	47.0657	1.2890	28.3142
SOST KO 6 Month WT	R	M	9240	M	1554.8789	1374.7121	50.4210	1.4077	30.0393
SOST KO 6 Month WT	R	M	9240	CA	1546.1450	1354.0756	54.6663	1.5693	30.9918
SOST KO 6 Month WT	L	M	9240	M	1476.2302	1277.1818	58.5921	1.8981	34.0945
SOST KO 6 Month WT	L	M	9240	CR	1462.6277	1293.6099	51.7717	1.6403	34.6778
SOST KO 6 Month WT	L	M	9240	L	1448.5761	1270.0071	53.8302	1.7591	35.0146
SOST KO 6 Month WT	L	M	9239	CR	1568.9152	1388.8410	52.3801	1.4333	30.9418
SOST KO 6 Month WT	R	M	9239	CA	1546.5118	1361.9945	54.7059	1.5533	32.1048
SOST KO 6 Month WT	R	M	9239	L	1545.1729	1363.6471	48.8161	1.4022	29.4664
SOST KO 6 Month WT	R	M	9239	M	1543.5797	1355.4058	58.8066	1.6852	33.9819
SOST KO 6 Month WT	L	M	9239	M	1524.8380	1358.0307	43.2839	1.2355	28.1478
SOST KO 6 Month WT	L	M	9239	L	1510.2712	1339.9046	49.4609	1.4529	31.9116
SOST KO 6 Month WT	R	M	9239	CR	1507.4383	1324.9873	53.8202	1.6091	32.8102
SOST KO 6 Month WT	L	M	9239	CA	1435.2545	1263.4769	51.7081	1.7067	35.2000
SOST KO 6 Month WT	R	M	9238	M	1513.8213	1341.9452	48.1041	1.4097	30.7776
SOST KO 6 Month WT	R	M	9238	L	1499.5197	1332.3200	50.3444	1.4918	33.3633
SOST KO 6 Month WT	L	M	9238	CA	1499.2091	1315.7102	57.5396	1.7461	35.1703
SOST KO 6 Month WT	L	M	9238	M	1494.5946	1320.6686	50.1549	1.5206	32.2131

SOST KO 6 Month WT	R	M	9238	CA	1488.7474	1287.3508	56.9257	1.8037	32.1928
SOST KO 6 Month WT	L	M	9238	L	1477.0428	1282.9041	59.1318	1.8785	34.9018
SOST KO 6 Month WT	R	M	9238	CR	1464.0636	1281.3758	49.4075	1.5738	31.0489
SOST KO 6 Month WT	L	M	9238	CR	1424.9480	1248.0466	55.6194	1.8624	37.1285
SOST KO 6 Month WT	L	M	9209	CA	-	-	-	-	-
SOST KO 6 Month WT	L	M	9209	L	-	-	-	-	-
SOST KO 6 Month WT	L	M	9209	M	-	-	-	-	-
SOST KO 6 Month WT	R	M	9209	CA	-	-	-	-	-
SOST KO 6 Month WT	R	M	9209	CR	-	-	-	-	-
SOST KO 6 Month WT	R	M	9209	M	-	-	-	-	-
SOST KO 6 Month WT	R	M	9209	L	1575.4595	1428.8359	30.4129	0.8882	23.5047
SOST KO 6 Month WT	L	M	9209	CR	1493.5258	1315.4724	53.4802	1.6239	33.6434
SOST KO 6 Month WT	R	M	9208	CA	-	-	-	-	-
SOST KO 6 Month WT	R	M	9208	L	-	-	-	-	-
SOST KO 6 Month WT	R	M	9208	M	-	-	-	-	-
SOST KO 6 Month WT	L	M	9208	M	1572.5767	1381.5867	53.8168	1.4877	30.1440
SOST KO 6 Month WT	R	M	9208	CR	1554.7006	1379.1100	40.3558	1.1197	24.5896
SOST KO 6 Month WT	L	M	9208	L	1552.5706	1353.8041	42.6399	1.2536	23.6749
SOST KO 6 Month WT	L	M	9208	CA	1552.5053	1374.3640	55.2348	1.5420	33.3210
SOST KO 6 Month WT	L	M	9208	CR	1540.7411	1360.6353	55.8379	1.5923	33.5507
SOST KO 6 Month WT	R	M	9207	CA	-	-	-	-	-
SOST KO 6 Month WT	R	M	9207	CR	-	-	-	-	-
SOST KO 6 Month WT	R	M	9207	M	-	-	-	-	-
SOST KO 6 Month WT	L	M	9207	M	1580.7064	1394.3379	60.2453	1.6374	34.2797
SOST KO 6 Month WT	L	M	9207	L	1557.2472	1375.4043	48.9205	1.3754	28.9145
SOST KO 6 Month WT	L	M	9207	CA	1546.5697	1347.8981	65.5808	1.8992	36.1219
SOST KO 6 Month WT	L	M	9207	CR	1528.6321	1339.8118	54.0816	1.5846	31.4918
SOST KO 6 Month WT	R	M	9207	L	1474.9670	1292.4387	51.8996	1.6332	32.4394
SOST TG 6 Month DEF	L	F	9530	CR	-	-	-	-	-
SOST TG 6 Month DEF	L	F	9530	L	-	-	-	-	-
SOST TG 6 Month DEF	R	F	9530	CA	-	-	-	-	-
SOST TG 6 Month DEF	R	F	9530	CR	-	-	-	-	-
SOST TG 6 Month DEF	R	F	9530	L	-	-	-	-	-
SOST TG 6 Month DEF	R	F	9530	M	-	-	-	-	-
SOST TG 6 Month DEF	L	F	9530	M	1721.5203	1539.4351	56.5647	1.2752	29.9971
SOST TG 6 Month DEF	L	F	9530	CA	1717.7636	1541.8585	52.3309	1.1878	29.1138
SOST TG 6 Month DEF	L	F	9528	CR	1749.4833	1591.5047	55.7236	1.1803	33.0675
SOST TG 6 Month DEF	L	F	9528	L	1723.6845	1565.7744	56.8865	1.2477	34.5461
SOST TG 6 Month DEF	L	F	9528	CA	1711.8813	1547.6096	51.6853	1.1580	30.7345
SOST TG 6 Month DEF	L	F	9528	M	1651.6658	1494.5138	47.7430	1.1385	30.2453

SOST TG 6 Month DEF	R	F	9528	CR	1650.4264	1431.0526	72.1869	1.8697	34.3750
SOST TG 6 Month DEF	R	F	9528	L	1585.2755	1387.1072	67.5864	1.8572	36.3093
SOST TG 6 Month DEF	R	F	9528	CA	1468.7755	1278.3682	60.0439	1.9243	36.3522
SOST TG 6 Month DEF	R	F	9528	M	1428.5964	1273.6413	53.0986	1.7172	39.9021
SOST TG 6 Month DEF	R	F	9295	CA	-	-	-	-	-
SOST TG 6 Month DEF	R	F	9295	L	-	-	-	-	-
SOST TG 6 Month DEF	R	F	9295	M	-	-	-	-	-
SOST TG 6 Month DEF	L	F	9295	M	1706.5851	1540.2068	56.6919	1.2758	32.8844
SOST TG 6 Month DEF	L	F	9295	CA	1702.9108	1556.6477	46.0318	1.0173	30.1903
SOST TG 6 Month DEF	L	F	9295	L	1651.5034	1488.0809	48.7246	1.1720	29.8840
SOST TG 6 Month DEF	L	F	9295	CR	1586.7709	1418.4918	51.6533	1.5065	32.7871
SOST TG 6 Month DEF	R	F	9295	CR	1375.4721	1216.8689	55.0883	1.9344	41.9303
SOST TG 6 Month DEF	L	F	9294	L	1765.9744	1630.7958	38.8024	0.7827	26.3246
SOST TG 6 Month DEF	R	F	9294	M	1713.5787	1522.4373	81.3542	1.8711	41.5679
SOST TG 6 Month DEF	R	F	9294	L	1696.6381	1489.1673	89.3986	2.1439	42.9694
SOST TG 6 Month DEF	R	F	9294	CA	1694.2648	1500.7605	73.7376	1.7419	37.6798
SOST TG 6 Month DEF	R	F	9294	CR	1662.8835	1479.3312	70.5465	1.7166	38.5811
SOST TG 6 Month DEF	L	F	9294	CR	1641.8307	1473.8419	54.0041	1.3388	33.1435
SOST TG 6 Month DEF	L	F	9294	CA	1638.0827	1493.5098	50.8833	1.2113	35.0199
SOST TG 6 Month DEF	L	F	9294	M	1601.8278	1436.6325	51.7690	1.3485	33.2102
SOST TG 6 Month DEF	R	M	8658	M	1753.9300	1636.2625	44.4177	0.8930	34.5791
SOST TG 6 Month DEF	R	M	8658	CA	1687.3727	1546.0809	48.9367	1.0975	33.7305
SOST TG 6 Month DEF	R	M	8658	L	1670.3532	1552.6530	50.3731	1.1199	41.3395
SOST TG 6 Month DEF	R	M	8658	CR	1608.4870	1457.4103	48.9742	1.2316	33.3775
SOST TG 6 Month DEF	L	M	8658	CR	1563.4553	1377.7291	37.2278	1.0340	21.4874
SOST TG 6 Month DEF	L	M	8658	CA	1557.1594	1366.2405	39.8248	1.1263	22.5454
SOST TG 6 Month DEF	L	M	8658	M	1542.1886	1347.5535	44.7696	1.2966	25.1758
SOST TG 6 Month DEF	L	M	8658	L	1485.4831	1308.4041	37.8289	1.1576	24.0112
SOST TG 6 Month DEF	R	M	8642	CA	1751.1217	1629.9167	51.5191	1.0413	38.9875
SOST TG 6 Month DEF	R	M	8642	L	1738.2145	1598.6611	46.7343	0.9838	31.3371
SOST TG 6 Month DEF	R	M	8642	M	1717.8339	1588.4451	50.7146	1.0806	36.8675
SOST TG 6 Month DEF	R	M	8642	CR	1717.5537	1576.3353	52.8766	1.1421	35.3482
SOST TG 6 Month DEF	L	M	8642	M	1550.0027	1369.4217	41.3554	1.1617	24.6885
SOST TG 6 Month DEF	L	M	8642	CR	1538.1065	1346.0740	42.0038	1.2188	23.9459
SOST TG 6 Month DEF	L	M	8642	L	1537.5296	1348.8857	39.2774	1.1361	22.7491
SOST TG 6 Month DEF	L	M	8642	CA	1524.7457	1329.1082	46.9363	1.3944	26.6115
SOST TG 6 Month DEF	R	M	8641	CR	-	-	-	-	-
SOST TG 6 Month DEF	R	M	8641	L	-	-	-	-	-
SOST TG 6 Month DEF	R	M	8641	M	-	-	-	-	-
SOST TG 6 Month DEF	R	M	8641	CA	1718.4117	1606.6887	42.9762	0.9016	36.8434

SOST TG 6 Month DEF	L	M	8641	L	1558.4904	1353.0845	46.3324	1.3305	24.5862
SOST TG 6 Month DEF	L	M	8641	CA	1534.7041	1328.5167	43.5979	1.2959	23.4371
SOST TG 6 Month DEF	L	M	8641	M	1520.3063	1346.9437	34.7739	1.0085	21.9756
SOST TG 6 Month DEF	L	M	8641	CR	1499.6806	1304.6919	43.4186	1.3363	25.1076
SOST TG 6 Month DEF	R	M	8601	CR	1828.6760	1724.6671	49.5187	0.9004	41.4003
SOST TG 6 Month DEF	R	M	8601	M	1749.2720	1626.2519	49.1521	1.0051	37.3968
SOST TG 6 Month DEF	R	M	8601	CA	1724.6979	1584.8841	52.8884	1.1272	35.5508
SOST TG 6 Month DEF	R	M	8601	L	1707.1618	1574.2294	58.1020	1.2609	41.4329
SOST TG 6 Month DEF	L	M	8601	CR	1614.0609	1400.4573	43.5802	1.1731	21.5408
SOST TG 6 Month DEF	L	M	8601	L	1587.0411	1379.2894	45.6135	1.2633	23.5087
SOST TG 6 Month DEF	L	M	8601	CA	1550.9850	1352.9697	45.2197	1.2978	24.8875
SOST TG 6 Month DEF	L	M	8601	M	1447.0292	1264.9028	39.5318	1.2878	25.1798
SOST TG 6 Month DEF	R	M	8587	L	1723.4862	1611.6921	41.7219	0.8689	34.6319
SOST TG 6 Month DEF	R	M	8587	CR	1707.4378	1587.6739	48.1822	1.0270	38.3031
SOST TG 6 Month DEF	R	M	8587	CA	1677.8937	1543.2642	55.3965	1.2439	39.9074
SOST TG 6 Month DEF	R	M	8587	M	1670.4254	1529.4557	52.1057	1.1899	35.9242
SOST TG 6 Month DEF	L	M	8587	L	1478.3050	1287.1748	36.4812	1.1519	21.8098
SOST TG 6 Month DEF	L	M	8587	M	1464.9925	1280.3470	37.3375	1.1955	23.2394
SOST TG 6 Month DEF	L	M	8587	CR	1460.3634	1277.2291	35.1410	1.1244	22.0969
SOST TG 6 Month DEF	L	M	8587	CA	1442.1621	1258.3824	37.7304	1.2524	24.0502
SOST TG 6 Month TG	L	F	9296	CR	1565.8334	1377.1740	47.0568	1.3069	26.7131
SOST TG 6 Month TG	R	F	9296	CA	1559.1299	1355.0095	58.6080	1.6786	31.2522
SOST TG 6 Month TG	L	F	9296	L	1549.6155	1371.4339	56.8331	1.5925	34.3016
SOST TG 6 Month TG	R	F	9296	L	1545.7687	1406.1567	53.2502	1.4265	40.3707
SOST TG 6 Month TG	L	F	9296	CA	1541.9696	1356.4587	56.6834	1.6213	33.2195
SOST TG 6 Month TG	R	F	9296	M	1517.5716	1321.9683	52.7726	1.5815	30.0282
SOST TG 6 Month TG	R	F	9296	CR	1475.9050	1300.3375	46.6370	1.4442	30.0640
SOST TG 6 Month TG	L	F	9296	M	1437.5681	1291.2141	54.4775	1.7067	42.3645
SOST TG 6 Month TG	R	F	9293	M	1555.5645	1369.0280	55.8037	1.5669	32.2452
SOST TG 6 Month TG	R	F	9293	CR	1527.9691	1352.9850	58.0992	1.6740	36.3533
SOST TG 6 Month TG	L	F	9293	L	1496.8844	1323.1983	46.1227	1.3850	29.6909
SOST TG 6 Month TG	L	F	9293	M	1475.6658	1302.5653	55.0903	1.7060	35.9904
SOST TG 6 Month TG	L	F	9293	CR	1472.7230	1300.7776	49.6733	1.5445	32.7249
SOST TG 6 Month TG	R	F	9293	CA	1466.3301	1298.5719	51.7613	1.6111	35.0815
SOST TG 6 Month TG	L	F	9293	CA	1457.1974	1277.9452	53.5374	1.7149	34.3619
SOST TG 6 Month TG	R	F	9293	L	1444.1591	1261.8468	56.6429	1.8557	36.1505
SOST TG 6 Month WT	R	F	9529	CA	-	-	-	-	-
SOST TG 6 Month WT	R	F	9529	CR	-	-	-	-	-
SOST TG 6 Month WT	L	F	9529	CA	1574.6176	1387.7667	44.1668	1.2117	25.1879
SOST TG 6 Month WT	L	F	9529	L	1552.7919	1368.2632	53.1077	1.4970	31.0719

SOST TG 6 Month WT	R	F	9529	M	1535.5966	1364.7764	61.0182	1.7256	38.6531
SOST TG 6 Month WT	L	F	9529	CR	1528.8196	1344.2297	51.7830	1.5056	30.7425
SOST TG 6 Month WT	L	F	9529	M	1507.4290	1324.1934	43.4258	1.3010	26.3834
SOST TG 6 Month WT	R	F	9529	L	1433.6502	1267.0974	62.3958	2.0282	43.4369
SOST TG 6 Month WT	L	F	9527	CR	-	-	-	-	-
SOST TG 6 Month WT	L	F	9527	M	-	-	-	-	-
SOST TG 6 Month WT	R	F	9527	CA	1702.9833	1535.9273	61.3297	1.3907	35.5224
SOST TG 6 Month WT	R	F	9527	L	1604.6383	1449.3509	63.0806	1.5955	41.7896
SOST TG 6 Month WT	R	F	9527	CR	1576.9384	1412.3849	60.5331	1.6045	38.4789
SOST TG 6 Month WT	R	F	9527	M	1536.0459	1389.7326	63.7229	1.7406	46.2916
SOST TG 6 Month WT	L	F	9527	CA	1532.4502	1313.5654	55.3480	1.6802	28.3112
SOST TG 6 Month WT	L	F	9527	L	1506.7520	1318.5135	49.5697	1.5010	29.2950
SOST TG 6 Month WT	L	F	9526	CA	1548.2405	1369.9062	45.0811	1.2682	27.2261
SOST TG 6 Month WT	R	F	9526	CA	1541.3944	1383.5475	62.6880	1.7289	42.4103
SOST TG 6 Month WT	R	F	9526	L	1537.5802	1365.5310	70.8464	1.9995	44.5098
SOST TG 6 Month WT	L	F	9526	M	1533.6523	1344.5190	51.7966	1.5061	30.0217
SOST TG 6 Month WT	L	F	9526	CR	1532.6139	1332.8873	54.7589	1.6180	30.2967
SOST TG 6 Month WT	L	F	9526	L	1530.9608	1335.3113	54.6200	1.6092	30.8115
SOST TG 6 Month WT	R	F	9526	CR	1514.7674	1356.2759	69.3750	1.9869	47.5673
SOST TG 6 Month WT	R	F	9526	M	1488.3611	1321.0248	70.7201	2.1266	47.1070
SOST TG 6 Month WT	R	M	8657	M	1705.1703	1560.8266	54.9585	1.2069	36.3571
SOST TG 6 Month WT	L	M	8657	L	1701.3675	1554.0354	54.2571	1.2051	35.8982
SOST TG 6 Month WT	R	M	8657	CR	1694.9371	1560.8755	43.5237	0.9594	31.2404
SOST TG 6 Month WT	R	M	8657	CA	1690.5764	1535.0886	55.7619	1.2654	34.8134
SOST TG 6 Month WT	L	M	8657	CA	1671.9439	1517.8613	56.7321	1.3156	36.2044
SOST TG 6 Month WT	R	M	8657	L	1632.7405	1482.4833	50.3616	1.2212	33.5495
SOST TG 6 Month WT	L	M	8657	CR	1615.8858	1468.9480	47.1209	1.1613	32.4177
SOST TG 6 Month WT	L	M	8657	M	1581.5573	1442.1399	46.8287	1.1949	34.5728
SOST TG 6 Month WT	R	M	8656	CR	-	-	-	-	-
SOST TG 6 Month WT	R	M	8656	L	1654.6135	1514.0225	45.1180	1.0497	31.5142
SOST TG 6 Month WT	R	M	8656	CA	1630.8907	1500.8031	43.2457	1.0239	33.2543
SOST TG 6 Month WT	R	M	8656	M	1559.4066	1444.0567	46.1446	1.1807	41.5064
SOST TG 6 Month WT	L	M	8656	M	1541.9812	1422.9115	43.6680	1.1482	38.2159
SOST TG 6 Month WT	L	M	8656	L	1516.6411	1376.1617	43.5758	1.2120	33.2681
SOST TG 6 Month WT	L	M	8656	CA	1453.4014	1299.5111	43.9597	1.3759	32.6882
SOST TG 6 Month WT	L	M	8656	CR	1440.4575	1310.4263	42.4426	1.2987	36.8094
SOST TG 6 Month WT	R	F	8643	CA	-	-	-	-	-
SOST TG 6 Month WT	R	F	8643	CR	-	-	-	-	-
SOST TG 6 Month WT	R	F	8643	L	-	-	-	-	-
SOST TG 6 Month WT	R	F	8643	M	1719.0226	1521.0627	77.1457	1.7772	38.1628

SOST TG 6 Month WT	L	F	8643	M	1599.7824	1407.3252	53.9873	1.4403	29.4778
SOST TG 6 Month WT	L	F	8643	CR	1595.9286	1402.2545	55.7573	1.4983	30.3338
SOST TG 6 Month WT	L	F	8643	L	1564.8825	1381.0196	55.7485	1.5407	32.3998
SOST TG 6 Month WT	L	F	8643	CA	1555.9548	1371.3628	49.1863	1.3785	28.6832
SOST TG 6 Month WT	L	M	8640	L	1712.3819	1598.9209	36.2338	0.7672	30.3299
SOST TG 6 Month WT	L	M	8640	CR	1663.9565	1535.0740	48.5436	1.1020	36.7162
SOST TG 6 Month WT	L	M	8640	M	1649.0981	1523.9281	41.5920	0.9583	32.6087
SOST TG 6 Month WT	L	M	8640	CA	1639.7973	1513.4828	44.5578	1.0450	34.8288
SOST TG 6 Month WT	R	M	8640	L	1638.8269	1498.6820	45.8055	1.0936	32.9306
SOST TG 6 Month WT	R	M	8640	CA	1577.1496	1430.3357	48.0883	1.2521	34.1446
SOST TG 6 Month WT	R	M	8640	M	1573.9859	1440.4755	37.7153	0.9649	29.1006
SOST TG 6 Month WT	R	M	8640	CR	1525.2411	1358.6637	49.3287	1.4296	32.8793
SOST TG 6 Month WT	R	F	8603	CR	-	-	-	-	-
SOST TG 6 Month WT	R	F	8603	L	1750.2237	1586.7395	70.6564	1.5021	40.7890
SOST TG 6 Month WT	R	F	8603	CA	1642.5431	1480.0548	60.6027	1.4685	37.3525
SOST TG 6 Month WT	L	F	8603	CA	1601.7630	1411.8277	46.4283	1.2323	25.5846
SOST TG 6 Month WT	L	F	8603	L	1595.9393	1394.6557	44.3289	1.2025	23.3278
SOST TG 6 Month WT	L	F	8603	CR	1561.0471	1367.6081	44.5016	1.2532	24.8112
SOST TG 6 Month WT	R	F	8603	M	1536.6424	1435.3076	59.3344	1.5307	60.9767
SOST TG 6 Month WT	L	F	8603	M	1530.3932	1344.4039	41.9382	1.2193	24.7442
SOST TG 6 Month WT	L	F	8602	L	1708.3488	1493.2533	64.1872	1.5298	29.6544
SOST TG 6 Month WT	L	F	8602	CA	1607.8686	1415.0700	54.5929	1.4428	29.5902
SOST TG 6 Month WT	R	F	8602	L	1603.8590	1440.5411	60.6802	1.5823	38.2251
SOST TG 6 Month WT	R	F	8602	M	1591.9688	1422.0510	63.3542	1.6616	38.9751
SOST TG 6 Month WT	L	F	8602	M	1554.0870	1366.4607	54.0972	1.5248	31.1287
SOST TG 6 Month WT	R	F	8602	CR	1552.1349	1386.8926	68.3329	1.8794	44.0572
SOST TG 6 Month WT	R	F	8602	CA	1524.6374	1337.3079	68.6854	2.0199	40.4739
SOST TG 6 Month WT	L	F	8602	CR	1487.9914	1299.1181	51.5882	1.5989	30.9323
SOST TG 6 Month WT	R	M	8600	CR	1726.9417	1590.3540	50.7862	1.0756	34.8560
SOST TG 6 Month WT	R	M	8600	L	1726.8170	1578.6165	53.9874	1.1616	34.5588
SOST TG 6 Month WT	R	M	8600	CA	1708.9999	1567.1742	48.8573	1.0700	32.8444
SOST TG 6 Month WT	R	M	8600	M	1704.1014	1563.6177	53.7772	1.1787	36.6492
SOST TG 6 Month WT	L	M	8600	L	1698.7728	1577.9450	51.5779	1.1085	40.2636
SOST TG 6 Month WT	L	M	8600	CA	1696.1794	1567.6134	53.2327	1.1663	39.9960
SOST TG 6 Month WT	L	M	8600	CR	1685.9572	1559.4347	39.1017	0.8641	29.6848
SOST TG 6 Month WT	L	M	8600	M	1676.3996	1546.6028	47.5771	1.0661	35.4470
SOST TG 6 Month WT	L	M	8599	CA	-	-	-	-	-
SOST TG 6 Month WT	L	M	8599	L	-	-	-	-	-
SOST TG 6 Month WT	L	M	8599	M	1755.0498	1637.5245	43.2942	0.8753	33.9972
SOST TG 6 Month WT	L	M	8599	CR	1671.4218	1531.7899	42.6664	0.9779	30.0482

SOST TG 6 Month WT	R	M	8599	L	1571.1919	1422.8379	52.8795	1.3821	37.1138
SOST TG 6 Month WT	R	M	8599	CA	1547.3398	1433.9914	40.6374	1.0589	37.7054
SOST TG 6 Month WT	R	M	8599	CR	1534.1797	1400.9429	42.6038	1.1621	34.2201
SOST TG 6 Month WT	R	M	8599	M	1451.5820	1319.3620	49.1702	1.4878	41.5647
SOST TG 8 Month DEF	L	F	8808	CA	1733.3864	1576.0663	34.1570	0.7352	20.4913
SOST TG 8 Month DEF	L	F	8808	L	1728.9747	1571.5596	37.8792	0.8199	22.7555
SOST TG 8 Month DEF	R	F	8808	CA	1712.4872	1577.0860	44.4968	0.9594	31.0473
SOST TG 8 Month DEF	L	F	8808	CR	1701.2361	1535.1161	37.8642	0.8562	22.0532
SOST TG 8 Month DEF	L	F	8808	M	1687.6374	1512.6903	31.6359	0.7364	17.8356
SOST TG 8 Month DEF	R	F	8808	L	1668.6393	1519.9606	54.6367	1.2641	35.9944
SOST TG 8 Month DEF	R	F	8808	CR	1647.3538	1502.6077	51.2090	1.2283	35.3400
SOST TG 8 Month DEF	R	F	8808	M	1637.0410	1487.3631	54.9520	1.3225	36.6852
SOST TG 8 Month DEF	R	F	8807	CA	-	-	-	-	-
SOST TG 8 Month DEF	R	F	8807	L	-	-	-	-	-
SOST TG 8 Month DEF	R	F	8807	M	-	-	-	-	-
SOST TG 8 Month DEF	R	F	8807	CR	1708.1144	1540.1771	57.4851	1.2982	33.1403
SOST TG 8 Month DEF	L	F	8807	CR	1617.9204	1456.5179	30.2307	0.7554	19.0145
SOST TG 8 Month DEF	L	F	8807	M	1617.0133	1435.5894	31.3206	0.8128	17.7372
SOST TG 8 Month DEF	L	F	8807	L	1591.7164	1421.8836	35.5591	0.9325	21.7661
SOST TG 8 Month DEF	L	F	8807	CA	1577.0643	1387.1759	33.2809	0.9120	18.6459
SOST TG 8 Month DEF	L	F	5589	CA	-	-	-	-	-
SOST TG 8 Month DEF	R	F	5589	CA	1835.6344	1715.7969	44.0338	0.8074	32.0325
SOST TG 8 Month DEF	R	F	5589	L	1731.6407	1582.3567	54.6041	1.1757	34.7896
SOST TG 8 Month DEF	R	F	5589	CR	1687.6061	1555.2163	43.4027	0.9560	31.5857
SOST TG 8 Month DEF	L	F	5589	M	1649.7299	1455.0009	40.7309	1.0196	21.2677
SOST TG 8 Month DEF	L	F	5589	CR	1633.6106	1474.7826	49.9289	1.2484	32.6748
SOST TG 8 Month DEF	L	F	5589	L	1592.3023	1384.8415	35.7072	0.9962	18.5659
SOST TG 8 Month DEF	R	F	5589	M	1552.7652	1415.1605	50.4760	1.3461	39.0625
SOST TG 8 Month DEF	R	F	5588	M	1706.7757	1552.5425	45.3647	1.0106	28.3864
SOST TG 8 Month DEF	L	F	5588	CA	1677.9727	1470.7040	35.5217	0.8730	17.2346
SOST TG 8 Month DEF	R	F	5588	CR	1671.6063	1546.1665	40.0553	0.8935	30.8257
SOST TG 8 Month DEF	L	F	5588	CR	1669.3365	1470.1730	38.7435	0.9514	19.5925
SOST TG 8 Month DEF	L	F	5588	M	1664.7068	1473.9576	38.7269	0.9469	20.3992
SOST TG 8 Month DEF	L	F	5588	L	1651.9839	1438.8609	42.9700	1.0993	20.7411
SOST TG 8 Month DEF	R	F	5588	CA	1637.0892	1479.4261	48.3771	1.1888	31.4003
SOST TG 8 Month DEF	R	F	5588	L	1612.2733	1467.2087	54.4344	1.3431	37.9299
SOST TG 8 Month TG	R	F	5599	L	1935.5478	1770.1792	61.8295	1.0741	32.0798
SOST TG 8 Month TG	R	F	5599	CR	1889.9383	1738.1245	63.8661	1.1422	36.2343
SOST TG 8 Month TG	R	F	5599	CA	1880.2778	1732.6345	54.1063	0.9757	31.9270
SOST TG 8 Month TG	R	F	5599	M	1769.4795	1620.6599	54.1009	1.1063	33.4102

SOST TG 8 Month TG	L	F	5599	CR	1588.0665	1393.7305	42.7683	1.1631	23.3295
SOST TG 8 Month TG	L	F	5599	M	1555.6919	1381.5818	44.2986	1.2245	27.2096
SOST TG 8 Month TG	L	F	5599	L	1528.0722	1360.1303	34.5700	0.9836	22.3910
SOST TG 8 Month TG	L	F	5599	CA	1517.1827	1335.5173	46.3645	1.3643	28.1022
SOST TG 8 Month TG	L	F	5592	L	-	-	-	-	-
SOST TG 8 Month TG	R	F	5592	L	1783.3010	1661.2815	43.6862	0.8516	32.2989
SOST TG 8 Month TG	R	F	5592	M	1750.0135	1588.8968	55.9608	1.1928	32.7325
SOST TG 8 Month TG	R	F	5592	CR	1721.2482	1583.3288	47.8423	1.0298	32.7396
SOST TG 8 Month TG	R	F	5592	CA	1702.8635	1559.6243	42.7279	0.9437	28.4965
SOST TG 8 Month TG	L	F	5592	CR	1625.3040	1438.0655	30.8870	0.7931	16.9763
SOST TG 8 Month TG	L	F	5592	M	1594.3356	1388.6840	33.7752	0.9319	17.5277
SOST TG 8 Month TG	L	F	5592	CA	1563.0958	1372.4739	39.2527	1.0978	22.1443
SOST TG 8 Month WT	L	F	5598	CR	-	-	-	-	-
SOST TG 8 Month WT	R	F	5598	CR	-	-	-	-	-
SOST TG 8 Month WT	R	F	5598	M	-	-	-	-	-
SOST TG 8 Month WT	L	F	5598	L	1825.2574	1641.0177	52.7729	1.0546	26.0931
SOST TG 8 Month WT	L	F	5598	M	1814.9461	1627.3506	47.1347	0.9637	23.1555
SOST TG 8 Month WT	L	F	5598	CA	1747.0489	1573.5451	52.9089	1.1458	28.8591
SOST TG 8 Month WT	R	F	5598	CA	1744.8216	1593.0765	40.3102	0.8516	24.7842
SOST TG 8 Month WT	R	F	5598	L	1684.0681	1518.4219	48.3727	1.1225	28.5963
SOST TG 8 Month WT	R	F	5597	CA	-	-	-	-	-
SOST TG 8 Month WT	R	F	5597	CR	-	-	-	-	-
SOST TG 8 Month WT	R	F	5597	L	-	-	-	-	-
SOST TG 8 Month WT	R	F	5597	M	-	-	-	-	-
SOST TG 8 Month WT	L	F	5597	M	1725.4077	1591.9165	56.0046	1.1951	39.4621
SOST TG 8 Month WT	L	F	5597	CR	1678.3826	1537.1107	53.0504	1.2082	36.9143
SOST TG 8 Month WT	L	F	5597	L	1601.0887	1460.0054	44.1495	1.0918	31.9442
SOST TG 8 Month WT	L	F	5597	CA	1590.7970	1431.0346	50.1281	1.2968	32.5684
SOST TG 8 Month WT	L	F	5593	CR	-	-	-	-	-
SOST TG 8 Month WT	L	F	5593	L	-	-	-	-	-
SOST TG 8 Month WT	R	F	5593	CA	1808.2273	1656.2082	35.0102	0.6869	20.8258
SOST TG 8 Month WT	L	F	5593	CA	1724.9959	1583.3888	49.5230	1.0593	32.9241
SOST TG 8 Month WT	R	F	5593	L	1723.9744	1573.3538	41.5865	0.9030	26.2718
SOST TG 8 Month WT	R	F	5593	CR	1722.6154	1572.5582	39.2784	0.8599	24.7620
SOST TG 8 Month WT	L	F	5593	M	1691.2885	1541.1557	52.5923	1.1857	33.8956
SOST TG 8 Month WT	R	F	5593	M	1671.0699	1497.0231	45.4131	1.0988	26.1535
SOST TG 8 Month WT	R	F	5590	CA	1729.6278	1568.6400	36.9978	0.8113	21.9379
SOST TG 8 Month WT	R	F	5590	M	1719.3005	1552.7814	46.1645	1.0229	26.5442
SOST TG 8 Month WT	L	F	5590	CA	1681.9512	1536.1365	43.7280	1.0044	29.4812
SOST TG 8 Month WT	R	F	5590	CR	1681.0196	1528.0728	42.3868	0.9687	26.9243

SOST TG 8 Month WT	R	F	5590	L	1664.1632	1499.6896	41.7271	0.9979	25.1858
SOST TG 8 Month WT	L	F	5590	L	1615.0289	1468.9273	46.4319	1.1559	32.3116
SOST TG 8 Month WT	L	F	5590	CR	1558.8297	1384.0338	54.3740	1.5151	33.4471
SOST TG 8 Month WT	L	F	5590	M	1525.8872	1343.7271	52.7848	1.5540	32.1435

Appendix D: Minitab Statistical Outputs

********MAX LOAD:**

General Linear Model: Max Load (mN versus Genotype+Phe, Limb, Region

Factor	Type	Levels	Values
Genotype+Phenotype	fixed	10	SOST KO 12 Month KO, SOST KO 12 Month WT, SOST KO 6 Month KO, SOST KO 6 Month WT, SOST TG 6 Month DEF, SOST TG 6 Month TG, SOST TG 6 Month WT, SOST TG 8 Month DEF, SOST TG 8 Month TG, SOST TG 8 Month WT
Limb	fixed	2	L, R
Region	fixed	4	CA, CR, L, M

Analysis of Variance for Max Load (mN), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	6399.36	6254.68	694.96	11.55	0.000
Limb	1	481.69	114.79	114.79	1.91	0.168
Region	3	93.61	58.36	19.45	0.32	0.808
Genotype+Phenotype*Limb	9	7330.95	7191.45	799.05	13.29	0.000
Genotype+Phenotype*Region	27	956.93	1018.16	37.71	0.63	0.927
Limb*Region	3	113.81	137.67	45.89	0.76	0.516
Genotype+Phenotype*Limb*Region	27	718.60	718.60	26.61	0.44	0.993
Error	261	15698.27	15698.27	60.15		
Total	340	31793.24				

S = 7.75543   R-Sq = 50.62%   R-Sq(adj) = 35.68%

Unusual Observations for Max Load (mN)

Obs	Max Load (mN)	Fit	SE Fit	Residual	St Resid	
31	34.5700	34.5700	7.7554	0.0000	*	X
48	56.7652	76.0907	3.8777	-19.3255	-2.88	R
263	41.7219	58.9860	3.1661	-17.2641	-2.44	R
288	37.7153	57.9147	2.3383	-20.1994	-2.73	R
300	77.1457	57.9147	2.3383	19.2310	2.60	R
351	30.4129	45.2304	3.4683	-14.8175	-2.14	R
369	73.7376	55.0712	2.9313	18.6664	2.60	R
371	89.3986	58.9860	3.1661	30.4125	4.30	R
372	81.3542	55.1405	3.1661	26.2137	3.70	R
382	69.3750	54.9262	2.9313	14.4488	2.01	R
390	72.1869	56.7676	2.9313	15.4192	2.15	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	N	Mean	Grouping
SOST KO 12 Month KO	34	61.1	A
SOST KO 12 Month WT	16	53.7	A B
SOST TG 6 Month TG	16	53.3	B
SOST TG 6 Month WT	77	52.4	B
SOST KO 6 Month WT	36	51.9	B
SOST KO 6 Month KO	36	51.7	B

SOST TG 6 Month DEF	60	50.8	B
SOST TG 8 Month WT	23	46.3	B C
SOST TG 8 Month TG	15	45.7	B C
SOST TG 8 Month DEF	28	43.2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
R	162	51.7	A
L	179	50.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
M	86	51.5	A
L	86	51.3	A
CA	85	51.0	A
CR	84	50.2	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	69.2	A
SOST TG 6 Month DEF	R	26	56.5	B
SOST TG 6 Month WT	R	37	56.2	B
SOST KO 12 Month WT	L	8	55.9	B C
SOST TG 6 Month TG	R	8	54.2	B C D
SOST KO 6 Month WT	L	21	53.6	B C D
SOST TG 8 Month TG	R	8	53.0	B C D
SOST KO 12 Month KO	R	17	52.9	B C D
SOST KO 6 Month KO	L	16	52.7	B C D
SOST TG 6 Month TG	L	8	52.4	B C D E
SOST KO 12 Month WT	R	8	51.5	B C D E
SOST KO 6 Month KO	R	20	50.7	B C D E
SOST TG 8 Month WT	L	13	50.7	B C D E
SOST KO 6 Month WT	R	15	50.2	B C D E
SOST TG 8 Month DEF	R	13	49.6	B C D E
SOST TG 6 Month WT	L	40	48.7	C D E
SOST TG 6 Month DEF	L	34	45.2	C D E F
SOST TG 8 Month WT	R	10	42.0	D E F
SOST TG 8 Month TG	L	7	38.3	E F
SOST TG 8 Month DEF	L	15	36.8	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	CA	8	63.5	A
SOST KO 12 Month KO	M	8	63.4	A
SOST KO 12 Month KO	L	9	60.5	A B

SOST KO 12 Month WT	L	4	58.8	A B C
SOST KO 12 Month KO	CR	9	57.0	A B C
SOST KO 6 Month WT	CA	8	56.0	A B C
SOST TG 6 Month TG	CA	4	55.1	A B C
SOST TG 6 Month TG	M	4	54.5	A B C
SOST TG 6 Month WT	L	20	54.2	A B C
SOST KO 12 Month WT	CR	4	53.6	A B C
SOST TG 6 Month TG	L	4	53.2	A B C
SOST KO 6 Month WT	M	8	52.8	A B C
SOST TG 6 Month WT	M	21	52.4	A B C
SOST KO 6 Month KO	CA	9	52.2	A B C
SOST KO 12 Month WT	CA	4	52.1	A B C
SOST KO 6 Month KO	M	9	52.1	A B C
SOST TG 6 Month WT	CA	19	51.9	A B C
SOST KO 6 Month KO	L	9	51.5	A B C
SOST TG 6 Month WT	CR	17	51.4	A B C
SOST TG 6 Month DEF	L	14	51.4	A B C
SOST KO 6 Month KO	CR	9	51.1	A B C
SOST TG 6 Month DEF	CR	15	51.1	A B C
SOST KO 6 Month WT	CR	10	50.8	A B C
SOST TG 6 Month DEF	CA	16	50.5	A B C
SOST KO 12 Month WT	M	4	50.4	A B C
SOST TG 6 Month DEF	M	15	50.4	A B C
SOST TG 6 Month TG	CR	4	50.4	A B C
SOST TG 8 Month WT	M	6	49.0	A B C
SOST KO 6 Month WT	L	10	48.0	B C
SOST TG 8 Month WT	CR	4	47.3	A B C
SOST TG 8 Month TG	M	4	47.0	A B C
SOST TG 8 Month TG	CR	4	46.3	A B C
SOST TG 8 Month DEF	L	7	46.3	B C
SOST TG 8 Month WT	L	6	45.8	B C
SOST TG 8 Month TG	CA	4	45.6	A B C
SOST TG 8 Month TG	L	3	43.7	A B C
SOST TG 8 Month DEF	CR	8	43.6	C
SOST TG 8 Month WT	CA	7	43.3	C
SOST TG 8 Month DEF	M	7	42.9	C
SOST TG 8 Month DEF	CA	6	40.0	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	Region	N	Mean	Grouping
R	L	42	53.0	A
R	M	40	52.0	A
L	CA	45	51.4	A
R	CR	40	51.1	A
L	M	46	50.9	A
R	CA	40	50.6	A
L	L	44	49.6	A
L	CR	44	49.4	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	M	4	76.1	A
SOST KO 12 Month KO	L	CA	4	69.9	A B

SOST KO 12 Month KO	L	L	5	67.4	A B C	
SOST KO 12 Month KO	L	CR	4	63.6	A B C D	
SOST KO 12 Month WT	L	L	2	60.9	A B C D E	
SOST TG 6 Month DEF	R	L	6	59.0	A B C D E	
SOST TG 6 Month WT	R	M	11	57.9	A B C D E	
SOST TG 6 Month WT	R	L	10	57.6	A B C D E	
SOST KO 12 Month WT	L	CA	2	57.5	A B C D E	
SOST KO 12 Month KO	R	CA	4	57.1	A B C D E	
SOST TG 6 Month DEF	R	CR	7	56.8	A B C D E	
SOST KO 12 Month WT	R	L	2	56.7	A B C D E	
SOST KO 6 Month WT	L	CA	5	56.5	A B C D E	
SOST TG 8 Month TG	R	CR	2	55.9	A B C D E	
SOST KO 6 Month WT	R	CA	3	55.4	A B C D E	
SOST TG 6 Month TG	R	CA	2	55.2	A B C D E	
SOST TG 6 Month DEF	R	M	6	55.1	A B C D E	
SOST TG 6 Month TG	L	CA	2	55.1	A B C D E	
SOST TG 6 Month DEF	R	CA	7	55.1	B C D E	
SOST TG 8 Month TG	R	M	2	55.0	A B C D E	
SOST TG 6 Month TG	R	L	2	54.9	A B C D E	
SOST TG 6 Month WT	R	CR	7	54.9	B C D E	
SOST TG 6 Month TG	L	M	2	54.8	A B C D E	
SOST KO 6 Month KO	L	M	4	54.6	A B C D E	
SOST TG 8 Month DEF	R	L	3	54.6	A B C D E	
SOST TG 6 Month WT	R	CA	9	54.4	B C D E	
SOST TG 6 Month TG	R	M	2	54.3	A B C D E	
SOST KO 6 Month WT	L	CR	6	53.9	B C D E	
SOST TG 8 Month WT	L	CR	2	53.7	A B C D E	
SOST KO 12 Month WT	L	CR	2	53.6	A B C D E	
SOST KO 12 Month KO	R	L	4	53.6	A B C D E	
SOST KO 6 Month KO	L	CA	4	53.5	A B C D E	
SOST KO 12 Month WT	R	CR	2	53.5	A B C D E	
SOST KO 6 Month WT	L	M	5	53.2	B C D E	
SOST TG 8 Month TG	R	L	2	52.8	A B C D E	
SOST KO 6 Month WT	R	M	3	52.4	A B C D E	
SOST TG 6 Month TG	R	CR	2	52.4	A B C D E	
SOST TG 8 Month WT	L	M	4	52.1	B C D E	
SOST KO 6 Month KO	R	L	5	51.9	B C D E	
SOST KO 12 Month WT	L	M	2	51.6	A B C D E	
SOST KO 6 Month KO	L	CR	4	51.5	B C D E	
SOST TG 6 Month TG	L	L	2	51.5	A B C D E	
SOST KO 6 Month KO	L	L	4	51.1	B C D E	
SOST KO 6 Month KO	R	CA	5	50.9	B C D E	
SOST KO 6 Month WT	L	L	5	50.8	B C D E	
SOST TG 6 Month WT	L	L	10	50.7	B C D E	
SOST KO 6 Month KO	R	CR	5	50.7	B C D E	
SOST KO 12 Month KO	R	M	4	50.6	B C D E	
SOST KO 12 Month KO	R	CR	5	50.3	B C D E	
SOST TG 8 Month DEF	R	M	3	50.3	B C D E	
SOST KO 6 Month KO	R	M	5	49.6	B C D E	
SOST TG 6 Month WT	L	CA	10	49.3	D E	
SOST KO 12 Month WT	R	M	2	49.3	A B C D E	
SOST TG 8 Month WT	L	CA	4	49.1	B C D E	
SOST TG 8 Month TG	R	CA	2	48.4	A B C D E	
SOST TG 6 Month TG	L	CR	2	48.4	A B C D E	
SOST TG 8 Month DEF	R	CR	4	48.0	B C D E	
SOST TG 6 Month WT	L	CR	10	47.8	D E	
SOST TG 8 Month WT	L	L	3	47.8	B C D E	
SOST KO 6 Month WT	R	CR	4	47.7	B C D E	
SOST TG 6 Month WT	L	M	10	46.8	D E	
SOST KO 12 Month WT	R	CA	2	46.7	B C D E	
SOST TG 6 Month DEF	L	CA	9	46.0	D E	

SOST TG 8 Month WT	R	M	2	45.8	B C D E
SOST TG 8 Month DEF	R	CA	3	45.6	B C D E
SOST TG 6 Month DEF	L	M	9	45.6	D E
SOST TG 6 Month DEF	L	CR	8	45.3	D E
SOST KO 6 Month WT	R	L	5	45.2	D E
SOST TG 8 Month WT	R	L	3	43.9	C D E
SOST TG 6 Month DEF	L	L	8	43.7	D E
SOST TG 8 Month TG	L	CA	2	42.8	B C D E
SOST TG 8 Month WT	R	CR	2	40.8	C D E
SOST TG 8 Month DEF	L	CR	4	39.2	E
SOST TG 8 Month TG	L	M	2	39.0	D E
SOST TG 8 Month DEF	L	L	4	38.0	E
SOST TG 8 Month WT	R	CA	3	37.4	E
SOST TG 8 Month TG	L	CR	2	36.8	D E
SOST TG 8 Month DEF	L	M	4	35.6	E
SOST TG 8 Month TG	L	L	1	34.6	B C D E
SOST TG 8 Month DEF	L	CA	3	34.3	E

Means that do not share a letter are significantly different.

Residual Plots for Max Load (mN)

Test for Equal Variances: Max Load (mN) versus Genotype+Phenotype, Limb, Region

95% Bonferroni confidence intervals for standard deviations

Genotype+Phenotype	Limb	Region	N	Lower	StDev	Upper
SOST KO 12 Month KO	L	CA	4	3.72893	9.3081	152.0
SOST KO 12 Month KO	L	CR	4	2.04473	5.1040	83.3
SOST KO 12 Month KO	L	L	5	4.16162	9.5358	84.7
SOST KO 12 Month KO	L	M	4	5.55373	13.8631	226.3
SOST KO 12 Month KO	R	CA	4	2.54105	6.3429	103.6
SOST KO 12 Month KO	R	CR	5	3.93847	9.0245	80.1
SOST KO 12 Month KO	R	L	4	2.59492	6.4774	105.8
SOST KO 12 Month KO	R	M	4	3.54462	8.8480	144.5
SOST KO 12 Month WT	L	CA	2	1.70157	6.1281	15450.9
SOST KO 12 Month WT	L	CR	2	1.40271	5.0518	12737.2
SOST KO 12 Month WT	L	L	2	1.85341	6.6750	16829.7
SOST KO 12 Month WT	L	M	2	2.12746	7.6619	19318.2
SOST KO 12 Month WT	R	CA	2	0.58560	2.1090	5317.5
SOST KO 12 Month WT	R	CR	2	1.46861	5.2891	13335.6
SOST KO 12 Month WT	R	L	2	3.63890	13.1053	33042.6
SOST KO 12 Month WT	R	M	2	4.11141	14.8070	37333.1
SOST KO 6 Month KO	L	CA	4	1.77646	4.4344	72.4
SOST KO 6 Month KO	L	CR	4	0.77685	1.9392	31.7
SOST KO 6 Month KO	L	L	4	1.55535	3.8824	63.4
SOST KO 6 Month KO	L	M	4	0.79505	1.9846	32.4
SOST KO 6 Month KO	R	CA	5	2.69221	6.1689	54.8
SOST KO 6 Month KO	R	CR	5	2.53766	5.8147	51.6
SOST KO 6 Month KO	R	L	5	3.47328	7.9586	70.7
SOST KO 6 Month KO	R	M	5	2.19072	5.0197	44.6
SOST KO 6 Month WT	L	CA	5	2.43813	5.5867	49.6
SOST KO 6 Month WT	L	CR	6	0.77080	1.6585	10.2
SOST KO 6 Month WT	L	L	5	2.67709	6.1342	54.5
SOST KO 6 Month WT	L	M	5	2.98153	6.8318	60.7
SOST KO 6 Month WT	R	CA	3	0.45555	1.2932	72.7
SOST KO 6 Month WT	R	CR	4	2.25089	5.6186	91.7

SOST KO 6 Month WT	R	L	5	3.80057	8.7085	77.3
SOST KO 6 Month WT	R	M	3	1.98354	5.6307	316.5
SOST TG 6 Month DEF	L	CA	9	2.69087	5.1237	18.3
SOST TG 6 Month DEF	L	CR	8	3.89016	7.6578	31.2
SOST TG 6 Month DEF	L	L	8	3.53955	6.9676	28.4
SOST TG 6 Month DEF	L	M	9	4.26215	8.1156	29.0
SOST TG 6 Month DEF	R	CA	7	4.77704	9.7858	47.4
SOST TG 6 Month DEF	R	CR	7	5.01233	10.2678	49.7
SOST TG 6 Month DEF	R	L	6	8.10895	17.4471	107.7
SOST TG 6 Month DEF	R	M	6	6.13417	13.1982	81.5
SOST TG 6 Month TG	L	CA	2	0.61769	2.2246	5608.9
SOST TG 6 Month TG	L	CR	2	0.51371	1.8501	4664.7
SOST TG 6 Month TG	L	L	2	2.10286	7.5733	19094.8
SOST TG 6 Month TG	L	M	2	0.12032	0.4333	1092.6
SOST TG 6 Month TG	R	CA	2	1.34428	4.8414	12206.6
SOST TG 6 Month TG	R	CR	2	2.25050	8.1050	20435.4
SOST TG 6 Month TG	R	L	2	0.66613	2.3990	6048.7
SOST TG 6 Month TG	R	M	2	0.59514	2.1434	5404.1
SOST TG 6 Month WT	L	CA	10	2.78250	5.1500	16.6
SOST TG 6 Month WT	L	CR	10	3.03805	5.6230	18.1
SOST TG 6 Month WT	L	L	10	4.20004	7.7737	25.0
SOST TG 6 Month WT	L	M	10	2.63902	4.8844	15.7
SOST TG 6 Month WT	R	CA	9	5.06973	9.6533	34.4
SOST TG 6 Month WT	R	CR	7	5.45881	11.1824	54.2
SOST TG 6 Month WT	R	L	10	5.06549	9.3755	30.2
SOST TG 6 Month WT	R	M	11	6.21002	11.2145	33.2
SOST TG 8 Month DEF	L	CA	3	0.39780	1.1292	63.5
SOST TG 8 Month DEF	L	CR	4	3.25090	8.1148	132.5
SOST TG 8 Month DEF	L	L	4	1.38634	3.4606	56.5
SOST TG 8 Month DEF	L	M	4	1.93695	4.8350	78.9
SOST TG 8 Month DEF	R	CA	3	0.84025	2.3852	134.1
SOST TG 8 Month DEF	R	CR	4	3.14180	7.8425	128.0
SOST TG 8 Month DEF	R	L	3	0.03828	0.1087	6.1
SOST TG 8 Month DEF	R	M	3	1.68990	4.7971	269.6
SOST TG 8 Month TG	L	CA	2	1.39632	5.0288	12679.1
SOST TG 8 Month TG	L	CR	2	2.33277	8.4013	21182.4
SOST TG 8 Month TG	L	L	1	*	*	*
SOST TG 8 Month TG	L	M	2	2.06616	7.4412	18761.5
SOST TG 8 Month TG	R	CA	2	2.23403	8.0457	20285.8
SOST TG 8 Month TG	R	CR	2	3.14612	11.3306	28568.0
SOST TG 8 Month TG	R	L	2	3.56225	12.8292	32346.6
SOST TG 8 Month TG	R	M	2	0.36517	1.3151	3315.9
SOST TG 8 Month WT	L	CA	4	1.54464	3.8557	63.0
SOST TG 8 Month WT	L	CR	2	0.25988	0.9360	2359.8
SOST TG 8 Month WT	L	L	3	1.57396	4.4680	251.1
SOST TG 8 Month WT	L	M	4	1.47390	3.6791	60.1
SOST TG 8 Month WT	R	CA	3	0.94319	2.6774	150.5
SOST TG 8 Month WT	R	CR	2	0.61030	2.1980	5541.8
SOST TG 8 Month WT	R	L	3	1.36612	3.8780	218.0
SOST TG 8 Month WT	R	M	2	0.14754	0.5314	1339.8

Bartlett's Test (Normal Distribution)
Test statistic = 119.10, p-value = 0.002

Levene's Test (Any Continuous Distribution)
Test statistic = 1.23, p-value = 0.122

Test for Equal Variances: Max Load (mN) versus Genotype+Phenotype, Limb, Region

*****Outliers removed: 9294R/CR, 9294R/L

General Linear Model: Max Load (mN versus Genotype+Phe, Limb, Region

Factor	Type	Levels	Values
Genotype+Phenotype	fixed	10	SOST KO 12 Month KO, SOST KO 12 Month WT, SOST KO 6 Month KO, SOST KO 6 Month WT, SOST TG 6 Month DEF, SOST TG 6 Month TG, SOST TG 6 Month WT, SOST TG 8 Month DEF, SOST TG 8 Month TG, SOST TG 8 Month WT
Limb	fixed	2	L, R
Region	fixed	4	CA, CR, L, M

Analysis of Variance for Max Load (mN)_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	6600.54	6388.84	709.87	12.80	0.000
Limb	1	331.46	81.72	81.72	1.47	0.226
Region	3	114.52	61.80	20.60	0.37	0.774
Genotype+Phenotype*Limb	9	6820.65	6667.28	740.81	13.36	0.000
Genotype+Phenotype*Region	27	986.26	1039.06	38.48	0.69	0.872
Limb*Region	3	56.98	100.48	33.49	0.60	0.613
Genotype+Phenotype*Limb*Region	27	691.32	691.32	25.60	0.46	0.991
Error	259	14366.86	14366.86	55.47		
Total	338	29968.59				

S = 7.44785   R-Sq = 52.06%   R-Sq(adj) = 37.44%

Unusual Observations for Max Load (mN)_1

Obs	Max Load (mN)_1	Fit	SE Fit	Residual	St Resid	
31	34.5700	34.5700	7.4479	-0.0000	*	X
48	56.7652	76.0907	3.7239	-19.3255	-3.00	R
51	81.1935	67.3517	3.3308	13.8418	2.08	R
87	36.2338	50.7207	2.3552	-14.4869	-2.05	R
234	63.9092	50.3142	3.3308	13.5951	2.04	R
277	68.6854	54.4329	2.4826	14.2525	2.03	R
288	37.7153	57.9147	2.2456	-20.1994	-2.84	R
300	77.1457	57.9147	2.2456	19.2310	2.71	R
351	30.4129	45.2304	3.3308	-14.8175	-2.22	R
369	73.7376	55.0712	2.8150	18.6664	2.71	R
372	81.3542	55.1405	3.0406	26.2137	3.86	R
382	69.3750	54.9262	2.8150	14.4488	2.10	R
390	72.1869	54.4711	3.0406	17.7157	2.61	R
391	67.5864	52.9035	3.3308	14.6828	2.20	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype   N Mean Grouping

SOST KO 12 Month KO	34	61.1	A
SOST KO 12 Month WT	16	53.7	B
SOST TG 6 Month TG	16	53.3	B
SOST TG 6 Month WT	77	52.4	B
SOST KO 6 Month WT	36	51.9	B
SOST KO 6 Month KO	36	51.7	B
SOST TG 6 Month DEF	58	49.8	B
SOST TG 8 Month WT	23	46.3	B C
SOST TG 8 Month TG	15	45.7	B C
SOST TG 8 Month DEF	28	43.2	C

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
R	160	51.5	A
L	179	50.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
M	86	51.5	A
L	85	51.0	A
CA	85	51.0	A
CR	83	50.1	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	69.2	A
SOST TG 6 Month WT	R	37	56.2	B
SOST KO 12 Month WT	L	8	55.9	B C
SOST TG 6 Month DEF	R	24	54.4	B C
SOST TG 6 Month TG	R	8	54.2	B C D
SOST KO 6 Month WT	L	21	53.6	B C D
SOST TG 8 Month TG	R	8	53.0	B C D
SOST KO 12 Month KO	R	17	52.9	B C D
SOST KO 6 Month KO	L	16	52.7	B C D
SOST TG 6 Month TG	L	8	52.4	B C D
SOST KO 12 Month WT	R	8	51.5	B C D E
SOST KO 6 Month KO	R	20	50.7	B C D E
SOST TG 8 Month WT	L	13	50.7	B C D E
SOST KO 6 Month WT	R	15	50.2	B C D E
SOST TG 8 Month DEF	R	13	49.6	B C D E
SOST TG 6 Month WT	L	40	48.7	C D E
SOST TG 6 Month DEF	L	34	45.2	D E
SOST TG 8 Month WT	R	10	42.0	D E F
SOST TG 8 Month TG	L	7	38.3	E F
SOST TG 8 Month DEF	L	15	36.8	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	CA	8	63.5	A
SOST KO 12 Month KO	M	8	63.4	A
SOST KO 12 Month KO	L	9	60.5	A B
SOST KO 12 Month WT	L	4	58.8	A B C
SOST KO 12 Month KO	CR	9	57.0	A B C
SOST KO 6 Month WT	CA	8	56.0	A B C
SOST TG 6 Month TG	CA	4	55.1	A B C D
SOST TG 6 Month TG	M	4	54.5	A B C D
SOST TG 6 Month WT	L	20	54.2	A B C D
SOST KO 12 Month WT	CR	4	53.6	A B C D
SOST TG 6 Month TG	L	4	53.2	A B C D
SOST KO 6 Month WT	M	8	52.8	A B C D
SOST TG 6 Month WT	M	21	52.4	A B C D
SOST KO 6 Month KO	CA	9	52.2	A B C D
SOST KO 12 Month WT	CA	4	52.1	A B C D
SOST KO 6 Month KO	M	9	52.1	A B C D
SOST TG 6 Month WT	CA	19	51.9	A B C D
SOST KO 6 Month KO	L	9	51.5	A B C D
SOST TG 6 Month WT	CR	17	51.4	A B C D
SOST KO 6 Month KO	CR	9	51.1	A B C D
SOST KO 6 Month WT	CR	10	50.8	A B C D
SOST TG 6 Month DEF	CA	16	50.5	B C D
SOST KO 12 Month WT	M	4	50.4	A B C D
SOST TG 6 Month DEF	M	15	50.4	B C D
SOST TG 6 Month TG	CR	4	50.4	A B C D
SOST TG 6 Month DEF	CR	14	49.9	B C D
SOST TG 8 Month WT	M	6	49.0	A B C D
SOST TG 6 Month DEF	L	13	48.3	B C D
SOST KO 6 Month WT	L	10	48.0	B C D
SOST TG 8 Month WT	CR	4	47.3	A B C D
SOST TG 8 Month TG	M	4	47.0	A B C D
SOST TG 8 Month TG	CR	4	46.3	A B C D
SOST TG 8 Month DEF	L	7	46.3	B C D
SOST TG 8 Month WT	L	6	45.8	B C D
SOST TG 8 Month TG	CA	4	45.6	B C D
SOST TG 8 Month TG	L	3	43.7	A B C D
SOST TG 8 Month DEF	CR	8	43.6	C D
SOST TG 8 Month WT	CA	7	43.3	C D
SOST TG 8 Month DEF	M	7	42.9	C D
SOST TG 8 Month DEF	CA	6	40.0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	Region	N	Mean	Grouping
R	L	41	52.4	A
R	M	40	52.0	A
L	CA	45	51.4	A
L	M	46	50.9	A
R	CR	39	50.9	A
R	CA	40	50.6	A
L	L	44	49.6	A
L	CR	44	49.4	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	M	4	76.1	A
SOST KO 12 Month KO	L	CA	4	69.9	A B
SOST KO 12 Month KO	L	L	5	67.4	A B C
SOST KO 12 Month KO	L	CR	4	63.6	A B C D
SOST KO 12 Month WT	L	L	2	60.9	A B C D E
SOST TG 6 Month WT	R	M	11	57.9	A B C D E
SOST TG 6 Month WT	R	L	10	57.6	A B C D E
SOST KO 12 Month WT	L	CA	2	57.5	A B C D E
SOST KO 12 Month KO	R	CA	4	57.1	A B C D E
SOST KO 12 Month WT	R	L	2	56.7	A B C D E
SOST KO 6 Month WT	L	CA	5	56.5	A B C D E
SOST TG 8 Month TG	R	CR	2	55.9	A B C D E
SOST KO 6 Month WT	R	CA	3	55.4	A B C D E
SOST TG 6 Month TG	R	CA	2	55.2	A B C D E
SOST TG 6 Month DEF	R	M	6	55.1	B C D E
SOST TG 6 Month TG	L	CA	2	55.1	A B C D E
SOST TG 6 Month DEF	R	CA	7	55.1	B C D E
SOST TG 8 Month TG	R	M	2	55.0	A B C D E
SOST TG 6 Month TG	R	L	2	54.9	A B C D E
SOST TG 6 Month WT	R	CR	7	54.9	B C D E
SOST TG 6 Month TG	L	M	2	54.8	A B C D E
SOST KO 6 Month KO	L	M	4	54.6	A B C D E
SOST TG 8 Month DEF	R	L	3	54.6	A B C D E
SOST TG 6 Month DEF	R	CR	6	54.5	B C D E
SOST TG 6 Month WT	R	CA	9	54.4	B C D E
SOST TG 6 Month TG	R	M	2	54.3	A B C D E
SOST KO 6 Month WT	L	CR	6	53.9	B C D E
SOST TG 8 Month WT	L	CR	2	53.7	A B C D E
SOST KO 12 Month WT	L	CR	2	53.6	A B C D E
SOST KO 12 Month KO	R	L	4	53.6	B C D E
SOST KO 6 Month KO	L	CA	4	53.5	B C D E
SOST KO 12 Month WT	R	CR	2	53.5	A B C D E
SOST KO 6 Month WT	L	M	5	53.2	B C D E
SOST TG 6 Month DEF	R	L	5	52.9	B C D E
SOST TG 8 Month TG	R	L	2	52.8	A B C D E
SOST KO 6 Month WT	R	M	3	52.4	A B C D E
SOST TG 6 Month TG	R	CR	2	52.4	A B C D E
SOST TG 8 Month WT	L	M	4	52.1	B C D E
SOST KO 6 Month KO	R	L	5	51.9	B C D E
SOST KO 12 Month WT	L	M	2	51.6	A B C D E
SOST KO 6 Month KO	L	CR	4	51.5	B C D E
SOST TG 6 Month TG	L	L	2	51.5	A B C D E
SOST KO 6 Month KO	L	L	4	51.1	B C D E
SOST KO 6 Month KO	R	CA	5	50.9	B C D E
SOST KO 6 Month WT	L	L	5	50.8	B C D E
SOST TG 6 Month WT	L	L	10	50.7	C D E
SOST KO 6 Month KO	R	CR	5	50.7	B C D E
SOST KO 12 Month KO	R	M	4	50.6	B C D E
SOST KO 12 Month KO	R	CR	5	50.3	B C D E
SOST TG 8 Month DEF	R	M	3	50.3	B C D E
SOST KO 6 Month KO	R	M	5	49.6	B C D E
SOST TG 6 Month WT	L	CA	10	49.3	D E
SOST KO 12 Month WT	R	M	2	49.3	A B C D E
SOST TG 8 Month WT	L	CA	4	49.1	B C D E
SOST TG 8 Month TG	R	CA	2	48.4	B C D E
SOST TG 6 Month TG	L	CR	2	48.4	B C D E
SOST TG 8 Month DEF	R	CR	4	48.0	B C D E

SOST TG 6 Month WT	L	CR	10	47.8		D E
SOST TG 8 Month WT	L	L	3	47.8		B C D E
SOST KO 6 Month WT	R	CR	4	47.7		C D E
SOST TG 6 Month WT	L	M	10	46.8		D E
SOST KO 12 Month WT	R	CA	2	46.7		B C D E
SOST TG 6 Month DEF	L	CA	9	46.0		D E
SOST TG 8 Month WT	R	M	2	45.8		B C D E
SOST TG 8 Month DEF	R	CA	3	45.6		C D E
SOST TG 6 Month DEF	L	M	9	45.6		D E
SOST TG 6 Month DEF	L	CR	8	45.3		D E
SOST KO 6 Month WT	R	L	5	45.2		D E
SOST TG 8 Month WT	R	L	3	43.9		D E
SOST TG 6 Month DEF	L	L	8	43.7		E
SOST TG 8 Month TG	L	CA	2	42.8		B C D E
SOST TG 8 Month WT	R	CR	2	40.8		D E
SOST TG 8 Month DEF	L	CR	4	39.2		E
SOST TG 8 Month TG	L	M	2	39.0		D E
SOST TG 8 Month DEF	L	L	4	38.0		E
SOST TG 8 Month WT	R	CA	3	37.4		E
SOST TG 8 Month TG	L	CR	2	36.8		D E
SOST TG 8 Month DEF	L	M	4	35.6		E
SOST TG 8 Month TG	L	L	1	34.6		C D E
SOST TG 8 Month DEF	L	CA	3	34.3		E

Means that do not share a letter are significantly different.

Residual Plots for Max Load (mN)_1

Test for Equal Variances: Max Load (mN)_1 versus Genotype+Phenoty, Limb, Region

95% Bonferroni confidence intervals for standard deviations

Genotype+Phenotype	Limb	Region	N	Lower	StDev	Upper
SOST KO 12 Month KO	L	CA	4	3.72893	9.3081	152.0
SOST KO 12 Month KO	L	CR	4	2.04473	5.1040	83.3
SOST KO 12 Month KO	L	L	5	4.16162	9.5358	84.7
SOST KO 12 Month KO	L	M	4	5.55373	13.8631	226.3
SOST KO 12 Month KO	R	CA	4	2.54105	6.3429	103.6
SOST KO 12 Month KO	R	CR	5	3.93847	9.0245	80.1
SOST KO 12 Month KO	R	L	4	2.59492	6.4774	105.8
SOST KO 12 Month KO	R	M	4	3.54462	8.8480	144.5
SOST KO 12 Month WT	L	CA	2	1.70157	6.1281	15450.9
SOST KO 12 Month WT	L	CR	2	1.40271	5.0518	12737.2
SOST KO 12 Month WT	L	L	2	1.85341	6.6750	16829.7
SOST KO 12 Month WT	L	M	2	2.12746	7.6619	19318.2
SOST KO 12 Month WT	R	CA	2	0.58560	2.1090	5317.5
SOST KO 12 Month WT	R	CR	2	1.46861	5.2891	13335.6
SOST KO 12 Month WT	R	L	2	3.63890	13.1053	33042.6
SOST KO 12 Month WT	R	M	2	4.11141	14.8070	37333.1
SOST KO 6 Month KO	L	CA	4	1.77646	4.4344	72.4
SOST KO 6 Month KO	L	CR	4	0.77685	1.9392	31.7
SOST KO 6 Month KO	L	L	4	1.55535	3.8824	63.4
SOST KO 6 Month KO	L	M	4	0.79505	1.9846	32.4
SOST KO 6 Month KO	R	CA	5	2.69221	6.1689	54.8
SOST KO 6 Month KO	R	CR	5	2.53766	5.8147	51.6
SOST KO 6 Month KO	R	L	5	3.47328	7.9586	70.7
SOST KO 6 Month KO	R	M	5	2.19072	5.0197	44.6

SOST KO 6 Month WT	L	CA	5	2.43813	5.5867	49.6
SOST KO 6 Month WT	L	CR	6	0.77080	1.6585	10.2
SOST KO 6 Month WT	L	L	5	2.67709	6.1342	54.5
SOST KO 6 Month WT	L	M	5	2.98153	6.8318	60.7
SOST KO 6 Month WT	R	CA	3	0.45555	1.2932	72.7
SOST KO 6 Month WT	R	CR	4	2.25089	5.6186	91.7
SOST KO 6 Month WT	R	L	5	3.80057	8.7085	77.3
SOST KO 6 Month WT	R	M	3	1.98354	5.6307	316.5
SOST TG 6 Month DEF	L	CA	9	2.69087	5.1237	18.3
SOST TG 6 Month DEF	L	CR	8	3.89016	7.6578	31.2
SOST TG 6 Month DEF	L	L	8	3.53955	6.9676	28.4
SOST TG 6 Month DEF	L	M	9	4.26215	8.1156	29.0
SOST TG 6 Month DEF	R	CA	7	4.77704	9.7858	47.4
SOST TG 6 Month DEF	R	CR	6	4.21416	9.0671	56.0
SOST TG 6 Month DEF	R	L	5	4.42972	10.1501	90.1
SOST TG 6 Month DEF	R	M	6	6.13417	13.1982	81.5
SOST TG 6 Month TG	L	CA	2	0.61769	2.2246	5608.9
SOST TG 6 Month TG	L	CR	2	0.51371	1.8501	4664.7
SOST TG 6 Month TG	L	L	2	2.10286	7.5733	19094.8
SOST TG 6 Month TG	L	M	2	0.12032	0.4333	1092.6
SOST TG 6 Month TG	R	CA	2	1.34428	4.8414	12206.6
SOST TG 6 Month TG	R	CR	2	2.25050	8.1050	20435.4
SOST TG 6 Month TG	R	L	2	0.66613	2.3990	6048.7
SOST TG 6 Month TG	R	M	2	0.59514	2.1434	5404.1
SOST TG 6 Month WT	L	CA	10	2.78250	5.1500	16.6
SOST TG 6 Month WT	L	CR	10	3.03805	5.6230	18.1
SOST TG 6 Month WT	L	L	10	4.20004	7.7737	25.0
SOST TG 6 Month WT	L	M	10	2.63902	4.8844	15.7
SOST TG 6 Month WT	R	CA	9	5.06973	9.6533	34.4
SOST TG 6 Month WT	R	CR	7	5.45881	11.1824	54.2
SOST TG 6 Month WT	R	L	10	5.06549	9.3755	30.2
SOST TG 6 Month WT	R	M	11	6.21002	11.2145	33.2
SOST TG 8 Month DEF	L	CA	3	0.39780	1.1292	63.5
SOST TG 8 Month DEF	L	CR	4	3.25090	8.1148	132.5
SOST TG 8 Month DEF	L	L	4	1.38634	3.4606	56.5
SOST TG 8 Month DEF	L	M	4	1.93695	4.8350	78.9
SOST TG 8 Month DEF	R	CA	3	0.84025	2.3852	134.1
SOST TG 8 Month DEF	R	CR	4	3.14180	7.8425	128.0
SOST TG 8 Month DEF	R	L	3	0.03828	0.1087	6.1
SOST TG 8 Month DEF	R	M	3	1.68990	4.7971	269.6
SOST TG 8 Month TG	L	CA	2	1.39632	5.0288	12679.1
SOST TG 8 Month TG	L	CR	2	2.33277	8.4013	21182.4
SOST TG 8 Month TG	L	L	1	*	*	*
SOST TG 8 Month TG	L	M	2	2.06616	7.4412	18761.5
SOST TG 8 Month TG	R	CA	2	2.23403	8.0457	20285.8
SOST TG 8 Month TG	R	CR	2	3.14612	11.3306	28568.0
SOST TG 8 Month TG	R	L	2	3.56225	12.8292	32346.6
SOST TG 8 Month TG	R	M	2	0.36517	1.3151	3315.9
SOST TG 8 Month WT	L	CA	4	1.54464	3.8557	63.0
SOST TG 8 Month WT	L	CR	2	0.25988	0.9360	2359.8
SOST TG 8 Month WT	L	L	3	1.57396	4.4680	251.1
SOST TG 8 Month WT	L	M	4	1.47390	3.6791	60.1
SOST TG 8 Month WT	R	CA	3	0.94319	2.6774	150.5
SOST TG 8 Month WT	R	CR	2	0.61030	2.1980	5541.8
SOST TG 8 Month WT	R	L	3	1.36612	3.8780	218.0
SOST TG 8 Month WT	R	M	2	0.14754	0.5314	1339.8

Bartlett's Test (Normal Distribution)
Test statistic = 107.84, p-value = 0.014

Levene's Test (Any Continuous Distribution)
Test statistic = 1.12, p-value = 0.250

Test for Equal Variances: Max Load (mN)_1 versus Genotype+Phenotype, Limb, Region

****HARDNESS:

General Linear Model: Hardness (Gp versus Genotype+Phe, Limb, Region

Factor	Type	Levels	Values
Genotype+Phenotype	fixed	10	SOST KO 12 Month KO, SOST KO 12 Month WT, SOST KO 6 Month KO, SOST KO 6 Month WT, SOST TG 6 Month DEF, SOST TG 6 Month TG, SOST TG 6 Month WT, SOST TG 8 Month DEF, SOST TG 8 Month TG, SOST TG 8 Month WT
Limb	fixed	2	L, R
Region	fixed	4	CA, CR, L, M

Analysis of Variance for Hardness (Gpa), using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	13.12737	12.54053	1.39339	23.49	0.000
Limb	1	0.20470	0.38800	0.38800	6.54	0.011
Region	3	0.03037	0.02250	0.00750	0.13	0.944
Genotype+Phenotype*Limb	9	8.04214	7.83406	0.87045	14.68	0.000
Genotype+Phenotype*Region	27	1.15760	1.24073	0.04595	0.77	0.783
Limb*Region	3	0.10440	0.10656	0.03552	0.60	0.616
Genotype+Phenotype*Limb*Region	27	0.81443	0.81443	0.03016	0.51	0.981
Error	265	15.71659	15.71659	0.05931		
Total	344	39.19759				

S = 0.243532   R-Sq = 59.90%   R-Sq(adj) = 47.95%

Unusual Observations for Hardness (Gpa)

Hardness

Obs	(Gpa)	Fit	SE Fit	Residual	St Resid	
31	0.98364	0.98364	0.24353	-0.00000	*	X
87	0.76719	1.31730	0.07701	-0.55012	-2.38	R
236	1.68593	1.14883	0.12177	0.53710	2.55	R
263	0.86893	1.37243	0.09942	-0.50350	-2.26	R
274	0.90036	1.40309	0.09205	-0.50273	-2.23	R
277	2.01990	1.36427	0.08118	0.65563	2.86	R
288	0.96488	1.50740	0.07343	-0.54252	-2.34	R
306	0.95936	1.44249	0.09205	-0.48312	-2.14	R
351	0.88823	1.32687	0.10891	-0.43864	-2.01	R
371	2.14387	1.37243	0.09942	0.77144	3.47	R
372	1.87111	1.29284	0.09942	0.57827	2.60	R
374	1.93437	1.40309	0.09205	0.53128	2.36	R
382	1.98688	1.44249	0.09205	0.54439	2.41	R
383	1.99948	1.46156	0.07701	0.53792	2.33	R
384	2.12659	1.50740	0.07343	0.61919	2.67	R
389	1.92426	1.29680	0.09205	0.62746	2.78	R
390	1.86966	1.40309	0.09205	0.46657	2.07	R
391	1.85720	1.37243	0.09942	0.48477	2.18	R
395	2.02824	1.46156	0.07701	0.56667	2.45	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	N	Mean	Grouping
SOST KO 12 Month KO	34	1.6	A
SOST TG 6 Month TG	16	1.6	A
SOST KO 12 Month WT	16	1.6	A
SOST KO 6 Month WT	36	1.5	A
SOST KO 6 Month KO	40	1.5	A B
SOST TG 6 Month WT	77	1.4	B C
SOST TG 6 Month DEF	60	1.3	C D
SOST TG 8 Month WT	23	1.1	E
SOST TG 8 Month TG	15	1.1	D E
SOST TG 8 Month DEF	28	1.0	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
L	183	1.4	A
R	162	1.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
CA	86	1.4	A
M	87	1.4	A
L	87	1.4	A
CR	85	1.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	2.1	A
SOST TG 6 Month TG	R	8	1.6	B
SOST KO 6 Month WT	L	21	1.6	B
SOST KO 12 Month WT	R	8	1.6	B C
SOST KO 12 Month WT	L	8	1.6	B C
SOST TG 6 Month TG	L	8	1.6	B C
SOST KO 6 Month KO	L	20	1.5	B C
SOST KO 6 Month KO	R	20	1.5	B C
SOST KO 6 Month WT	R	15	1.5	B C D
SOST TG 6 Month WT	R	37	1.4	B C D
SOST TG 6 Month DEF	R	26	1.3	B C D E
SOST TG 6 Month WT	L	40	1.3	B C D E
SOST TG 6 Month DEF	L	34	1.2	D E F
SOST TG 8 Month WT	L	13	1.2	C D E F G
SOST KO 12 Month KO	R	17	1.2	D E F G
SOST TG 8 Month DEF	R	13	1.1	D E F G
SOST TG 8 Month TG	L	7	1.1	D E F G
SOST TG 8 Month TG	R	8	1.0	E F G
SOST TG 8 Month WT	R	10	0.9	F G
SOST TG 8 Month DEF	L	15	0.9	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	CA	8	1.7	A
SOST KO 12 Month WT	L	4	1.7	A B
SOST KO 12 Month KO	M	8	1.7	A B
SOST TG 6 Month TG	CA	4	1.7	A B C
SOST KO 6 Month WT	CA	8	1.7	A B C
SOST TG 6 Month TG	M	4	1.6	A B C D
SOST KO 12 Month KO	L	9	1.6	A B C D
SOST KO 12 Month WT	CR	4	1.6	A B C D
SOST KO 6 Month KO	CA	10	1.6	A B C D
SOST TG 6 Month TG	L	4	1.6	A B C D E
SOST KO 12 Month WT	CA	4	1.5	A B C D E F
SOST KO 6 Month WT	M	8	1.5	A B C D E F
SOST KO 6 Month WT	CR	10	1.5	A B C D E F
SOST KO 6 Month KO	CR	10	1.5	A B C D E F
SOST TG 6 Month TG	CR	4	1.5	A B C D E F G
SOST KO 6 Month KO	M	10	1.5	A B C D E F G
SOST KO 6 Month KO	L	10	1.5	A B C D E F G
SOST KO 12 Month KO	CR	9	1.5	A B C D E F G
SOST KO 12 Month WT	M	4	1.4	A B C D E F G
SOST KO 6 Month WT	L	10	1.4	A B C D E F G
SOST TG 6 Month WT	L	20	1.4	A B C D E F G
SOST TG 6 Month WT	M	21	1.4	A B C D E F G
SOST TG 6 Month WT	CR	17	1.4	A B C D E F G
SOST TG 6 Month WT	CA	19	1.3	B C D E F G
SOST TG 6 Month DEF	CR	15	1.3	B C D E F G
SOST TG 6 Month DEF	L	14	1.3	B C D E F G
SOST TG 6 Month DEF	M	15	1.3	B C D E F G
SOST TG 6 Month DEF	CA	16	1.3	B C D E F G
SOST TG 8 Month WT	M	6	1.1	B C D E F G
SOST TG 8 Month WT	CR	4	1.1	B C D E F G

SOST TG 8 Month TG	M	4	1.1	B C D E F G
SOST TG 8 Month DEF	L	7	1.1	C D E F G
SOST TG 8 Month TG	CA	4	1.1	B C D E F G
SOST TG 8 Month WT	L	6	1.1	C D E F G
SOST TG 8 Month DEF	M	7	1.1	D E F G
SOST TG 8 Month TG	CR	4	1.0	C D E F G
SOST TG 8 Month DEF	CR	8	1.0	E F G
SOST TG 8 Month TG	L	3	1.0	C D E F G
SOST TG 8 Month WT	CA	7	1.0	F G
SOST TG 8 Month DEF	CA	6	0.9	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	Region	N	Mean	Grouping
L	CA	46	1.4	A
L	M	47	1.4	A
L	L	45	1.4	A
L	CR	45	1.4	A
R	L	42	1.3	A
R	M	40	1.3	A
R	CR	40	1.3	A
R	CA	40	1.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	M	4	2.2	A
SOST KO 12 Month KO	L	L	5	2.2	A
SOST KO 12 Month KO	L	CA	4	2.1	A B
SOST KO 12 Month KO	L	CR	4	1.8	A B C
SOST KO 12 Month WT	R	L	2	1.8	A B C D
SOST KO 12 Month WT	L	L	2	1.7	A B C D
SOST TG 6 Month TG	L	M	2	1.7	A B C D E
SOST KO 12 Month WT	R	CR	2	1.7	A B C D E
SOST TG 6 Month TG	L	CA	2	1.7	A B C D E
SOST KO 6 Month WT	L	CA	5	1.7	A B C D E
SOST TG 6 Month TG	R	CA	2	1.6	A B C D E
SOST KO 6 Month WT	R	CA	3	1.6	A B C D E
SOST TG 6 Month TG	R	L	2	1.6	A B C D E
SOST KO 6 Month KO	L	CA	5	1.6	A B C D E
SOST KO 6 Month WT	L	CR	6	1.6	A B C D E
SOST KO 12 Month WT	L	CA	2	1.6	A B C D E
SOST TG 6 Month TG	R	M	2	1.6	A B C D E
SOST KO 12 Month WT	L	CR	2	1.6	A B C D E
SOST TG 6 Month TG	R	CR	2	1.6	A B C D E
SOST KO 6 Month WT	L	M	5	1.6	A B C D E
SOST KO 6 Month WT	L	L	5	1.5	A B C D E
SOST KO 6 Month KO	L	M	5	1.5	A B C D E
SOST KO 6 Month KO	L	CR	5	1.5	A B C D E
SOST KO 6 Month KO	R	CA	5	1.5	A B C D E
SOST TG 6 Month WT	R	M	11	1.5	C D E
SOST KO 6 Month WT	R	M	3	1.5	A B C D E
SOST KO 6 Month KO	R	L	5	1.5	A B C D E
SOST KO 12 Month WT	R	M	2	1.5	A B C D E
SOST TG 6 Month TG	L	L	2	1.5	A B C D E

SOST KO 6 Month KO	L	L	5	1.5		B C D E
SOST KO 6 Month KO	R	CR	5	1.5		B C D E
SOST TG 6 Month WT	R	L	10	1.5		C D E
SOST TG 6 Month WT	R	CR	7	1.4		C D E
SOST KO 6 Month KO	R	M	5	1.4		C D E
SOST KO 12 Month WT	R	CA	2	1.4		A B C D E
SOST TG 6 Month TG	L	CR	2	1.4		A B C D E
SOST TG 6 Month DEF	R	CR	7	1.4		C D E
SOST KO 12 Month WT	L	M	2	1.4		A B C D E
SOST KO 6 Month WT	R	CR	4	1.4		C D E
SOST TG 6 Month DEF	R	L	6	1.4		C D E
SOST TG 6 Month WT	R	CA	9	1.4		C D E
SOST TG 8 Month WT	L	CR	2	1.4		A B C D E
SOST KO 12 Month KO	R	CA	4	1.3		C D E
SOST KO 6 Month WT	R	L	5	1.3		C D E
SOST TG 6 Month WT	L	L	10	1.3		C D E
SOST TG 6 Month WT	L	CA	10	1.3		C D E
SOST TG 6 Month DEF	R	CA	7	1.3		C D E
SOST TG 6 Month DEF	R	M	6	1.3		C D E
SOST TG 6 Month WT	L	CR	10	1.3		C D E
SOST TG 8 Month DEF	R	L	3	1.3		C D E
SOST TG 6 Month DEF	L	CR	8	1.2		C D E
SOST TG 8 Month TG	L	CA	2	1.2		C D E
SOST TG 8 Month DEF	R	M	3	1.2		C D E
SOST TG 8 Month WT	L	M	4	1.2		C D E
SOST TG 6 Month WT	L	M	10	1.2		C D E
SOST TG 6 Month DEF	L	M	9	1.2		C D E
SOST TG 6 Month DEF	L	CA	9	1.2		C D E
SOST KO 12 Month KO	R	CR	5	1.2		C D E
SOST TG 6 Month DEF	L	L	8	1.2		C D E
SOST TG 8 Month TG	R	M	2	1.1		C D E
SOST KO 12 Month KO	R	M	4	1.1		C D E
SOST TG 8 Month WT	L	CA	4	1.1		C D E
SOST KO 12 Month KO	R	L	4	1.1		C D E
SOST TG 8 Month WT	L	L	3	1.1		C D E
SOST TG 8 Month DEF	R	CR	4	1.1		C D E
SOST TG 8 Month TG	R	CR	2	1.1		C D E
SOST TG 8 Month TG	L	M	2	1.1		C D E
SOST TG 8 Month WT	R	M	2	1.1		C D E
SOST TG 8 Month WT	R	L	3	1.0		C D E
SOST TG 8 Month DEF	R	CA	3	1.0		C D E
SOST TG 8 Month TG	L	L	1	1.0		C D E
SOST TG 8 Month TG	L	CR	2	1.0		C D E
SOST TG 8 Month TG	R	L	2	1.0		C D E
SOST TG 8 Month DEF	L	L	4	1.0		D E
SOST TG 8 Month TG	R	CA	2	1.0		C D E
SOST TG 8 Month DEF	L	CR	4	1.0		D E
SOST TG 8 Month WT	R	CR	2	0.9		C D E
SOST TG 8 Month DEF	L	M	4	0.9		D E
SOST TG 8 Month DEF	L	CA	3	0.8		D E
SOST TG 8 Month WT	R	CA	3	0.8		E

Means that do not share a letter are significantly different.

Test for Equal Variances: Hardness (Gpa) versus Genotype+Phenoty, Limb, Region

95% Bonferroni confidence intervals for standard deviations

Genotype+Phenotype	Limb	Region	N	Lower	StDev	Upper
SOST KO 12 Month KO	L	CA	4	0.089428	0.223229	3.645
SOST KO 12 Month KO	L	CR	4	0.059818	0.149315	2.438
SOST KO 12 Month KO	L	L	5	0.062959	0.144263	1.281
SOST KO 12 Month KO	L	M	4	0.078442	0.195804	3.197
SOST KO 12 Month KO	R	CA	4	0.057521	0.143582	2.344
SOST KO 12 Month KO	R	CR	5	0.108807	0.249318	2.214
SOST KO 12 Month KO	R	L	4	0.048419	0.120863	1.973
SOST KO 12 Month KO	R	M	4	0.144477	0.360640	5.888
SOST KO 12 Month WT	L	CA	2	0.046050	0.165846	418.149
SOST KO 12 Month WT	L	CR	2	0.020651	0.074373	187.518
SOST KO 12 Month WT	L	L	2	0.061439	0.221268	557.886
SOST KO 12 Month WT	L	M	2	0.068503	0.246711	622.037
SOST KO 12 Month WT	R	CA	2	0.039304	0.141550	356.893
SOST KO 12 Month WT	R	CR	2	0.010214	0.036784	92.743
SOST KO 12 Month WT	R	L	2	0.070707	0.254649	642.050
SOST KO 12 Month WT	R	M	2	0.101892	0.366957	925.214
SOST KO 6 Month KO	L	CA	5	0.068757	0.157548	1.399
SOST KO 6 Month KO	L	CR	5	0.046302	0.106094	0.942
SOST KO 6 Month KO	L	L	5	0.060791	0.139295	1.237
SOST KO 6 Month KO	L	M	5	0.056701	0.129922	1.154
SOST KO 6 Month KO	R	CA	5	0.074950	0.171737	1.525
SOST KO 6 Month KO	R	CR	5	0.074653	0.171057	1.519
SOST KO 6 Month KO	R	L	5	0.131889	0.302206	2.683
SOST KO 6 Month KO	R	M	5	0.040991	0.093926	0.834
SOST KO 6 Month WT	L	CA	5	0.078360	0.179551	1.594
SOST KO 6 Month WT	L	CR	6	0.064416	0.138597	0.856
SOST KO 6 Month WT	L	L	5	0.115302	0.264200	2.346
SOST KO 6 Month WT	L	M	5	0.105182	0.241010	2.140
SOST KO 6 Month WT	R	CA	3	0.049386	0.140192	7.880
SOST KO 6 Month WT	R	CR	4	0.093891	0.234368	3.827
SOST KO 6 Month WT	R	L	5	0.125504	0.287575	2.553
SOST KO 6 Month WT	R	M	3	0.056229	0.159620	8.972
SOST TG 6 Month DEF	L	CA	9	0.058102	0.110632	0.395
SOST TG 6 Month DEF	L	CR	8	0.075425	0.148473	0.604
SOST TG 6 Month DEF	L	L	8	0.083790	0.164942	0.671
SOST TG 6 Month DEF	L	M	9	0.055242	0.105186	0.375
SOST TG 6 Month DEF	R	CA	7	0.187441	0.383974	1.860
SOST TG 6 Month DEF	R	CR	7	0.208062	0.426216	2.065
SOST TG 6 Month DEF	R	L	6	0.237979	0.512032	3.161
SOST TG 6 Month DEF	R	M	6	0.187386	0.403177	2.489
SOST TG 6 Month TG	L	CA	2	0.018382	0.066201	166.913
SOST TG 6 Month TG	L	CR	2	0.046649	0.168005	423.593
SOST TG 6 Month TG	L	L	2	0.040734	0.146702	369.881
SOST TG 6 Month TG	L	M	2	0.000136	0.000489	1.232
SOST TG 6 Month TG	R	CA	2	0.013267	0.047781	120.471
SOST TG 6 Month TG	R	CR	2	0.045124	0.162510	409.740
SOST TG 6 Month TG	R	L	2	0.084273	0.303504	765.229
SOST TG 6 Month TG	R	M	2	0.002868	0.010330	26.045
SOST TG 6 Month WT	L	CA	10	0.093930	0.173851	0.559
SOST TG 6 Month WT	L	CR	10	0.142379	0.263523	0.848
SOST TG 6 Month WT	L	L	10	0.142881	0.264453	0.851
SOST TG 6 Month WT	L	M	10	0.120350	0.222751	0.717
SOST TG 6 Month WT	R	CA	9	0.175407	0.333994	1.192
SOST TG 6 Month WT	R	CR	7	0.195302	0.400077	1.938
SOST TG 6 Month WT	R	L	10	0.189213	0.350207	1.127
SOST TG 6 Month WT	R	M	11	0.190400	0.343838	1.017
SOST TG 8 Month DEF	L	CA	3	0.032713	0.092862	5.220
SOST TG 8 Month DEF	L	CR	4	0.085194	0.212659	3.472
SOST TG 8 Month DEF	L	L	4	0.046885	0.117034	1.911
SOST TG 8 Month DEF	L	M	4	0.051248	0.127924	2.089

SOST TG 8 Month DEF	R	CA	3	0.067638	0.192005	10.793
SOST TG 8 Month DEF	R	CR	4	0.079777	0.199137	3.251
SOST TG 8 Month DEF	R	L	3	0.029496	0.083732	4.707
SOST TG 8 Month DEF	R	M	3	0.065950	0.187212	10.523
SOST TG 8 Month TG	L	CA	2	0.052334	0.188477	475.209
SOST TG 8 Month TG	L	CR	2	0.072643	0.261620	659.627
SOST TG 8 Month TG	L	L	1	*	*	*
SOST TG 8 Month TG	L	M	2	0.057456	0.206924	521.720
SOST TG 8 Month TG	R	CA	2	0.006276	0.022602	56.986
SOST TG 8 Month TG	R	CR	2	0.022080	0.079518	200.491
SOST TG 8 Month TG	R	L	2	0.043690	0.157346	396.718
SOST TG 8 Month TG	R	M	2	0.016980	0.061153	154.185
SOST TG 8 Month WT	L	CA	4	0.051090	0.127529	2.082
SOST TG 8 Month WT	L	CR	2	0.060259	0.217019	547.172
SOST TG 8 Month WT	L	L	3	0.018067	0.051288	2.883
SOST TG 8 Month WT	L	M	4	0.097855	0.244262	3.988
SOST TG 8 Month WT	R	CA	3	0.030241	0.085846	4.825
SOST TG 8 Month WT	R	CR	2	0.021355	0.076910	193.915
SOST TG 8 Month WT	R	L	3	0.038793	0.110123	6.190
SOST TG 8 Month WT	R	M	2	0.014906	0.053685	135.356

```
Bartlett's Test (Normal Distribution)
Test statistic = 117.59, p-value = 0.003


Levene's Test (Any Continuous Distribution)
Test statistic = 1.36, p-value = 0.039
```

Test for Equal Variances: Hardness (Gpa) versus Genotype+Phenotype, Limb, Regio

OUTLIERS REMOVED: 9294R/L,8602R/CA

General Linear Model: Hardness (Gp versus Genotype+Phe, Limb, Region

Factor	Type	Levels	Values
Genotype+Phenotype	fixed	10	SOST KO 12 Month KO, SOST KO 12 Month WT, SOST KO 6 Month KO, SOST KO 6 Month WT, SOST TG 6 Month DEF, SOST TG 6 Month TG, SOST TG 6 Month WT, SOST TG 8 Month DEF, SOST TG 8 Month TG, SOST TG 8 Month WT
Limb	fixed	2	L, R
Region	fixed	4	CA, CR, L, M

Analysis of Variance for Hardness (Gpa)_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	13.31455	12.76804	1.41867	25.70	0.000
Limb	1	0.29855	0.44806	0.44806	8.12	0.005
Region	3	0.02191	0.01911	0.00637	0.12	0.951
Genotype+Phenotype*Limb	9	7.73976	7.47065	0.83007	15.04	0.000
Genotype+Phenotype*Region	27	1.28030	1.38367	0.05125	0.93	0.571
Limb*Region	3	0.13861	0.11446	0.03815	0.69	0.558
Genotype+Phenotype*Limb*Region	27	0.85215	0.85215	0.03156	0.57	0.958
Error	263	14.51887	14.51887	0.05520		
Total	342	38.16469				

S = 0.234957   R-Sq = 61.96%   R-Sq(adj) = 50.53%

Unusual Observations for Hardness (Gpa)_1

Obs	Hardness (Gpa)_1	Fit	SE Fit	Residual	St Resid	
31	0.98364	0.98364	0.23496	0.00000	*	X
87	0.76719	1.31730	0.07430	-0.55012	-2.47	R
236	1.68593	1.14883	0.11748	0.53710	2.64	R
274	0.90036	1.40309	0.08881	-0.50273	-2.31	R
278	1.87943	1.44249	0.08881	0.43695	2.01	R
288	0.96488	1.50740	0.07084	-0.54252	-2.42	R
306	0.95936	1.44249	0.08881	-0.48312	-2.22	R
351	0.88823	1.32687	0.10508	-0.43864	-2.09	R
369	1.74190	1.29680	0.08881	0.44511	2.05	R
372	1.87111	1.29284	0.09592	0.57827	2.70	R
374	1.93437	1.40309	0.08881	0.53128	2.44	R
381	1.72892	1.28232	0.08307	0.44660	2.03	R
382	1.98688	1.44249	0.08881	0.54439	2.50	R
383	1.99948	1.46156	0.07430	0.53792	2.41	R
384	2.12659	1.50740	0.07084	0.61919	2.76	R
389	1.92426	1.29680	0.08881	0.62746	2.88	R
390	1.86966	1.40309	0.08881	0.46657	2.14	R
391	1.85720	1.21815	0.10508	0.63906	3.04	R
395	2.02824	1.46156	0.07430	0.56667	2.54	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	N	Mean	Grouping
SOST KO 12 Month KO	34	1.6	A
SOST TG 6 Month TG	16	1.6	A
SOST KO 12 Month WT	16	1.6	A
SOST KO 6 Month WT	36	1.5	A
SOST KO 6 Month KO	40	1.5	A
SOST TG 6 Month WT	76	1.4	B
SOST TG 6 Month DEF	59	1.3	B C
SOST TG 8 Month WT	23	1.1	C D
SOST TG 8 Month TG	15	1.1	C D
SOST TG 8 Month DEF	28	1.0	D

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
L	183	1.4	A
R	160	1.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
M	87	1.4	A
CA	85	1.4	A
L	86	1.4	A
CR	85	1.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	2.1	A
SOST TG 6 Month TG	R	8	1.6	B
SOST KO 6 Month WT	L	21	1.6	B
SOST KO 12 Month WT	R	8	1.6	B
SOST KO 12 Month WT	L	8	1.6	B C
SOST TG 6 Month TG	L	8	1.6	B C
SOST KO 6 Month KO	L	20	1.5	B C
SOST KO 6 Month KO	R	20	1.5	B C
SOST KO 6 Month WT	R	15	1.5	B C D
SOST TG 6 Month WT	R	36	1.4	B C D E
SOST TG 6 Month DEF	R	25	1.3	B C D E F
SOST TG 6 Month WT	L	40	1.3	B C D E F
SOST TG 6 Month DEF	L	34	1.2	D F G
SOST TG 8 Month WT	L	13	1.2	C D E F G H
SOST KO 12 Month KO	R	17	1.2	D E F G H
SOST TG 8 Month DEF	R	13	1.1	F G H
SOST TG 8 Month TG	L	7	1.1	E F G H
SOST TG 8 Month TG	R	8	1.0	F G H
SOST TG 8 Month WT	R	10	0.9	G H
SOST TG 8 Month DEF	L	15	0.9	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	CA	8	1.7	A
SOST KO 12 Month WT	L	4	1.7	A B
SOST KO 12 Month KO	M	8	1.7	A B
SOST TG 6 Month TG	CA	4	1.7	A B C
SOST KO 6 Month WT	CA	8	1.7	A B C
SOST TG 6 Month TG	M	4	1.6	A B C D
SOST KO 12 Month KO	L	9	1.6	A B C D
SOST KO 12 Month WT	CR	4	1.6	A B C D
SOST KO 6 Month KO	CA	10	1.6	A B C D
SOST TG 6 Month TG	L	4	1.6	A B C D E
SOST KO 12 Month WT	CA	4	1.5	A B C D E F
SOST KO 6 Month WT	M	8	1.5	A B C D E F
SOST KO 6 Month WT	CR	10	1.5	A B C D E F
SOST KO 6 Month KO	CR	10	1.5	A B C D E F
SOST TG 6 Month TG	CR	4	1.5	A B C D E F G
SOST KO 6 Month KO	M	10	1.5	A B C D E F G
SOST KO 6 Month KO	L	10	1.5	A B C D E F G
SOST KO 12 Month KO	CR	9	1.5	A B C D E F G
SOST KO 12 Month WT	M	4	1.4	A B C D E F G
SOST KO 6 Month WT	L	10	1.4	A B C D E F G
SOST TG 6 Month WT	L	20	1.4	A B C D E F G
SOST TG 6 Month WT	M	21	1.4	A B C D E F G

SOST TG 6 Month WT	CR	17	1.4	A B C D E F G
SOST TG 6 Month DEF	CR	15	1.3	B C D E F G
SOST TG 6 Month WT	CA	18	1.3	B C D E F G
SOST TG 6 Month DEF	M	15	1.3	B C D E F G
SOST TG 6 Month DEF	CA	16	1.3	B C D E F G
SOST TG 6 Month DEF	L	13	1.2	C D E F G
SOST TG 8 Month WT	M	6	1.1	B C D E F G
SOST TG 8 Month WT	CR	4	1.1	B C D E F G
SOST TG 8 Month TG	M	4	1.1	B C D E F G
SOST TG 8 Month DEF	L	7	1.1	C D E F G
SOST TG 8 Month TG	CA	4	1.1	B C D E F G
SOST TG 8 Month WT	L	6	1.1	D E F G
SOST TG 8 Month DEF	M	7	1.1	E F G
SOST TG 8 Month TG	CR	4	1.0	C D E F G
SOST TG 8 Month DEF	CR	8	1.0	E F G
SOST TG 8 Month TG	L	3	1.0	C D E F G
SOST TG 8 Month WT	CA	7	1.0	F G
SOST TG 8 Month DEF	CA	6	0.9	G

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	Region	N	Mean	Grouping
L	CA	46	1.4	A
L	M	47	1.4	A
L	L	45	1.4	A
L	CR	45	1.4	A
R	M	40	1.3	A
R	L	41	1.3	A
R	CR	40	1.3	A
R	CA	39	1.3	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	M	4	2.2	A
SOST KO 12 Month KO	L	L	5	2.2	A
SOST KO 12 Month KO	L	CA	4	2.1	A B
SOST KO 12 Month KO	L	CR	4	1.8	A B C
SOST KO 12 Month WT	R	L	2	1.8	A B C D
SOST KO 12 Month WT	L	L	2	1.7	A B C D E
SOST TG 6 Month TG	L	M	2	1.7	A B C D E
SOST KO 12 Month WT	R	CR	2	1.7	A B C D E
SOST TG 6 Month TG	L	CA	2	1.7	A B C D E F
SOST KO 6 Month WT	L	CA	5	1.7	A B C D E F
SOST TG 6 Month TG	R	CA	2	1.6	A B C D E F
SOST KO 6 Month WT	R	CA	3	1.6	A B C D E F
SOST TG 6 Month TG	R	L	2	1.6	A B C D E F
SOST KO 6 Month KO	L	CA	5	1.6	A B C D E F
SOST KO 6 Month WT	L	CR	6	1.6	A B C D E F
SOST KO 12 Month WT	L	CA	2	1.6	A B C D E F
SOST TG 6 Month TG	R	M	2	1.6	A B C D E F
SOST KO 12 Month WT	L	CR	2	1.6	A B C D E F
SOST TG 6 Month TG	R	CR	2	1.6	A B C D E F
SOST KO 6 Month WT	L	M	5	1.6	A B C D E F
SOST KO 6 Month WT	L	L	5	1.5	A B C D E F

SOST KO 6 Month KO	L	M	5	1.5	A B C D E F
SOST KO 6 Month KO	L	CR	5	1.5	A B C D E F
SOST KO 6 Month KO	R	CA	5	1.5	A B C D E F
SOST TG 6 Month WT	R	M	11	1.5	C D E F
SOST KO 6 Month WT	R	M	3	1.5	A B C D E F
SOST KO 6 Month KO	R	L	5	1.5	B C D E F
SOST KO 12 Month WT	R	M	2	1.5	A B C D E F
SOST TG 6 Month TG	L	L	2	1.5	A B C D E F
SOST KO 6 Month KO	L	L	5	1.5	C D E F
SOST KO 6 Month KO	R	CR	5	1.5	C D E F
SOST TG 6 Month WT	R	L	10	1.5	C D E F
SOST TG 6 Month WT	R	CR	7	1.4	C D E F
SOST KO 6 Month KO	R	M	5	1.4	C D E F
SOST KO 12 Month WT	R	CA	2	1.4	A B C D E F
SOST TG 6 Month TG	L	CR	2	1.4	A B C D E F
SOST TG 6 Month DEF	R	CR	7	1.4	C D E F
SOST KO 12 Month WT	L	M	2	1.4	A B C D E F
SOST KO 6 Month WT	R	CR	4	1.4	C D E F
SOST TG 8 Month WT	L	CR	2	1.4	A B C D E F
SOST KO 12 Month KO	R	CA	4	1.3	C D E F
SOST KO 6 Month WT	R	L	5	1.3	C D E F
SOST TG 6 Month WT	L	L	10	1.3	C D E F
SOST TG 6 Month WT	L	CA	10	1.3	C D E F
SOST TG 6 Month DEF	R	CA	7	1.3	C D E F
SOST TG 6 Month DEF	R	M	6	1.3	C D E F
SOST TG 6 Month WT	L	CR	10	1.3	C D E F
SOST TG 6 Month WT	R	CA	8	1.3	C D E F
SOST TG 8 Month DEF	R	L	3	1.3	C D E F
SOST TG 6 Month DEF	L	CR	8	1.2	C D E F
SOST TG 8 Month TG	L	CA	2	1.2	C D E F
SOST TG 8 Month DEF	R	M	3	1.2	C D E F
SOST TG 8 Month WT	L	M	4	1.2	C D E F
SOST TG 6 Month WT	L	M	10	1.2	C D E F
SOST TG 6 Month DEF	L	M	9	1.2	C D E F
SOST TG 6 Month DEF	R	L	5	1.2	C D E F
SOST TG 6 Month DEF	L	CA	9	1.2	C D E F
SOST KO 12 Month KO	R	CR	5	1.2	C D E F
SOST TG 6 Month DEF	L	L	8	1.2	C D E F
SOST TG 8 Month TG	R	M	2	1.1	C D E F
SOST KO 12 Month KO	R	M	4	1.1	C D E F
SOST TG 8 Month WT	L	CA	4	1.1	C D E F
SOST KO 12 Month KO	R	L	4	1.1	C D E F
SOST TG 8 Month WT	L	L	3	1.1	C D E F
SOST TG 8 Month DEF	R	CR	4	1.1	C D E F
SOST TG 8 Month TG	R	CR	2	1.1	C D E F
SOST TG 8 Month TG	L	M	2	1.1	C D E F
SOST TG 8 Month WT	R	M	2	1.1	C D E F
SOST TG 8 Month WT	R	L	3	1.0	C D E F
SOST TG 8 Month DEF	R	CA	3	1.0	D E F
SOST TG 8 Month TG	L	L	1	1.0	C D E F
SOST TG 8 Month TG	L	CR	2	1.0	C D E F
SOST TG 8 Month TG	R	L	2	1.0	C D E F
SOST TG 8 Month DEF	L	L	4	1.0	D E F
SOST TG 8 Month TG	R	CA	2	1.0	C D E F
SOST TG 8 Month DEF	L	CR	4	1.0	D E F
SOST TG 8 Month WT	R	CR	2	0.9	C D E F
SOST TG 8 Month DEF	L	M	4	0.9	E F
SOST TG 8 Month DEF	L	CA	3	0.8	E F
SOST TG 8 Month WT	R	CA	3	0.8	F

Means that do not share a letter are significantly different.

Test for Equal Variances: Hardness (Gp versus Genotype+Phe, Limb, Region

95% Bonferroni confidence intervals for standard deviations

Genotype+Phenotype	Limb	Region	N	Lower	StDev	Upper
SOST KO 12 Month KO	L	CA	4	0.089428	0.223229	3.645
SOST KO 12 Month KO	L	CR	4	0.059818	0.149315	2.438
SOST KO 12 Month KO	L	L	5	0.062959	0.144263	1.281
SOST KO 12 Month KO	L	M	4	0.078442	0.195804	3.197
SOST KO 12 Month KO	R	CA	4	0.057521	0.143582	2.344
SOST KO 12 Month KO	R	CR	5	0.108807	0.249318	2.214
SOST KO 12 Month KO	R	L	4	0.048419	0.120863	1.973
SOST KO 12 Month KO	R	M	4	0.144477	0.360640	5.888
SOST KO 12 Month WT	L	CA	2	0.046050	0.165846	418.149
SOST KO 12 Month WT	L	CR	2	0.020651	0.074373	187.518
SOST KO 12 Month WT	L	L	2	0.061439	0.221268	557.886
SOST KO 12 Month WT	L	M	2	0.068503	0.246711	622.037
SOST KO 12 Month WT	R	CA	2	0.039304	0.141550	356.893
SOST KO 12 Month WT	R	CR	2	0.010214	0.036784	92.743
SOST KO 12 Month WT	R	L	2	0.070707	0.254649	642.050
SOST KO 12 Month WT	R	M	2	0.101892	0.366957	925.214
SOST KO 6 Month KO	L	CA	5	0.068757	0.157548	1.399
SOST KO 6 Month KO	L	CR	5	0.046302	0.106094	0.942
SOST KO 6 Month KO	L	L	5	0.060791	0.139295	1.237
SOST KO 6 Month KO	L	M	5	0.056701	0.129922	1.154
SOST KO 6 Month KO	R	CA	5	0.074950	0.171737	1.525
SOST KO 6 Month KO	R	CR	5	0.074653	0.171057	1.519
SOST KO 6 Month KO	R	L	5	0.131889	0.302206	2.683
SOST KO 6 Month KO	R	M	5	0.040991	0.093926	0.834
SOST KO 6 Month WT	L	CA	5	0.078360	0.179551	1.594
SOST KO 6 Month WT	L	CR	6	0.064416	0.138597	0.856
SOST KO 6 Month WT	L	L	5	0.115302	0.264200	2.346
SOST KO 6 Month WT	L	M	5	0.105182	0.241010	2.140
SOST KO 6 Month WT	R	CA	3	0.049386	0.140192	7.880
SOST KO 6 Month WT	R	CR	4	0.093891	0.234368	3.827
SOST KO 6 Month WT	R	L	5	0.125504	0.287575	2.553
SOST KO 6 Month WT	R	M	3	0.056229	0.159620	8.972
SOST TG 6 Month DEF	L	CA	9	0.058102	0.110632	0.395
SOST TG 6 Month DEF	L	CR	8	0.075425	0.148473	0.604
SOST TG 6 Month DEF	L	L	8	0.083790	0.164942	0.671
SOST TG 6 Month DEF	L	M	9	0.055242	0.105186	0.375
SOST TG 6 Month DEF	R	CA	7	0.187441	0.383974	1.860
SOST TG 6 Month DEF	R	CR	7	0.208062	0.426216	2.065
SOST TG 6 Month DEF	R	L	5	0.168566	0.386247	3.429
SOST TG 6 Month DEF	R	M	6	0.187386	0.403177	2.489
SOST TG 6 Month TG	L	CA	2	0.018382	0.066201	166.913
SOST TG 6 Month TG	L	CR	2	0.046649	0.168005	423.593
SOST TG 6 Month TG	L	L	2	0.040734	0.146702	369.881
SOST TG 6 Month TG	L	M	2	0.000136	0.000489	1.232
SOST TG 6 Month TG	R	CA	2	0.013267	0.047781	120.471
SOST TG 6 Month TG	R	CR	2	0.045124	0.162510	409.740
SOST TG 6 Month TG	R	L	2	0.084273	0.303504	765.229
SOST TG 6 Month TG	R	M	2	0.002868	0.010330	26.045
SOST TG 6 Month WT	L	CA	10	0.093930	0.173851	0.559
SOST TG 6 Month WT	L	CR	10	0.142379	0.263523	0.848
SOST TG 6 Month WT	L	L	10	0.142881	0.264453	0.851
SOST TG 6 Month WT	L	M	10	0.120350	0.222751	0.717
SOST TG 6 Month WT	R	CA	8	0.122769	0.241670	0.984

SOST TG 6 Month WT	R	CR	7	0.195302	0.400077	1.938
SOST TG 6 Month WT	R	L	10	0.189213	0.350207	1.127
SOST TG 6 Month WT	R	M	11	0.190400	0.343838	1.017
SOST TG 8 Month DEF	L	CA	3	0.032713	0.092862	5.220
SOST TG 8 Month DEF	L	CR	4	0.085194	0.212659	3.472
SOST TG 8 Month DEF	L	L	4	0.046885	0.117034	1.911
SOST TG 8 Month DEF	L	M	4	0.051248	0.127924	2.089
SOST TG 8 Month DEF	R	CA	3	0.067638	0.192005	10.793
SOST TG 8 Month DEF	R	CR	4	0.079777	0.199137	3.251
SOST TG 8 Month DEF	R	L	3	0.029496	0.083732	4.707
SOST TG 8 Month DEF	R	M	3	0.065950	0.187212	10.523
SOST TG 8 Month TG	L	CA	2	0.052334	0.188477	475.209
SOST TG 8 Month TG	L	CR	2	0.072643	0.261620	659.627
SOST TG 8 Month TG	L	L	1	*	*	*
SOST TG 8 Month TG	L	M	2	0.057456	0.206924	521.720
SOST TG 8 Month TG	R	CA	2	0.006276	0.022602	56.986
SOST TG 8 Month TG	R	CR	2	0.022080	0.079518	200.491
SOST TG 8 Month TG	R	L	2	0.043690	0.157346	396.718
SOST TG 8 Month TG	R	M	2	0.016980	0.061153	154.185
SOST TG 8 Month WT	L	CA	4	0.051090	0.127529	2.082
SOST TG 8 Month WT	L	CR	2	0.060259	0.217019	547.172
SOST TG 8 Month WT	L	L	3	0.018067	0.051288	2.883
SOST TG 8 Month WT	L	M	4	0.097855	0.244262	3.988
SOST TG 8 Month WT	R	CA	3	0.030241	0.085846	4.825
SOST TG 8 Month WT	R	CR	2	0.021355	0.076910	193.915
SOST TG 8 Month WT	R	L	3	0.038793	0.110123	6.190
SOST TG 8 Month WT	R	M	2	0.014906	0.053685	135.356

Bartlett's Test (Normal Distribution)
Test statistic = 109.09, p-value = 0.012

Levene's Test (Any Continuous Distribution)
Test statistic = 1.30, p-value = 0.068

Test for Equal Variances: Hardness (Gpa)_1 versus Genotype+Phenotype, Limb, Reg

******MODULUS:

General Linear Model: E_mod versus Genotype+Phenotype, Limb, Region

Factor	Type	Levels	Values
Genotype+Phenotype	fixed	10	SOST KO 12 Month KO, SOST KO 12 Month WT, SOST KO 6 Month KO, SOST KO 6 Month WT, SOST TG 6 Month DEF, SOST TG 6 Month TG, SOST TG

			6 Month WT, SOST TG 8 Month DEF, SOST TG 8 Month TG, SOST TG 8 Month WT
Limb	fixed	2	L, R
Region	fixed	4	CA, CR, L, M

Analysis of Variance for E_mod, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	6309.71	6000.83	666.76	40.83	0.000
Limb	1	727.68	224.44	224.44	13.74	0.000
Region	3	64.34	39.54	13.18	0.81	0.491
Genotype+Phenotype*Limb	9	5142.49	4971.31	552.37	33.82	0.000
Genotype+Phenotype*Region	27	426.53	424.41	15.72	0.96	0.522
Limb*Region	3	29.46	38.20	12.73	0.78	0.506
Genotype+Phenotype*Limb*Region	27	460.98	460.98	17.07	1.05	0.408
Error	265	4327.65	4327.65	16.33		
Total	344	17488.84				

S = 4.04113   R-Sq = 75.25%   R-Sq(adj) = 67.88%

Unusual Observations for E_mod

Obs	E_mod	Fit	SE Fit	Residual	St Resid	
6	32.6748	23.3337	2.0206	9.3411	2.67	R
24	39.4621	32.1641	2.0206	7.2979	2.09	R
28	23.1555	32.1641	2.0206	-9.0087	-2.57	R
31	22.3910	22.3910	4.0411	0.0000	*	X
43	63.6079	54.5187	1.8072	9.0892	2.51	R
69	39.9960	30.8301	1.2779	9.1660	2.39	R
71	40.2636	31.6320	1.2779	8.6316	2.25	R
83	23.3278	31.6320	1.2779	-8.3042	-2.17	R
169	35.0199	27.3989	1.3470	7.6210	2.00	R
191	34.5461	25.9275	1.4288	8.6186	2.28	R
284	60.9767	41.3949	1.2184	19.5817	5.08	R
288	29.1006	41.3949	1.2184	-12.2943	-3.19	R
382	47.5673	37.6142	1.5274	9.9531	2.66	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	N	Mean	Grouping
SOST KO 12 Month KO	34	43.6	A
SOST TG 6 Month WT	77	34.8	B
SOST TG 6 Month TG	16	33.8	B C
SOST TG 6 Month DEF	60	32.2	C
SOST KO 6 Month KO	40	31.9	C D
SOST KO 12 Month WT	16	31.6	B C D E
SOST KO 6 Month WT	36	31.4	C D E
SOST TG 8 Month WT	23	28.7	D E F
SOST TG 8 Month TG	15	27.5	E F
SOST TG 8 Month DEF	28	27.2	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
R	162	33.2	A
L	183	31.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
L	87	32.7	A
M	87	32.6	A
CA	86	32.0	A
CR	85	31.8	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	49.7	A
SOST TG 6 Month WT	R	37	38.3	B
SOST TG 6 Month DEF	R	26	37.6	B C
SOST KO 12 Month KO	R	17	37.5	B C D
SOST TG 6 Month TG	R	8	33.9	B C D E
SOST TG 8 Month DEF	R	13	33.8	B C D E
SOST TG 6 Month TG	L	8	33.7	B C D E
SOST TG 8 Month TG	R	8	32.5	C D E
SOST KO 6 Month WT	L	21	32.5	E
SOST KO 6 Month KO	L	20	32.2	E
SOST TG 8 Month WT	L	13	32.1	D E
SOST KO 12 Month WT	L	8	31.9	C D E F
SOST KO 6 Month KO	R	20	31.5	E F
SOST TG 6 Month WT	L	40	31.4	E F
SOST KO 12 Month WT	R	8	31.3	E F
SOST KO 6 Month WT	R	15	30.4	E F
SOST TG 6 Month DEF	L	34	26.8	F G
SOST TG 8 Month WT	R	10	25.3	F G H
SOST TG 8 Month TG	L	7	22.5	G H
SOST TG 8 Month DEF	L	15	20.6	H

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	9	45.4	A
SOST KO 12 Month KO	CA	8	44.9	A
SOST KO 12 Month KO	M	8	44.5	A B
SOST KO 12 Month KO	CR	9	39.6	A B C
SOST TG 6 Month WT	M	21	36.5	C D
SOST TG 6 Month TG	M	4	35.2	B C D E
SOST TG 6 Month TG	L	4	35.1	B C D E
SOST TG 6 Month WT	L	20	34.7	C D E
SOST TG 6 Month WT	CR	17	34.4	C D E
SOST KO 12 Month WT	L	4	34.2	C D E
SOST TG 6 Month WT	CA	19	33.7	C D E

SOST TG 6 Month TG	CA	4	33.5	C D E
SOST KO 6 Month WT	CA	8	32.7	C D E
SOST KO 6 Month KO	CA	10	32.7	C D E
SOST TG 6 Month DEF	M	15	32.6	D E
SOST TG 6 Month DEF	CA	16	32.2	D E
SOST TG 6 Month DEF	CR	15	32.1	D E
SOST TG 6 Month DEF	L	14	32.0	D E
SOST KO 6 Month KO	M	10	31.9	D E
SOST KO 6 Month KO	CR	10	31.8	D E
SOST KO 6 Month WT	M	8	31.7	D E
SOST TG 6 Month TG	CR	4	31.5	C D E
SOST KO 6 Month WT	CR	10	31.4	D E
SOST KO 6 Month KO	L	10	31.1	D E
SOST KO 12 Month WT	CR	4	31.1	C D E
SOST KO 12 Month WT	CA	4	30.8	C D E
SOST TG 8 Month WT	CR	4	30.5	C D E
SOST KO 12 Month WT	M	4	30.1	D E
SOST KO 6 Month WT	L	10	30.0	E
SOST TG 8 Month WT	M	6	29.3	D E
SOST TG 8 Month DEF	L	7	28.6	E
SOST TG 8 Month WT	L	6	28.4	E
SOST TG 8 Month DEF	CR	8	28.0	E
SOST TG 8 Month TG	M	4	27.7	E
SOST TG 8 Month TG	CA	4	27.7	E
SOST TG 8 Month TG	CR	4	27.3	E
SOST TG 8 Month TG	L	3	27.3	D E
SOST TG 8 Month DEF	M	7	27.0	E
SOST TG 8 Month WT	CA	7	26.7	E
SOST TG 8 Month DEF	CA	6	25.1	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	Region	N	Mean	Grouping
R	L	42	34.1	A
R	M	40	33.5	A B
R	CR	40	32.9	A B
R	CA	40	32.3	A B
L	M	47	31.8	A B
L	CA	46	31.7	A B
L	L	45	31.3	A B
L	CR	45	30.6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	L	5	54.5	A
SOST KO 12 Month KO	L	M	4	51.2	A B
SOST KO 12 Month KO	L	CA	4	49.6	A B C
SOST KO 12 Month KO	L	CR	4	43.6	A B C D
SOST TG 6 Month WT	R	M	11	41.4	B C D
SOST KO 12 Month KO	R	CA	4	40.2	B C D E
SOST TG 6 Month TG	L	M	2	39.2	B C D E F
SOST TG 6 Month TG	R	L	2	38.3	B C D E F
SOST TG 6 Month DEF	R	L	6	38.0	D E F
SOST TG 6 Month WT	R	L	10	37.8	D E F

SOST KO 12 Month KO	R	M	4	37.8	C D E F
SOST TG 6 Month DEF	R	M	6	37.7	D E F
SOST TG 6 Month DEF	R	CR	7	37.6	D E F
SOST TG 6 Month WT	R	CR	7	37.6	D E F
SOST TG 6 Month DEF	R	CA	7	37.0	D E F
SOST TG 6 Month WT	R	CA	9	36.5	D E F
SOST TG 8 Month DEF	R	L	3	36.2	D E F G
SOST KO 12 Month KO	R	L	4	36.2	D E F G
SOST KO 12 Month KO	R	CR	5	35.6	D E F G
SOST TG 8 Month WT	L	CR	2	35.2	C D E F G H
SOST TG 8 Month DEF	R	M	3	34.7	D E F G H
SOST TG 8 Month TG	R	CR	2	34.5	D E F G H
SOST KO 12 Month WT	L	L	2	34.3	D E F G H I
SOST KO 12 Month WT	R	L	2	34.2	D E F G H I
SOST TG 6 Month TG	L	CA	2	33.8	D E F G H I J
SOST KO 6 Month KO	L	CA	5	33.8	D E F G H I J
SOST KO 6 Month WT	L	CA	5	33.7	D E F G H I J
SOST KO 6 Month WT	L	CR	6	33.6	D E F G H I J
SOST TG 6 Month TG	R	CR	2	33.2	D E F G H I J
SOST TG 6 Month TG	R	CA	2	33.2	D E F G H I J
SOST TG 8 Month TG	R	M	2	33.1	D E F G H I J
SOST TG 8 Month DEF	R	CR	4	32.7	D E F G H I J
SOST KO 12 Month WT	L	CA	2	32.6	D E F G H I J
SOST KO 6 Month KO	L	M	5	32.5	D E F G H I J
SOST TG 8 Month TG	R	L	2	32.2	D E F G H I J
SOST TG 8 Month WT	L	M	4	32.2	D E F G H I J
SOST KO 6 Month KO	L	CR	5	32.2	E F G H I J
SOST TG 6 Month TG	L	L	2	32.0	D E F G H I J
SOST KO 6 Month KO	R	L	5	31.9	E F G H I J
SOST KO 6 Month WT	L	M	5	31.8	E F G H I J
SOST KO 6 Month WT	R	CA	3	31.8	D E F G H I J
SOST KO 12 Month WT	R	CR	2	31.7	D E F G H I J
SOST TG 6 Month WT	L	M	10	31.7	E F G H I J
SOST TG 6 Month WT	L	L	10	31.6	E F G H I J
SOST KO 6 Month WT	R	M	3	31.6	D E F G H I J
SOST KO 6 Month KO	R	CA	5	31.5	E F G H I J
SOST TG 8 Month DEF	R	CA	3	31.5	D E F G H I J
SOST KO 6 Month KO	R	CR	5	31.4	E F G H I J
SOST TG 6 Month WT	L	CR	10	31.3	E F G H I J
SOST KO 6 Month KO	R	M	5	31.3	E F G H I J
SOST TG 6 Month TG	R	M	2	31.1	D E F G H I J
SOST TG 8 Month WT	L	CA	4	31.0	E F G H I J
SOST KO 6 Month WT	L	L	5	30.9	E F G H I J
SOST TG 6 Month WT	L	CA	10	30.8	E F G H I J
SOST KO 12 Month WT	L	CR	2	30.6	D E F G H I J
SOST KO 6 Month KO	L	L	5	30.4	E F G H I J
SOST TG 8 Month TG	R	CA	2	30.2	D E F G H I J
SOST KO 12 Month WT	L	M	2	30.2	D E F G H I J
SOST TG 8 Month WT	L	L	3	30.1	E F G H I J
SOST KO 12 Month WT	R	M	2	30.1	D E F G H I J
SOST TG 6 Month TG	L	CR	2	29.7	D E F G H I J
SOST KO 6 Month WT	R	CR	4	29.2	E F G H I J
SOST KO 6 Month WT	R	L	5	29.1	E F G H I J
SOST KO 12 Month WT	R	CA	2	29.0	D E F G H I J
SOST TG 6 Month DEF	L	M	9	27.4	F G H I J
SOST TG 6 Month DEF	L	CA	9	27.4	F G H I J
SOST TG 8 Month WT	R	L	3	26.7	F G H I J
SOST TG 6 Month DEF	L	CR	8	26.6	F G H I J
SOST TG 8 Month WT	R	M	2	26.3	E F G H I J
SOST TG 6 Month DEF	L	L	8	25.9	F G H I J
SOST TG 8 Month WT	R	CR	2	25.8	E F G H I J

SOST TG 8 Month TG	L	CA	2	25.1		F G H I J
SOST TG 8 Month DEF	L	CR	4	23.3		G H I J
SOST TG 8 Month WT	R	CA	3	22.5		G H I J
SOST TG 8 Month TG	L	L	1	22.4		E F G H I J
SOST TG 8 Month TG	L	M	2	22.4		F G H I J
SOST TG 8 Month DEF	L	L	4	21.0		H I J
SOST TG 8 Month TG	L	CR	2	20.2		H I J
SOST TG 8 Month DEF	L	M	4	19.3		J
SOST TG 8 Month DEF	L	CA	3	18.8		I J

Means that do not share a letter are significantly different.

Residual Plots for E_mod

Test for Equal Variances: E_mod versus Genotype+Phenotype, Limb, Region

95% Bonferroni confidence intervals for standard deviations

Genotype+Phenotype	Limb	Region	N	Lower	StDev	Upper
SOST KO 12 Month KO	L	CA	4	0.95064	2.37297	38.7
SOST KO 12 Month KO	L	CR	4	1.37208	3.42496	55.9
SOST KO 12 Month KO	L	L	5	2.35427	5.39450	47.9
SOST KO 12 Month KO	L	M	4	1.10291	2.75305	44.9
SOST KO 12 Month KO	R	CA	4	1.86300	4.65037	75.9
SOST KO 12 Month KO	R	CR	5	1.32927	3.04585	27.0
SOST KO 12 Month KO	R	L	4	1.27211	3.17541	51.8
SOST KO 12 Month KO	R	M	4	1.71095	4.27083	69.7
SOST KO 12 Month WT	L	CA	2	0.81041	2.91864	7358.8
SOST KO 12 Month WT	L	CR	2	0.24885	0.89623	2259.7
SOST KO 12 Month WT	L	L	2	1.31077	4.72066	11902.3
SOST KO 12 Month WT	L	M	2	1.02919	3.70656	9345.4
SOST KO 12 Month WT	R	CA	2	1.08588	3.91074	9860.2
SOST KO 12 Month WT	R	CR	2	0.96112	3.46141	8727.3
SOST KO 12 Month WT	R	L	2	0.79062	2.84736	7179.1
SOST KO 12 Month WT	R	M	2	1.72145	6.19970	15631.4
SOST KO 6 Month KO	L	CA	5	1.30297	2.98558	26.5
SOST KO 6 Month KO	L	CR	5	1.46247	3.35106	29.8
SOST KO 6 Month KO	L	L	5	0.51190	1.17295	10.4
SOST KO 6 Month KO	L	M	5	0.99388	2.27735	20.2
SOST KO 6 Month KO	R	CA	5	1.19995	2.74952	24.4
SOST KO 6 Month KO	R	CR	5	1.24966	2.86343	25.4
SOST KO 6 Month KO	R	L	5	1.69314	3.87960	34.4
SOST KO 6 Month KO	R	M	5	1.08551	2.48730	22.1
SOST KO 6 Month WT	L	CA	5	1.33389	3.05643	27.1
SOST KO 6 Month WT	L	CR	6	1.04179	2.24151	13.8
SOST KO 6 Month WT	L	L	5	2.07140	4.74633	42.1
SOST KO 6 Month WT	L	M	5	1.14767	2.62972	23.3
SOST KO 6 Month WT	R	CA	3	0.23583	0.66947	37.6
SOST KO 6 Month WT	R	CR	4	1.43499	3.58198	58.5
SOST KO 6 Month WT	R	L	5	1.76411	4.04223	35.9
SOST KO 6 Month WT	R	M	3	0.73833	2.09591	117.8
SOST TG 6 Month DEF	L	CA	9	2.17596	4.14326	14.8
SOST TG 6 Month DEF	L	CR	8	2.74369	5.40095	22.0
SOST TG 6 Month DEF	L	L	8	2.17631	4.28406	17.4
SOST TG 6 Month DEF	L	M	9	2.21762	4.22258	15.1
SOST TG 6 Month DEF	R	CA	7	1.01812	2.08562	10.1
SOST TG 6 Month DEF	R	CR	7	1.64278	3.36525	16.3
SOST TG 6 Month DEF	R	L	6	2.14228	4.60929	28.5

SOST TG 6 Month DEF	R	M	6	1.20282	2.58798	16.0
SOST TG 6 Month TG	L	CA	2	0.22430	0.80781	2036.8
SOST TG 6 Month TG	L	CR	2	1.18036	4.25100	10718.1
SOST TG 6 Month TG	L	L	2	0.90527	3.26028	8220.2
SOST TG 6 Month TG	L	M	2	1.25149	4.50717	11364.0
SOST TG 6 Month TG	R	CA	2	0.75186	2.70778	6827.2
SOST TG 6 Month TG	R	CR	2	1.23484	4.44719	11212.8
SOST TG 6 Month TG	R	L	2	0.82859	2.98412	7523.9
SOST TG 6 Month TG	R	M	2	0.43530	1.56770	3952.7
SOST TG 6 Month WT	L	CA	10	2.65402	4.91221	15.8
SOST TG 6 Month WT	L	CR	10	1.88544	3.48967	11.2
SOST TG 6 Month WT	L	L	10	2.39594	4.43454	14.3
SOST TG 6 Month WT	L	M	10	2.24628	4.15754	13.4
SOST TG 6 Month WT	R	CA	9	1.72342	3.28158	11.7
SOST TG 6 Month WT	R	CR	7	2.97936	6.10323	29.6
SOST TG 6 Month WT	R	L	10	2.50896	4.64371	14.9
SOST TG 6 Month WT	R	M	11	4.50875	8.14224	24.1
SOST TG 8 Month DEF	L	CA	3	0.57531	1.63315	91.8
SOST TG 8 Month DEF	L	CR	4	2.54999	6.36522	103.9
SOST TG 8 Month DEF	L	L	4	0.71861	1.79378	29.3
SOST TG 8 Month DEF	L	M	4	0.71911	1.79504	29.3
SOST TG 8 Month DEF	R	CA	3	0.17583	0.49913	28.1
SOST TG 8 Month DEF	R	CR	4	0.79841	1.99298	32.5
SOST TG 8 Month DEF	R	L	3	0.55810	1.58428	89.1
SOST TG 8 Month DEF	R	M	3	1.97450	5.60506	315.1
SOST TG 8 Month TG	L	CA	2	1.16977	4.21287	10622.0
SOST TG 8 Month TG	L	CR	2	1.24740	4.49244	11326.9
SOST TG 8 Month TG	L	L	1	*	*	*
SOST TG 8 Month TG	L	M	2	1.90094	6.84613	17261.2
SOST TG 8 Month TG	R	CA	2	0.67354	2.42573	6116.0
SOST TG 8 Month TG	R	CR	2	0.68615	2.47112	6230.5
SOST TG 8 Month TG	R	L	2	0.04302	0.15492	390.6
SOST TG 8 Month TG	R	M	2	0.13306	0.47922	1208.3
SOST TG 8 Month WT	L	CA	4	0.83539	2.08527	34.0
SOST TG 8 Month WT	L	CR	2	0.68075	2.45168	6181.5
SOST TG 8 Month WT	L	L	3	1.22909	3.48905	196.1
SOST TG 8 Month WT	L	M	4	2.71132	6.76793	110.5
SOST TG 8 Month WT	R	CA	3	0.71918	2.04155	114.8
SOST TG 8 Month WT	R	CR	2	0.42454	1.52896	3855.0
SOST TG 8 Month WT	R	L	3	0.61376	1.74230	97.9
SOST TG 8 Month WT	R	M	2	0.07671	0.27625	696.5

Bartlett's Test (Normal Distribution)
Test statistic = 89.66, p-value = 0.173

Levene's Test (Any Continuous Distribution)
Test statistic = 0.78, p-value = 0.899

Test for Equal Variances: E_mod versus Genotype+Phenotype, Limb, Region

OUTLIERS REMOVED: 8603R/M,8640R/M

General Linear Model: E_mod_1 versus Genotype+Phenotype, Limb, Region

Factor	Type	Levels	Values
Genotype+Phenotype	fixed	10	SOST KO 12 Month KO, SOST KO 12 Month WT, SOST KO 6 Month KO, SOST KO 6 Month WT, SOST

			TG 6 Month DEF, SOST TG 6 Month TG, SOST TG 6 Month WT, SOST TG 8 Month DEF, SOST TG 8 Month TG, SOST TG 8 Month WT
Limb	fixed	2	L, R
Region	fixed	4	CA, CR, L, M

Analysis of Variance for E_mod_1, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Genotype+Phenotype	9	6227.51	5968.48	663.16	46.05	0.000
Limb	1	669.71	219.45	219.45	15.24	0.000
Region	3	46.41	37.69	12.56	0.87	0.456
Genotype+Phenotype*Limb	9	5080.54	4934.56	548.28	38.08	0.000
Genotype+Phenotype*Region	27	403.92	407.07	15.08	1.05	0.406
Limb*Region	3	25.24	38.60	12.87	0.89	0.445
Genotype+Phenotype*Limb*Region	27	442.88	442.88	16.40	1.14	0.295
Error	263	3787.15	3787.15	14.40		
Total	342	16683.35				

S = 3.79471   R-Sq = 77.30%   R-Sq(adj) = 70.48%

Unusual Observations for E_mod_1

Obs	E_mod_1	Fit	SE Fit	Residual	St Resid	
6	32.6748	23.3337	1.8974	9.3411	2.84	R
24	39.4621	32.1641	1.8974	7.2979	2.22	R
28	23.1555	32.1641	1.8974	-9.0087	-2.74	R
31	22.3910	22.3910	3.7947	-0.0000	*	X
43	63.6079	54.5187	1.6970	9.0892	2.68	R
69	39.9960	30.8301	1.2000	9.1660	2.55	R
71	40.2636	31.6320	1.2000	8.6316	2.40	R
83	23.3278	31.6320	1.2000	-8.3042	-2.31	R
147	23.6749	30.8835	1.6970	-7.2086	-2.12	R
169	35.0199	27.3989	1.2649	7.6210	2.13	R
191	34.5461	25.9275	1.3416	8.6186	2.43	R
204	28.3864	34.7114	2.1909	-6.3250	-2.04	R
249	47.2195	40.2454	1.8974	6.9741	2.12	R
382	47.5673	37.6142	1.4343	9.9531	2.83	R

R denotes an observation with a large standardized residual.
X denotes an observation whose X value gives it large leverage.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	N	Mean	Grouping
SOST KO 12 Month KO	34	43.6	A
SOST TG 6 Month WT	75	34.7	B
SOST TG 6 Month TG	16	33.8	B C
SOST TG 6 Month DEF	60	32.2	C
SOST KO 6 Month KO	40	31.9	C D
SOST KO 12 Month WT	16	31.6	B C D E
SOST KO 6 Month WT	36	31.4	C D E
SOST TG 8 Month WT	23	28.7	D E F
SOST TG 8 Month TG	15	27.5	E F
SOST TG 8 Month DEF	28	27.2	F

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	N	Mean	Grouping
R	160	33.2	A
L	183	31.3	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Region	N	Mean	Grouping
L	87	32.7	A
M	85	32.6	A
CA	86	32.0	A
CR	85	31.8	A

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	N	Mean	Grouping
SOST KO 12 Month KO	L	17	49.7	A
SOST TG 6 Month WT	R	35	38.1	B
SOST TG 6 Month DEF	R	26	37.6	B C
SOST KO 12 Month KO	R	17	37.5	B C D
SOST TG 6 Month TG	R	8	33.9	B C D E
SOST TG 8 Month DEF	R	13	33.8	B C D E
SOST TG 6 Month TG	L	8	33.7	B C D E
SOST TG 8 Month TG	R	8	32.5	C D E
SOST KO 6 Month WT	L	21	32.5	E
SOST KO 6 Month KO	L	20	32.2	E
SOST TG 8 Month WT	L	13	32.1	E
SOST KO 12 Month WT	L	8	31.9	D E F
SOST KO 6 Month KO	R	20	31.5	E F
SOST TG 6 Month WT	L	40	31.4	E F
SOST KO 12 Month WT	R	8	31.3	E F G
SOST KO 6 Month WT	R	15	30.4	E F G
SOST TG 6 Month DEF	L	34	26.8	F G H
SOST TG 8 Month WT	R	10	25.3	G H I
SOST TG 8 Month TG	L	7	22.5	H I
SOST TG 8 Month DEF	L	15	20.6	I

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	9	45.4	A
SOST KO 12 Month KO	CA	8	44.9	A
SOST KO 12 Month KO	M	8	44.5	A
SOST KO 12 Month KO	CR	9	39.6	A B
SOST TG 6 Month WT	M	19	36.1	B C
SOST TG 6 Month TG	M	4	35.2	B C D
SOST TG 6 Month TG	L	4	35.1	B C D
SOST TG 6 Month WT	L	20	34.7	B C D
SOST TG 6 Month WT	CR	17	34.4	B C D

SOST KO 12 Month WT	L	4	34.2	B C D E
SOST TG 6 Month WT	CA	19	33.7	B C D E
SOST TG 6 Month TG	CA	4	33.5	B C D E
SOST KO 6 Month WT	CA	8	32.7	B C D E
SOST KO 6 Month KO	CA	10	32.7	C D E
SOST TG 6 Month DEF	M	15	32.6	C D E
SOST TG 6 Month DEF	CA	16	32.2	C D E
SOST TG 6 Month DEF	CR	15	32.1	C D E
SOST TG 6 Month DEF	L	14	32.0	C D E
SOST KO 6 Month KO	M	10	31.9	C D E
SOST KO 6 Month KO	CR	10	31.8	C D E
SOST KO 6 Month WT	M	8	31.7	C D E
SOST TG 6 Month TG	CR	4	31.5	B C D E
SOST KO 6 Month WT	CR	10	31.4	C D E
SOST KO 6 Month KO	L	10	31.1	C D E
SOST KO 12 Month WT	CR	4	31.1	B C D E
SOST KO 12 Month WT	CA	4	30.8	B C D E
SOST TG 8 Month WT	CR	4	30.5	C D E
SOST KO 12 Month WT	M	4	30.1	C D E
SOST KO 6 Month WT	L	10	30.0	D E
SOST TG 8 Month WT	M	6	29.3	C D E
SOST TG 8 Month DEF	L	7	28.6	D E
SOST TG 8 Month WT	L	6	28.4	D E
SOST TG 8 Month DEF	CR	8	28.0	D E
SOST TG 8 Month TG	M	4	27.7	D E
SOST TG 8 Month TG	CA	4	27.7	D E
SOST TG 8 Month TG	CR	4	27.3	D E
SOST TG 8 Month TG	L	3	27.3	C D E
SOST TG 8 Month DEF	M	7	27.0	D E
SOST TG 8 Month WT	CA	7	26.7	D E
SOST TG 8 Month DEF	CA	6	25.1	E

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Limb	Region	N	Mean	Grouping
R	L	42	34.1	A
R	M	38	33.4	A B
R	CR	40	32.9	A B
R	CA	40	32.3	A B
L	M	47	31.8	A B
L	CA	46	31.7	A B
L	L	45	31.3	A B
L	CR	45	30.6	B

Means that do not share a letter are significantly different.

Grouping Information Using Tukey Method and 95.0% Confidence

Genotype+Phenotype	Limb	Region	N	Mean	Grouping
SOST KO 12 Month KO	L	L	5	54.5	A
SOST KO 12 Month KO	L	M	4	51.2	A B
SOST KO 12 Month KO	L	CA	4	49.6	A B C
SOST KO 12 Month KO	L	CR	4	43.6	B C D
SOST TG 6 Month WT	R	M	9	40.6	C D E
SOST KO 12 Month KO	R	CA	4	40.2	B C D E F
SOST TG 6 Month TG	L	M	2	39.2	B C D E F G
SOST TG 6 Month TG	R	L	2	38.3	B C D E F G H

SOST TG 6 Month DEF	R	L	6	38.0	D E F G H
SOST TG 6 Month WT	R	L	10	37.8	D E F G H
SOST KO 12 Month KO	R	M	4	37.8	D E F G H
SOST TG 6 Month DEF	R	M	6	37.7	D E F G H
SOST TG 6 Month DEF	R	CR	7	37.6	D E F G H
SOST TG 6 Month WT	R	CR	7	37.6	D E F G H
SOST TG 6 Month DEF	R	CA	7	37.0	D E F G H
SOST TG 6 Month WT	R	CA	9	36.5	D E F G H
SOST TG 8 Month DEF	R	L	3	36.2	D E F G H
SOST KO 12 Month KO	R	L	4	36.2	D E F G H
SOST KO 12 Month KO	R	CR	5	35.6	D E F G H
SOST TG 8 Month WT	L	CR	2	35.2	D E F G H I
SOST TG 8 Month DEF	R	M	3	34.7	D E F G H I
SOST TG 8 Month TG	R	CR	2	34.5	D E F G H I J
SOST KO 12 Month WT	L	L	2	34.3	D E F G H I J
SOST KO 12 Month WT	R	L	2	34.2	D E F G H I J
SOST TG 6 Month TG	L	CA	2	33.8	D E F G H I J
SOST KO 6 Month KO	L	CA	5	33.8	D E F G H I J
SOST KO 6 Month WT	L	CA	5	33.7	D E F G H I J
SOST KO 6 Month WT	L	CR	6	33.6	D E F G H I J
SOST TG 6 Month TG	R	CR	2	33.2	D E F G H I J K
SOST TG 6 Month TG	R	CA	2	33.2	D E F G H I J K
SOST TG 8 Month TG	R	M	2	33.1	D E F G H I J K L
SOST TG 8 Month DEF	R	CR	4	32.7	D E F G H I J K L
SOST KO 12 Month WT	L	CA	2	32.6	D E F G H I J K L
SOST KO 6 Month KO	L	M	5	32.5	E F G H I J K L
SOST TG 8 Month TG	R	L	2	32.2	D E F G H I J K L
SOST TG 8 Month WT	L	M	4	32.2	E F G H I J K L
SOST KO 6 Month KO	L	CR	5	32.2	E F G H I J K L
SOST TG 6 Month TG	L	L	2	32.0	D E F G H I J K L
SOST KO 6 Month KO	R	L	5	31.9	E F G H I J K L
SOST KO 6 Month WT	L	M	5	31.8	E F G H I J K L
SOST KO 6 Month WT	R	CA	3	31.8	D E F G H I J K L
SOST KO 12 Month WT	R	CR	2	31.7	D E F G H I J K L
SOST TG 6 Month WT	L	M	10	31.7	F G H I J K L
SOST TG 6 Month WT	L	L	10	31.6	F G H I J K L
SOST KO 6 Month WT	R	M	3	31.6	D E F G H I J K L
SOST KO 6 Month KO	R	CA	5	31.5	F G H I J K L
SOST TG 8 Month DEF	R	CA	3	31.5	D E F G H I J K L
SOST KO 6 Month KO	R	CR	5	31.4	F G H I J K L
SOST TG 6 Month WT	L	CR	10	31.3	F G H I J K L
SOST KO 6 Month KO	R	M	5	31.3	F G H I J K L
SOST TG 6 Month TG	R	M	2	31.1	D E F G H I J K L
SOST TG 8 Month WT	L	CA	4	31.0	F G H I J K L
SOST KO 6 Month WT	L	L	5	30.9	F G H I J K L
SOST TG 6 Month WT	L	CA	10	30.8	F G H I J K L
SOST KO 12 Month WT	L	CR	2	30.6	D E F G H I J K L
SOST KO 6 Month KO	L	L	5	30.4	F G H I J K L
SOST TG 8 Month TG	R	CA	2	30.2	D E F G H I J K L
SOST KO 12 Month WT	L	M	2	30.2	D E F G H I J K L
SOST TG 8 Month WT	L	L	3	30.1	E F G H I J K L
SOST KO 12 Month WT	R	M	2	30.1	D E F G H I J K L
SOST TG 6 Month TG	L	CR	2	29.7	E F G H I J K L
SOST KO 6 Month WT	R	CR	4	29.2	F G H I J K L
SOST KO 6 Month WT	R	L	5	29.1	G H I J K L
SOST KO 12 Month WT	R	CA	2	29.0	E F G H I J K L
SOST TG 6 Month DEF	L	M	9	27.4	G H I J K L
SOST TG 6 Month DEF	L	CA	9	27.4	G H I J K L
SOST TG 8 Month WT	R	L	3	26.7	G H I J K L
SOST TG 6 Month DEF	L	CR	8	26.6	G H I J K L
SOST TG 8 Month WT	R	M	2	26.3	G H I J K L

SOST TG 6 Month DEF	L	L	8	25.9	H I J K L
SOST TG 8 Month WT	R	CR	2	25.8	G H I J K L
SOST TG 8 Month TG	L	CA	2	25.1	G H I J K L
SOST TG 8 Month DEF	L	CR	4	23.3	I J K L
SOST TG 8 Month WT	R	CA	3	22.5	I J K L
SOST TG 8 Month TG	L	L	1	22.4	G H I J K L
SOST TG 8 Month TG	L	M	2	22.4	H I J K L
SOST TG 8 Month DEF	L	L	4	21.0	J K L
SOST TG 8 Month TG	L	CR	2	20.2	I J K L
SOST TG 8 Month DEF	L	M	4	19.3	L
SOST TG 8 Month DEF	L	CA	3	18.8	K L

Means that do not share a letter are significantly different.

Residual Plots for E_mod_1

Test for Equal Variances: E_mod_1 versus Genotype+Phenotype, Limb, Region

95% Bonferroni confidence intervals for standard deviations

Genotype+Phenotype	Limb	Region	N	Lower	StDev	Upper
SOST KO 12 Month KO	L	CA	4	0.95064	2.37297	38.7
SOST KO 12 Month KO	L	CR	4	1.37208	3.42496	55.9
SOST KO 12 Month KO	L	L	5	2.35427	5.39450	47.9
SOST KO 12 Month KO	L	M	4	1.10291	2.75305	44.9
SOST KO 12 Month KO	R	CA	4	1.86300	4.65037	75.9
SOST KO 12 Month KO	R	CR	5	1.32927	3.04585	27.0
SOST KO 12 Month KO	R	L	4	1.27211	3.17541	51.8
SOST KO 12 Month KO	R	M	4	1.71095	4.27083	69.7
SOST KO 12 Month WT	L	CA	2	0.81041	2.91864	7358.8
SOST KO 12 Month WT	L	CR	2	0.24885	0.89623	2259.7
SOST KO 12 Month WT	L	L	2	1.31077	4.72066	11902.3
SOST KO 12 Month WT	L	M	2	1.02919	3.70656	9345.4
SOST KO 12 Month WT	R	CA	2	1.08588	3.91074	9860.2
SOST KO 12 Month WT	R	CR	2	0.96112	3.46141	8727.3
SOST KO 12 Month WT	R	L	2	0.79062	2.84736	7179.1
SOST KO 12 Month WT	R	M	2	1.72145	6.19970	15631.4
SOST KO 6 Month KO	L	CA	5	1.30297	2.98558	26.5
SOST KO 6 Month KO	L	CR	5	1.46247	3.35106	29.8
SOST KO 6 Month KO	L	L	5	0.51190	1.17295	10.4
SOST KO 6 Month KO	L	M	5	0.99388	2.27735	20.2
SOST KO 6 Month KO	R	CA	5	1.19995	2.74952	24.4
SOST KO 6 Month KO	R	CR	5	1.24966	2.86343	25.4
SOST KO 6 Month KO	R	L	5	1.69314	3.87960	34.4
SOST KO 6 Month KO	R	M	5	1.08551	2.48730	22.1
SOST KO 6 Month WT	L	CA	5	1.33389	3.05643	27.1
SOST KO 6 Month WT	L	CR	6	1.04179	2.24151	13.8
SOST KO 6 Month WT	L	L	5	2.07140	4.74633	42.1
SOST KO 6 Month WT	L	M	5	1.14767	2.62972	23.3
SOST KO 6 Month WT	R	CA	3	0.23583	0.66947	37.6
SOST KO 6 Month WT	R	CR	4	1.43499	3.58198	58.5
SOST KO 6 Month WT	R	L	5	1.76411	4.04223	35.9
SOST KO 6 Month WT	R	M	3	0.73833	2.09591	117.8
SOST TG 6 Month DEF	L	CA	9	2.17596	4.14326	14.8
SOST TG 6 Month DEF	L	CR	8	2.74369	5.40095	22.0
SOST TG 6 Month DEF	L	L	8	2.17631	4.28406	17.4
SOST TG 6 Month DEF	L	M	9	2.21762	4.22258	15.1
SOST TG 6 Month DEF	R	CA	7	1.01812	2.08562	10.1

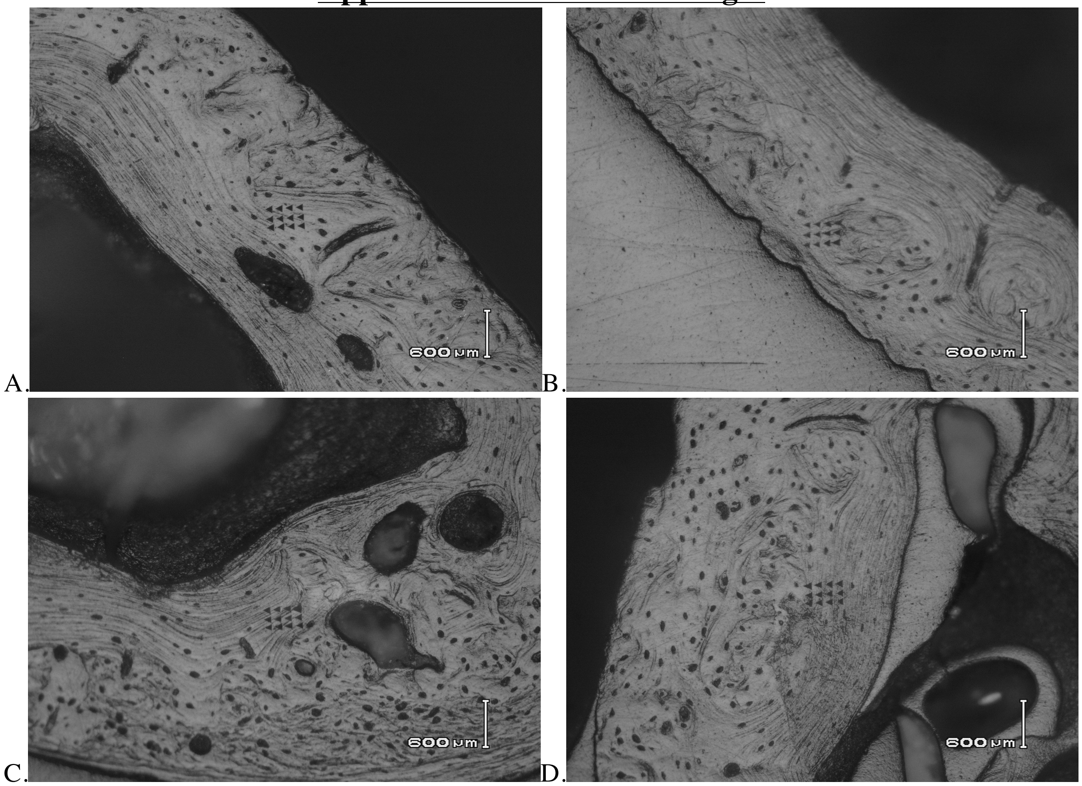
SOST TG 6 Month DEF	R	CR	7	1.64278	3.36525	16.3
SOST TG 6 Month DEF	R	L	6	2.14228	4.60929	28.5
SOST TG 6 Month DEF	R	M	6	1.20282	2.58798	16.0
SOST TG 6 Month TG	L	CA	2	0.22430	0.80781	2036.8
SOST TG 6 Month TG	L	CR	2	1.18036	4.25100	10718.1
SOST TG 6 Month TG	L	L	2	0.90527	3.26028	8220.2
SOST TG 6 Month TG	L	M	2	1.25149	4.50717	11364.0
SOST TG 6 Month TG	R	CA	2	0.75186	2.70778	6827.2
SOST TG 6 Month TG	R	CR	2	1.23484	4.44719	11212.8
SOST TG 6 Month TG	R	L	2	0.82859	2.98412	7523.9
SOST TG 6 Month TG	R	M	2	0.43530	1.56770	3952.7
SOST TG 6 Month WT	L	CA	10	2.65402	4.91221	15.8
SOST TG 6 Month WT	L	CR	10	1.88544	3.48967	11.2
SOST TG 6 Month WT	L	L	10	2.39594	4.43454	14.3
SOST TG 6 Month WT	L	M	10	2.24628	4.15754	13.4
SOST TG 6 Month WT	R	CA	9	1.72342	3.28158	11.7
SOST TG 6 Month WT	R	CR	7	2.97936	6.10323	29.6
SOST TG 6 Month WT	R	L	10	2.50896	4.64371	14.9
SOST TG 6 Month WT	R	M	9	2.05481	3.91259	14.0
SOST TG 8 Month DEF	L	CA	3	0.57531	1.63315	91.8
SOST TG 8 Month DEF	L	CR	4	2.54999	6.36522	103.9
SOST TG 8 Month DEF	L	L	4	0.71861	1.79378	29.3
SOST TG 8 Month DEF	L	M	4	0.71911	1.79504	29.3
SOST TG 8 Month DEF	R	CA	3	0.17583	0.49913	28.1
SOST TG 8 Month DEF	R	CR	4	0.79841	1.99298	32.5
SOST TG 8 Month DEF	R	L	3	0.55810	1.58428	89.1
SOST TG 8 Month DEF	R	M	3	1.97450	5.60506	315.1
SOST TG 8 Month TG	L	CA	2	1.16977	4.21287	10622.0
SOST TG 8 Month TG	L	CR	2	1.24740	4.49244	11326.9
SOST TG 8 Month TG	L	L	1	*	*	*
SOST TG 8 Month TG	L	M	2	1.90094	6.84613	17261.2
SOST TG 8 Month TG	R	CA	2	0.67354	2.42573	6116.0
SOST TG 8 Month TG	R	CR	2	0.68615	2.47112	6230.5
SOST TG 8 Month TG	R	L	2	0.04302	0.15492	390.6
SOST TG 8 Month TG	R	M	2	0.13306	0.47922	1208.3
SOST TG 8 Month WT	L	CA	4	0.83539	2.08527	34.0
SOST TG 8 Month WT	L	CR	2	0.68075	2.45168	6181.5
SOST TG 8 Month WT	L	L	3	1.22909	3.48905	196.1
SOST TG 8 Month WT	L	M	4	2.71132	6.76793	110.5
SOST TG 8 Month WT	R	CA	3	0.71918	2.04155	114.8
SOST TG 8 Month WT	R	CR	2	0.42454	1.52896	3855.0
SOST TG 8 Month WT	R	L	3	0.61376	1.74230	97.9
SOST TG 8 Month WT	R	M	2	0.07671	0.27625	696.5

Bartlett's Test (Normal Distribution)
Test statistic = 73.84, p-value = 0.612

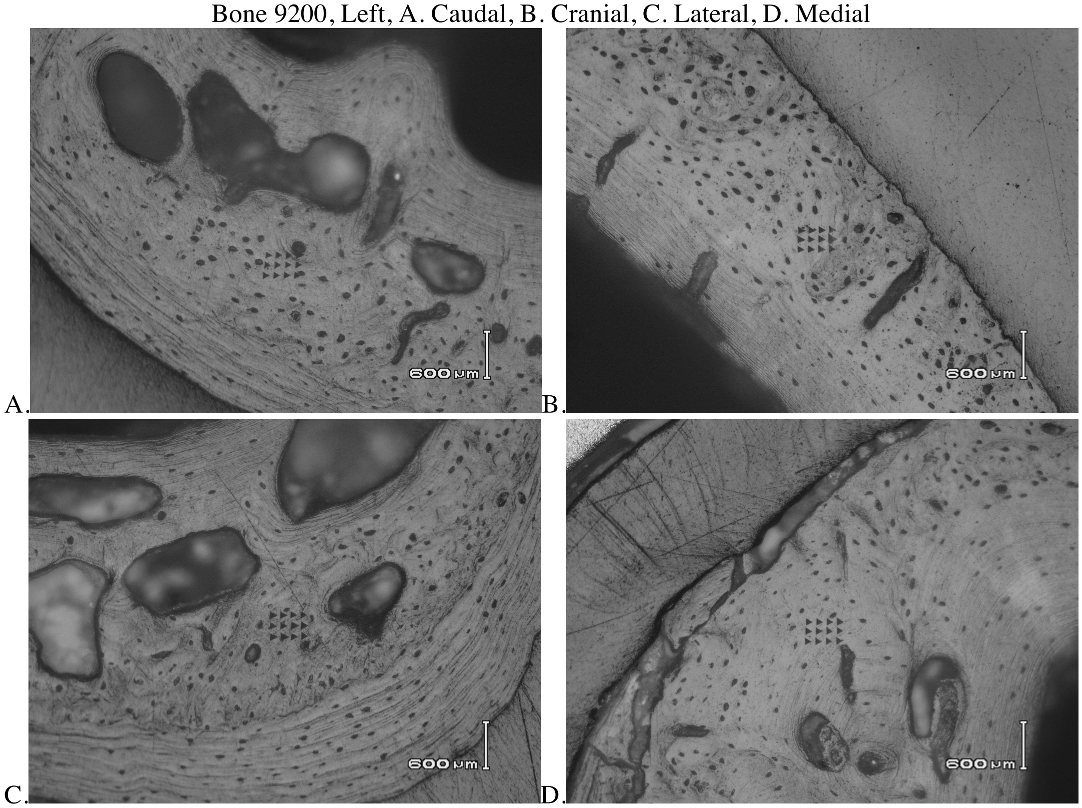
Levene's Test (Any Continuous Distribution)
Test statistic = 0.77, p-value = 0.909

Test for Equal Variances: E_mod_1 versus Genotype+Phenotype, Limb, Region

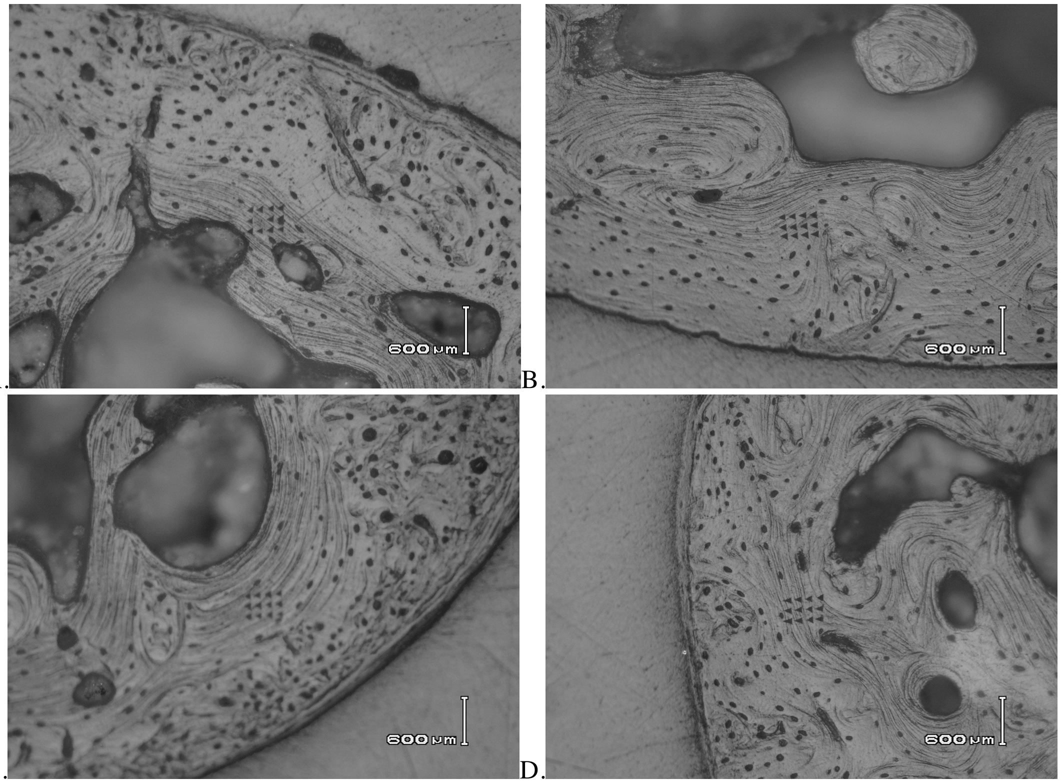
Appendix E: Indentation Images



Bone 9200, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

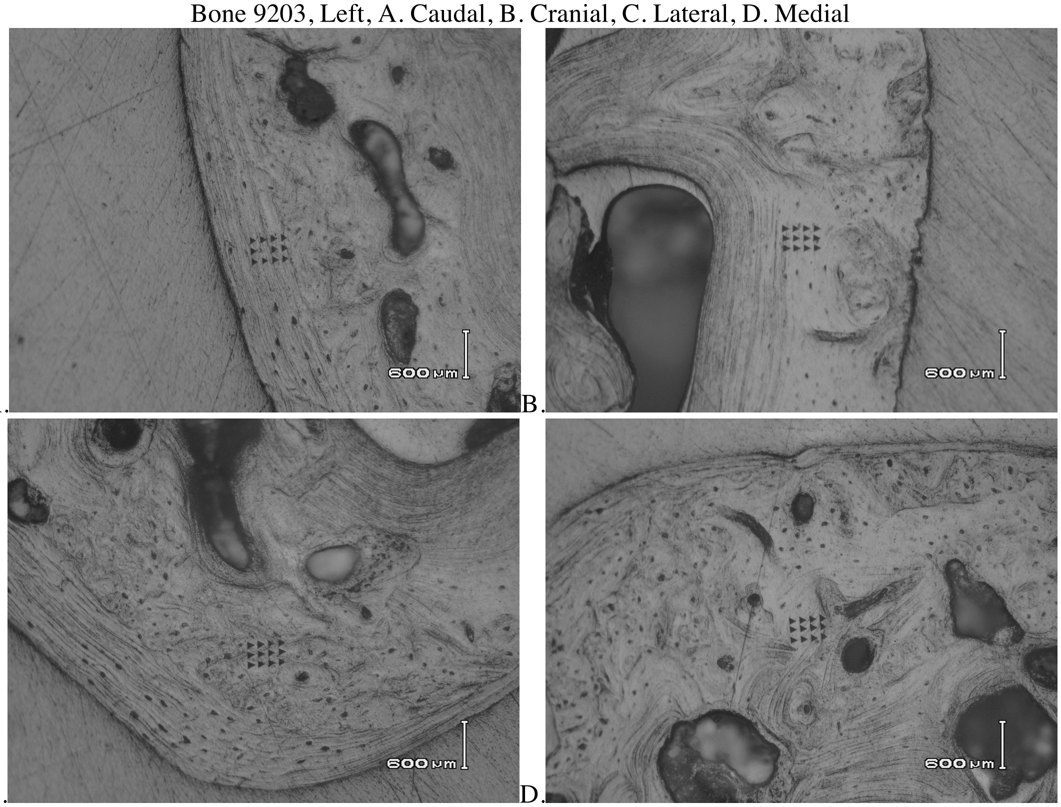


Bone 9200, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



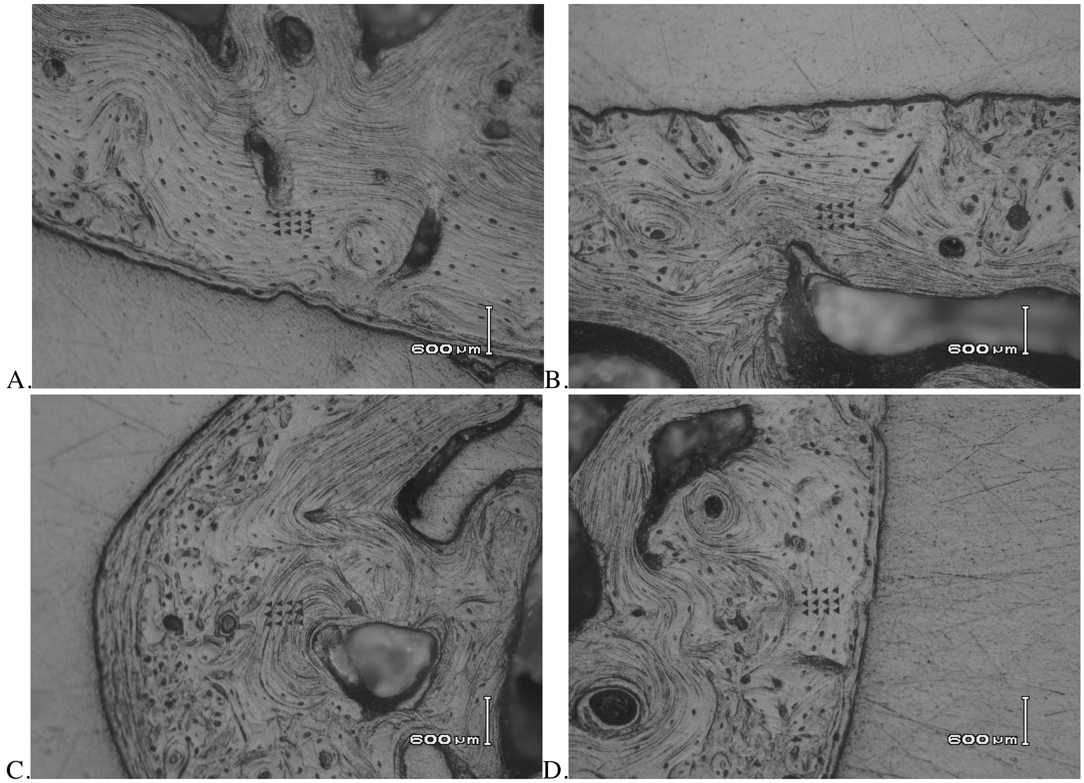
A. B. C. D.

Bone 9203, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

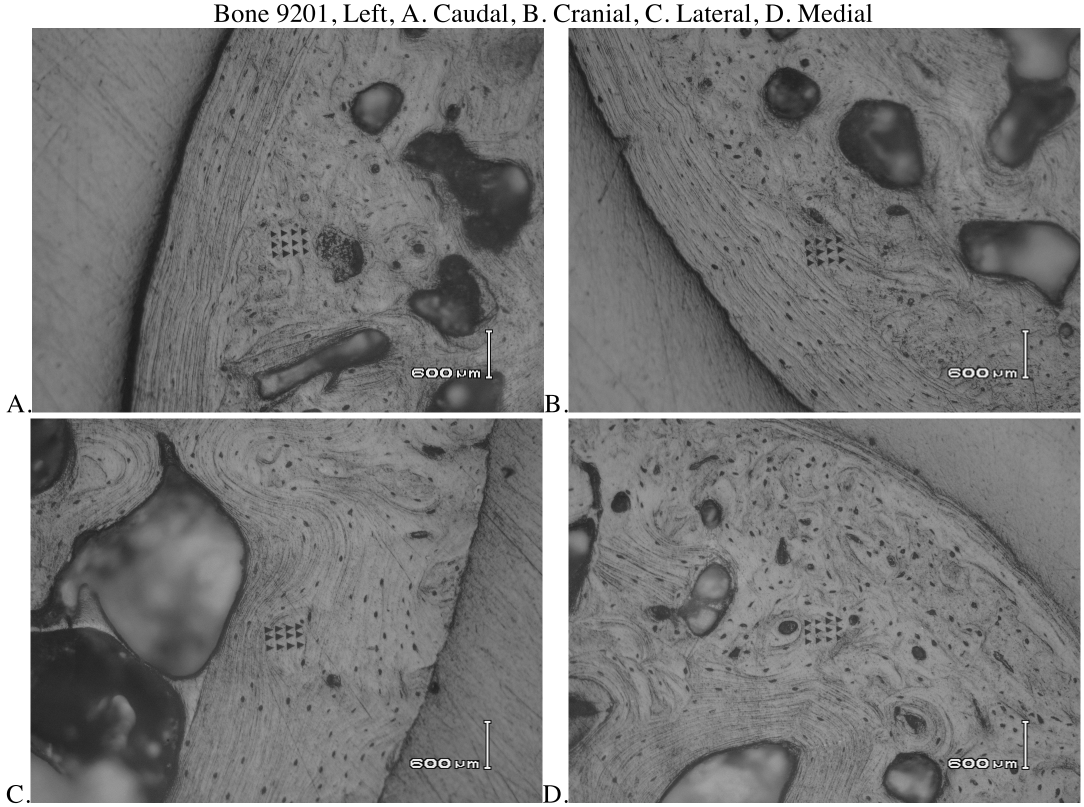


A. B. C. D.

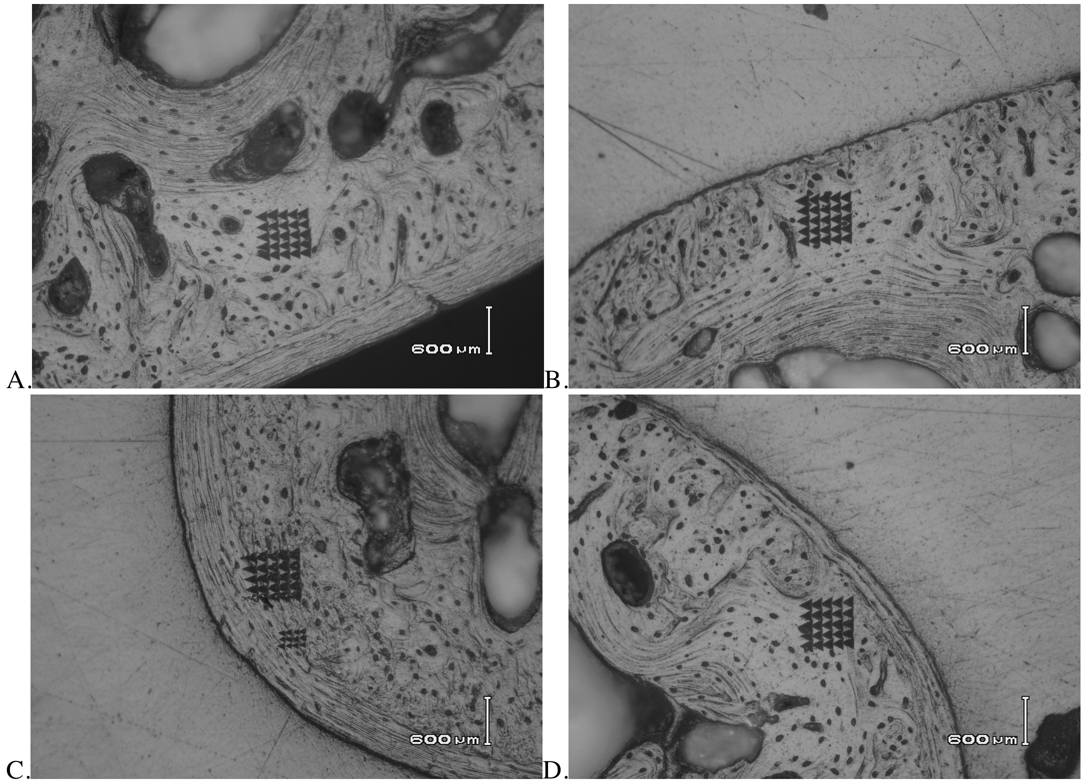
Bone 9203, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



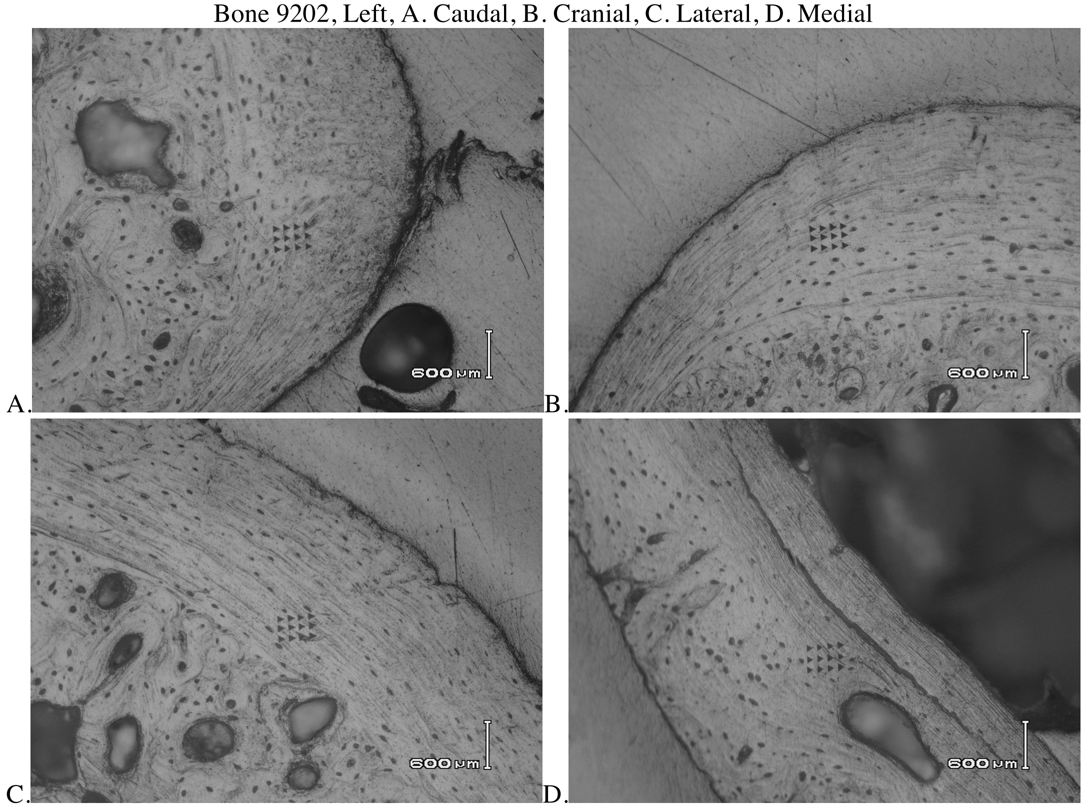
Bone 9201, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



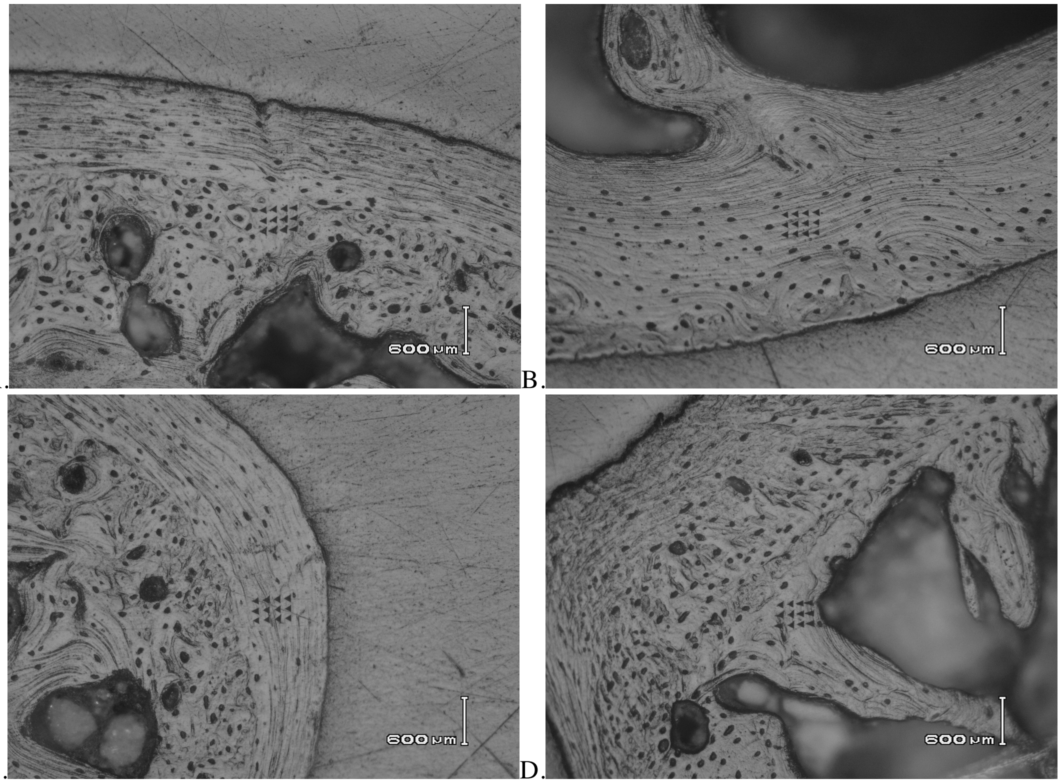
Bone 9201, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 9202, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

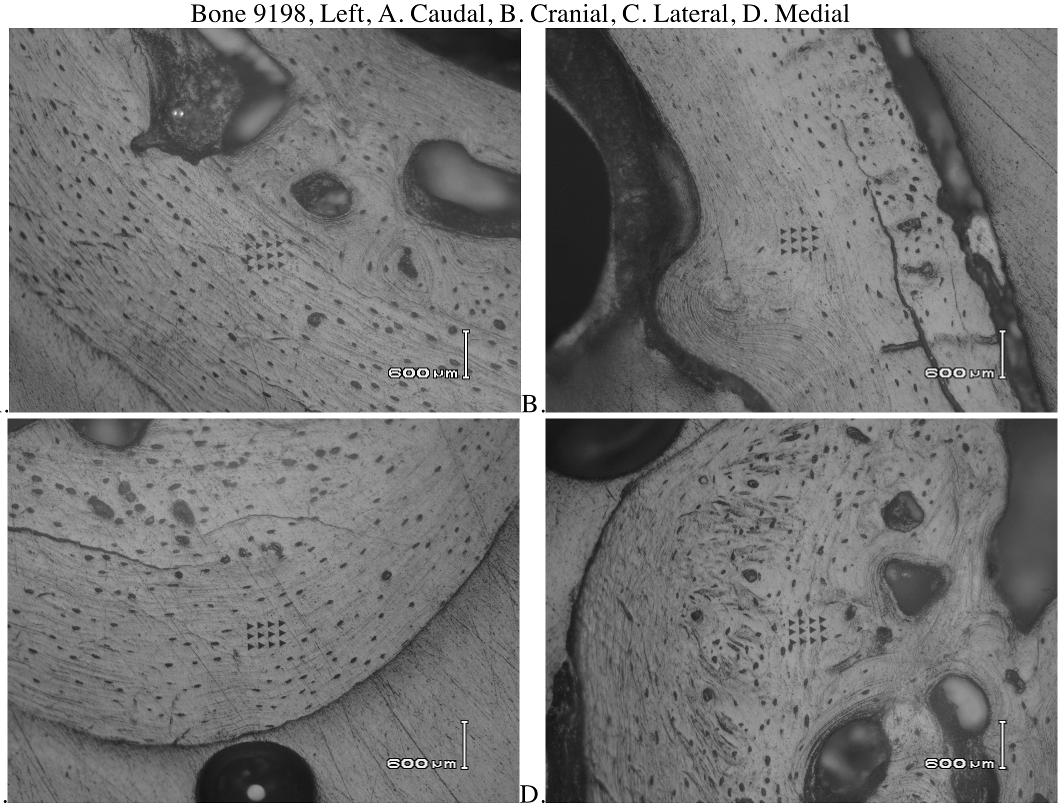


Bone 9202, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



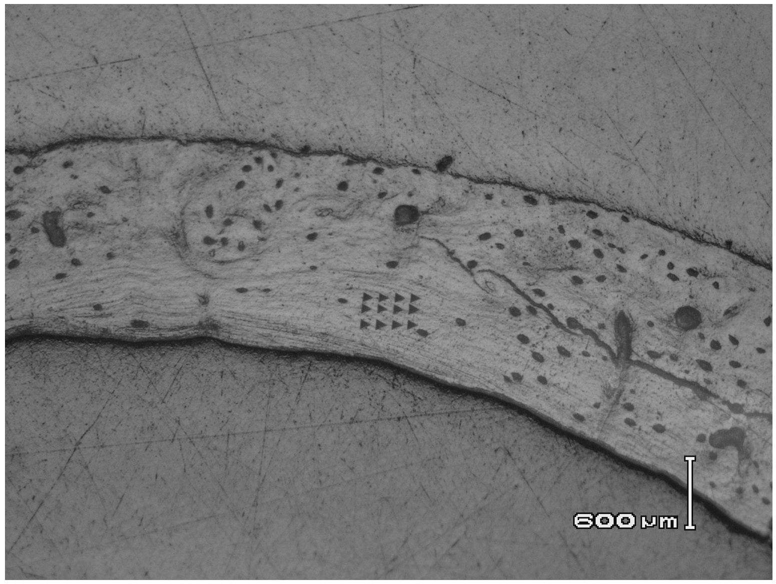
A. B. C. D.

Bone 9198, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

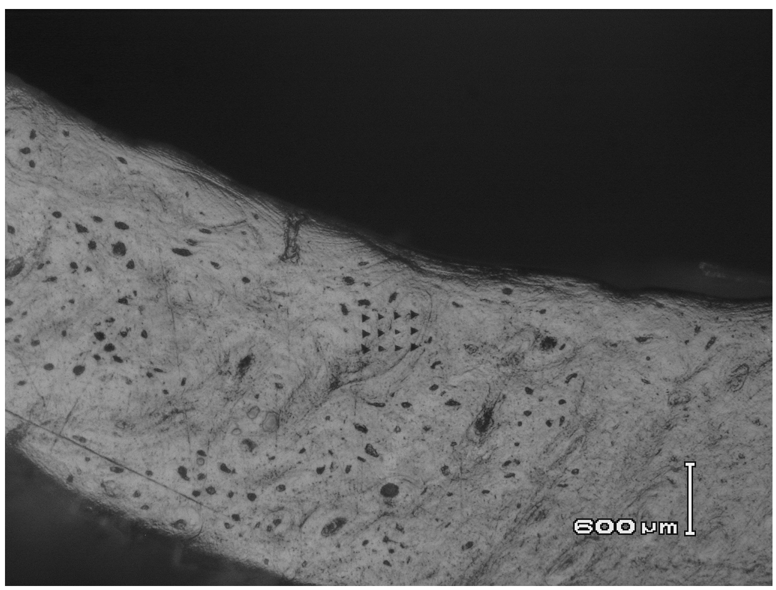


A. B. C. D.

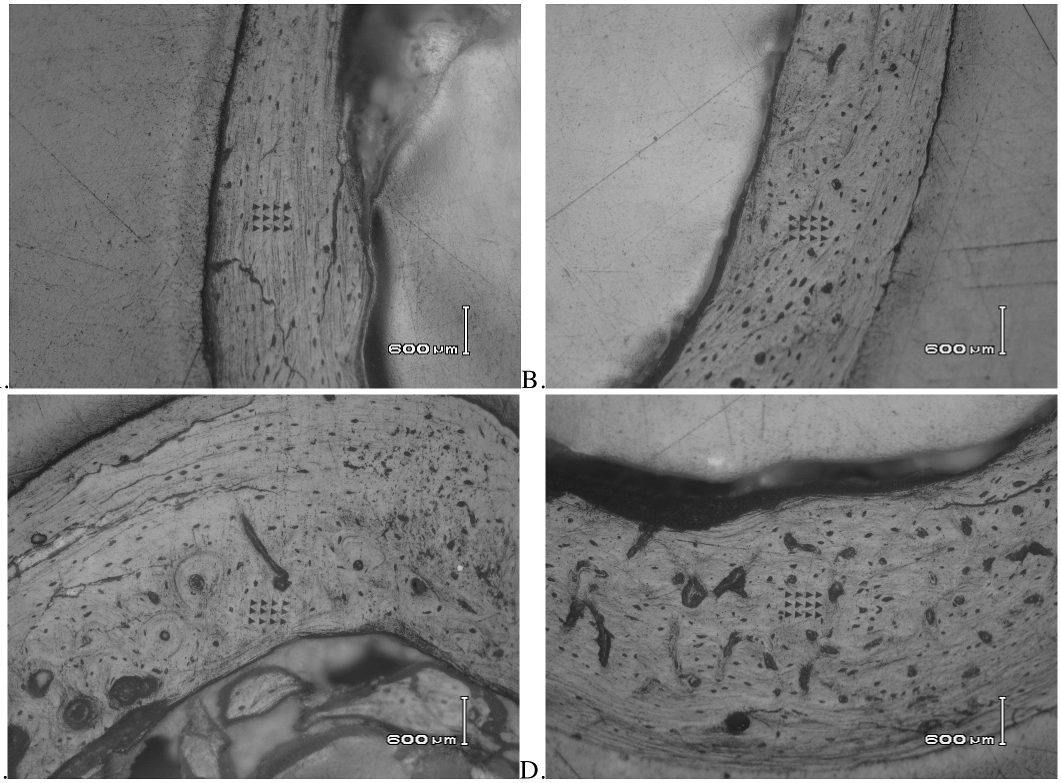
Bone 9198, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial

A. 

Bone 9209, Left, A. Cranial, Partial Exposure

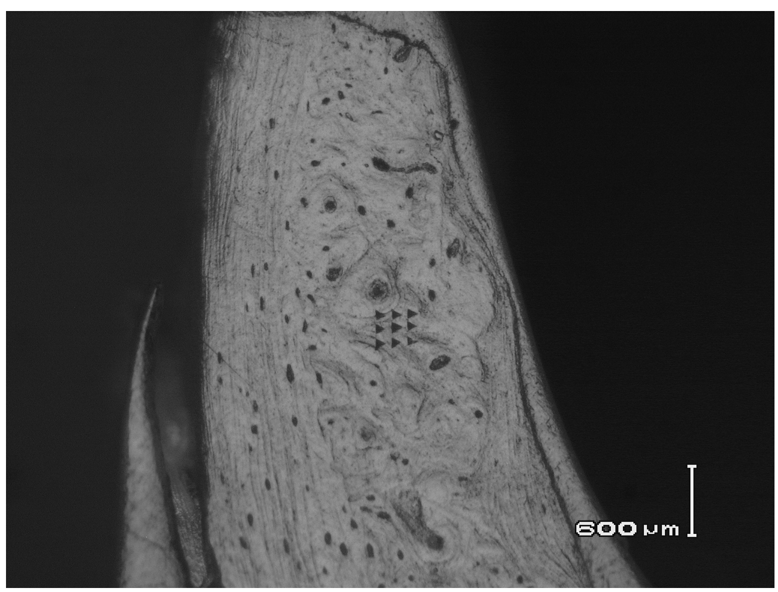
A. 

Bone 9209, Right, A. Lateral, Partial Exposure



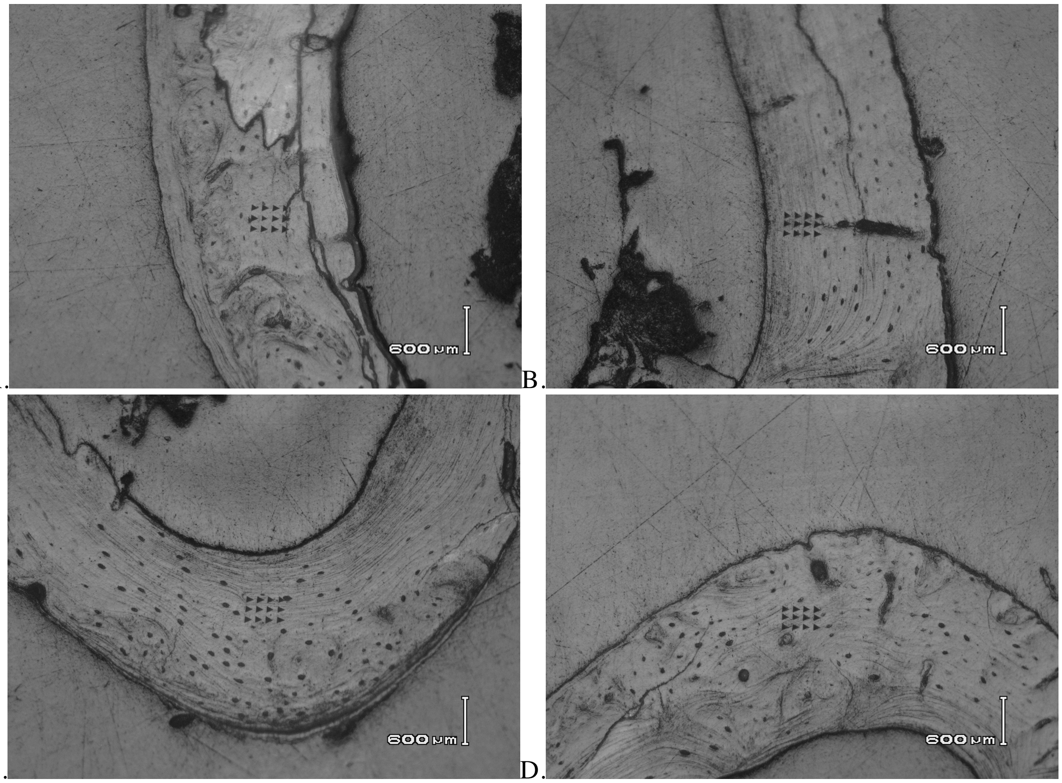
A. B. C. D.

Bone 9208, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

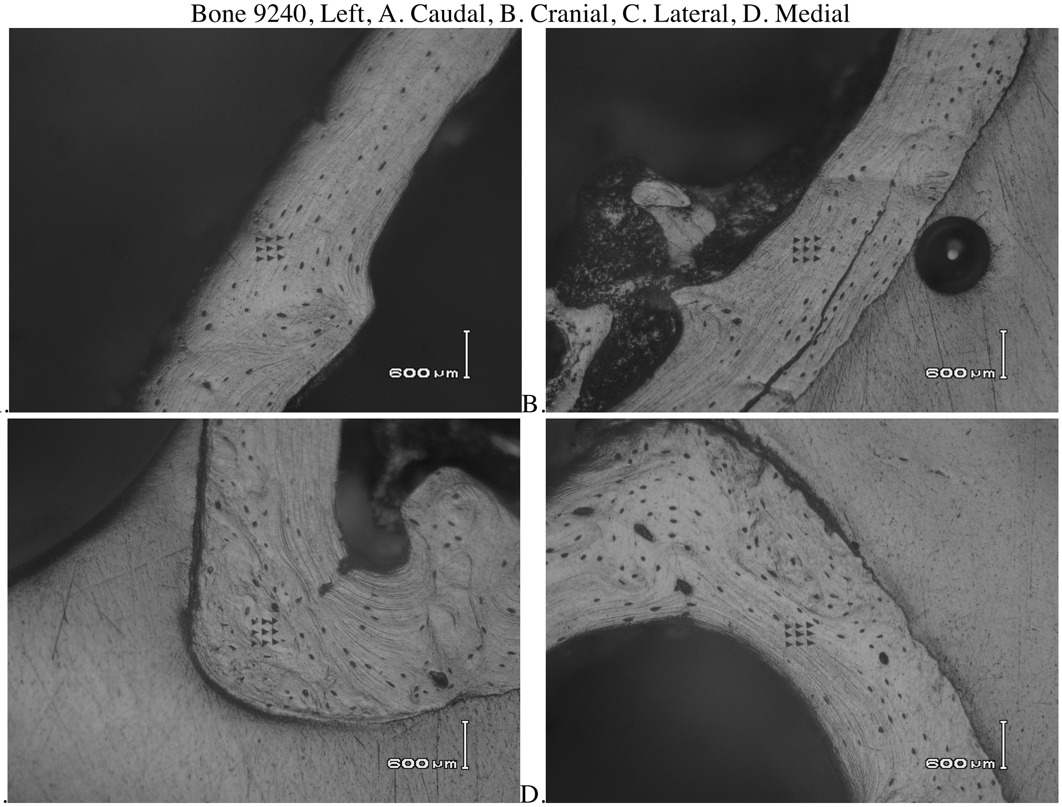


A.

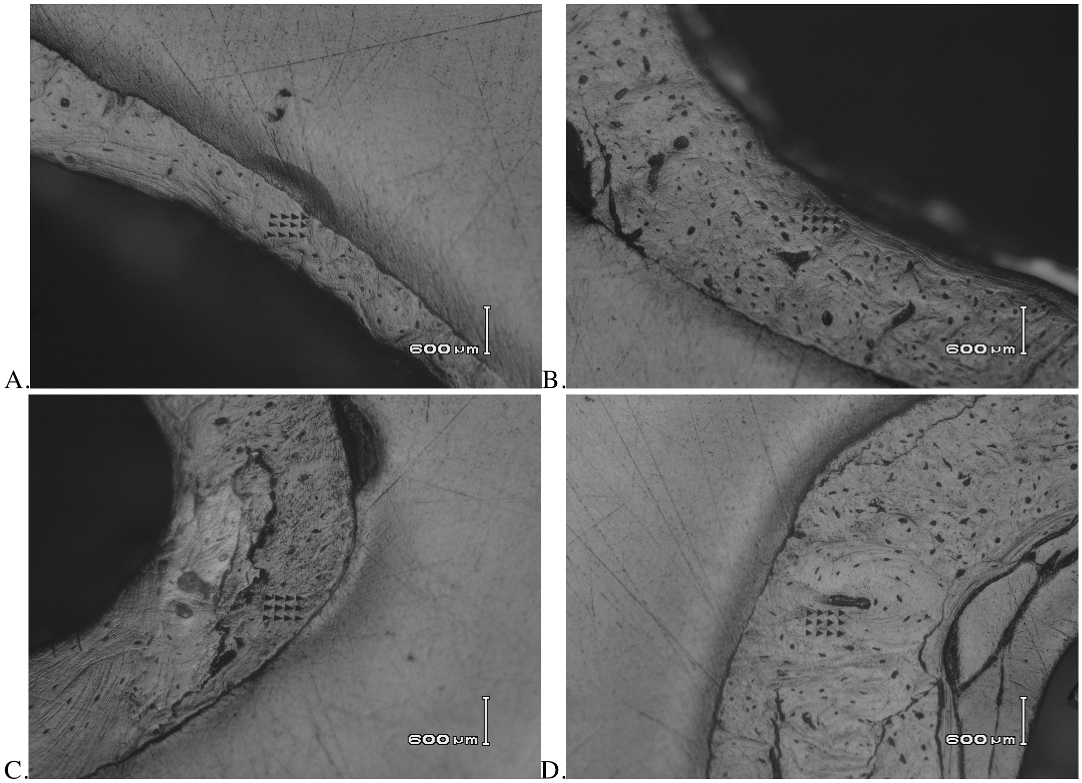
Bone 9208, Right, A. Cranial, Partial Exposure



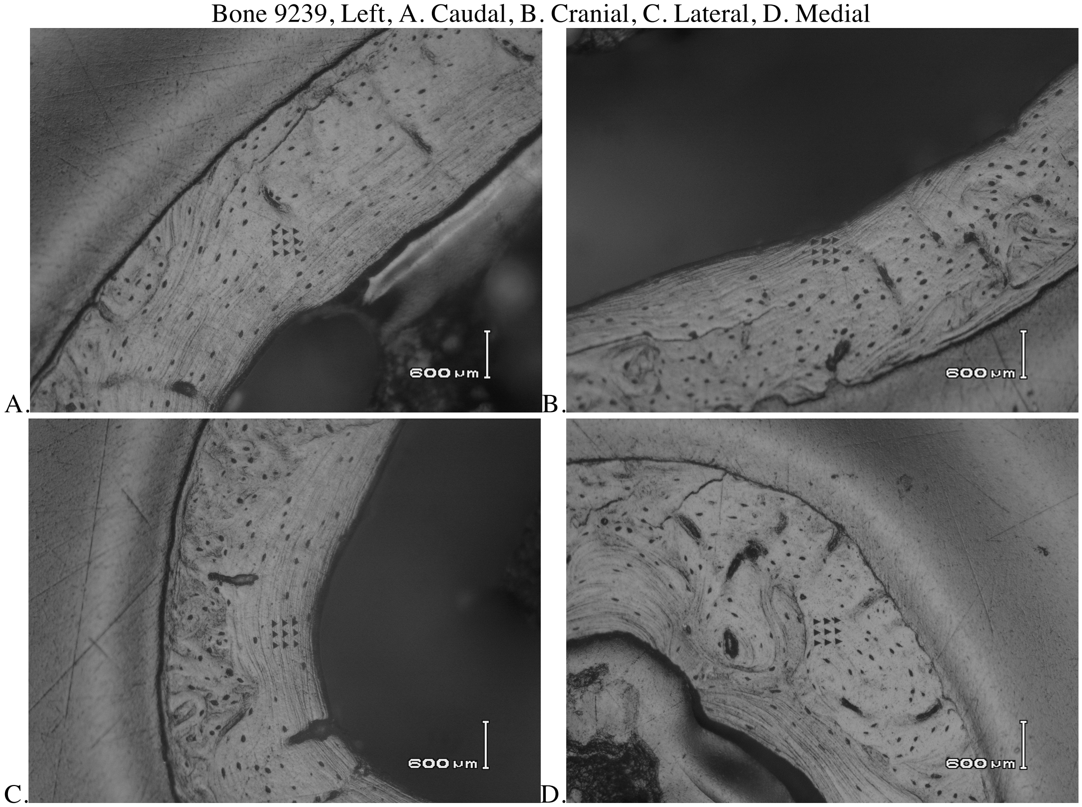
Bone 9240, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



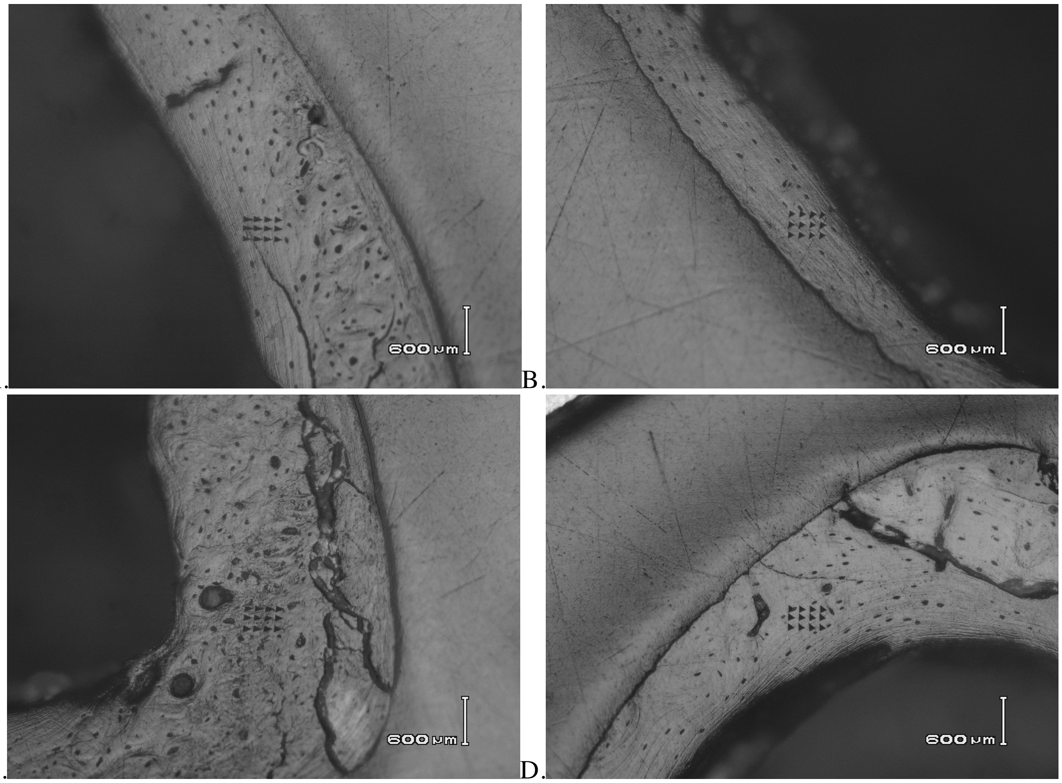
Bone 9240, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 9239, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

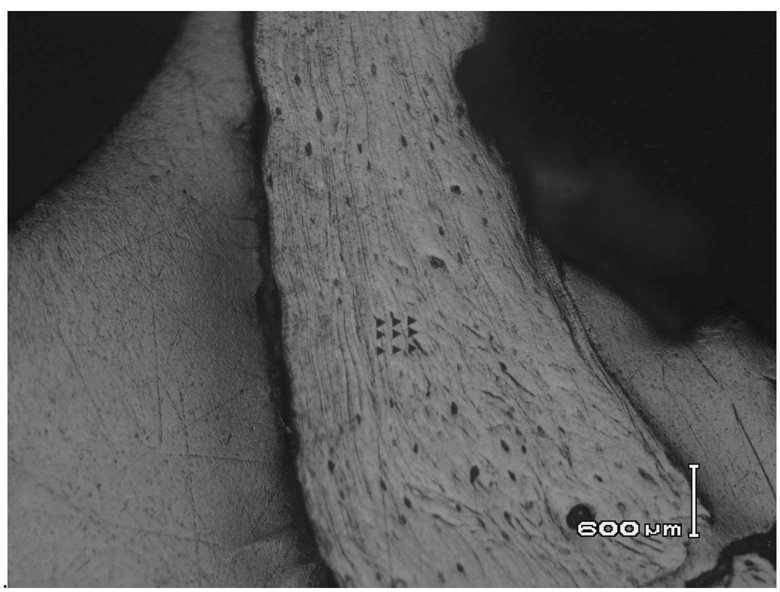


Bone 9239, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



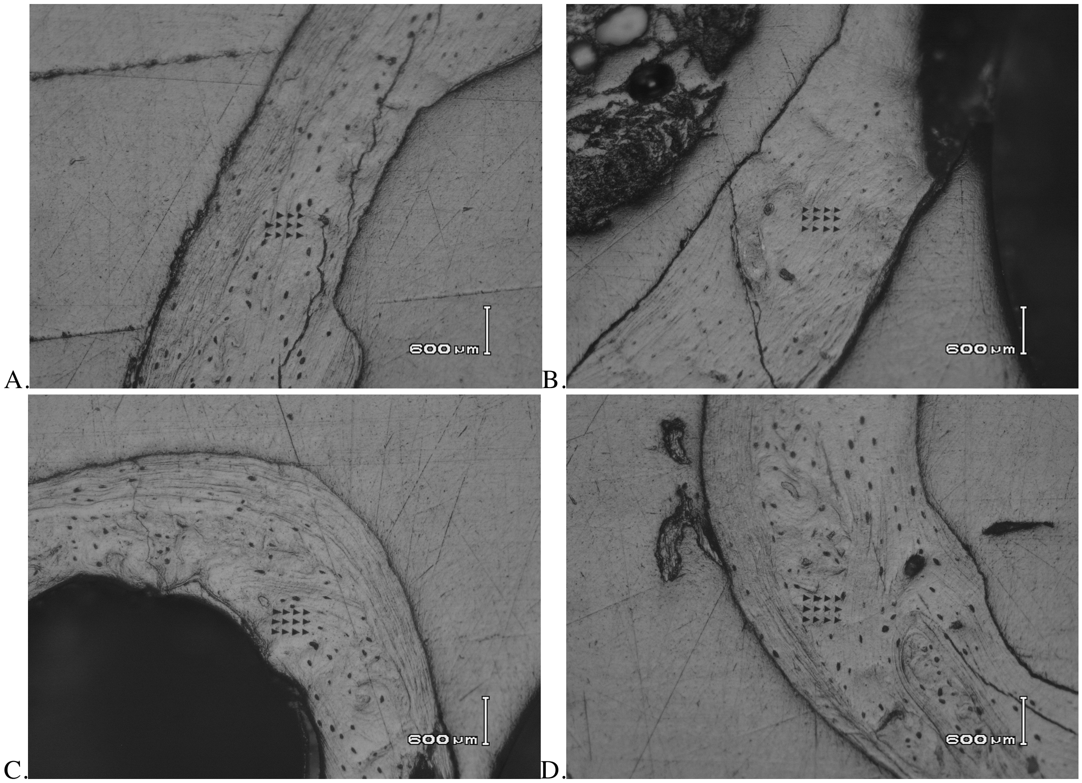
A. B. C. D.

Bone 9207, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

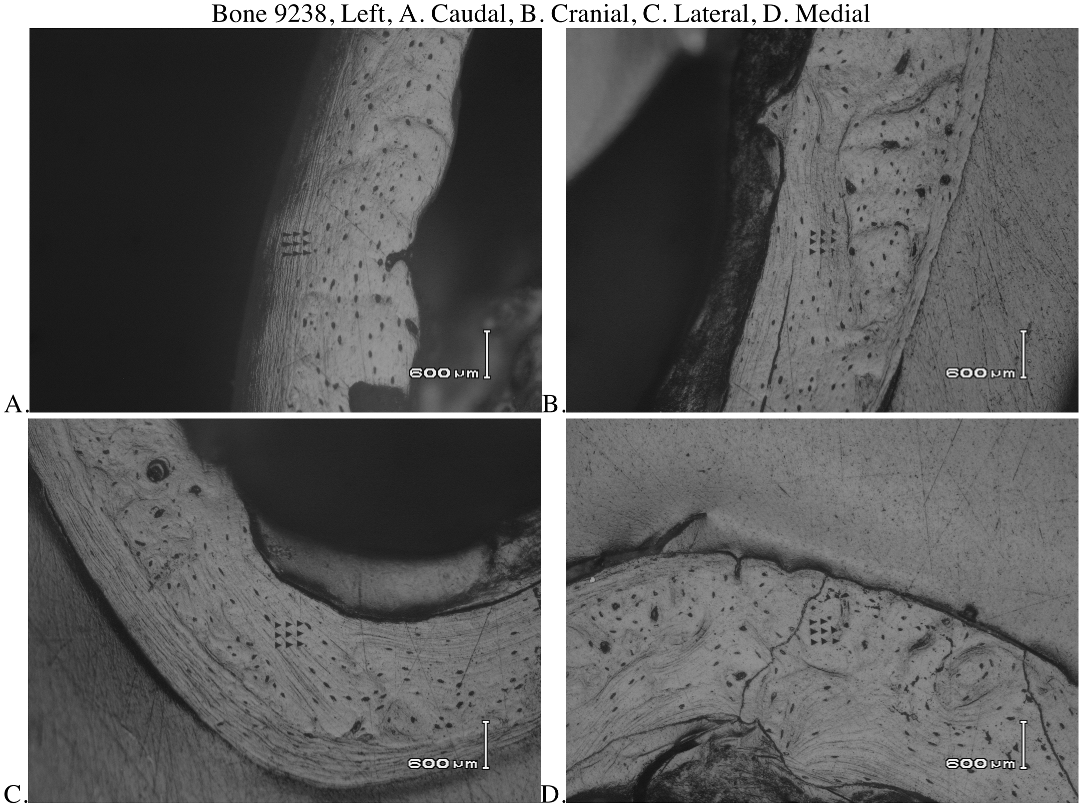


A.

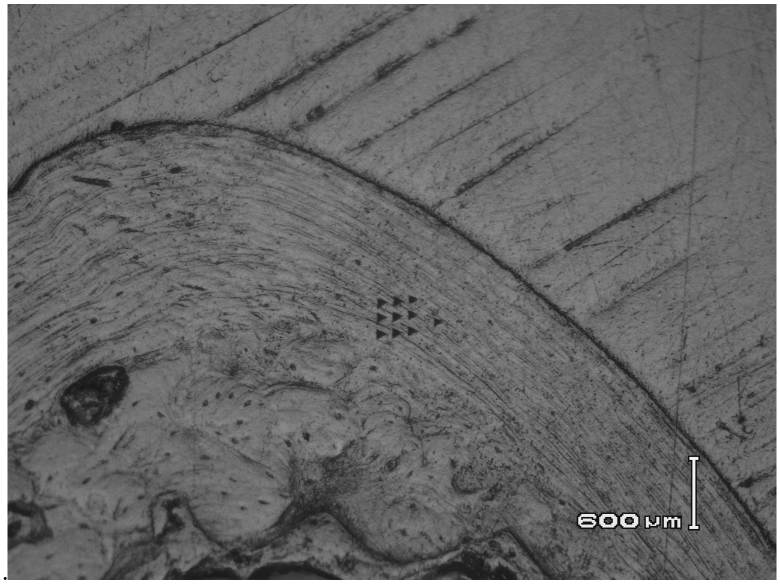
Bone 9207, Right, A. Lateral, Partial Exposure



Bone 9238, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

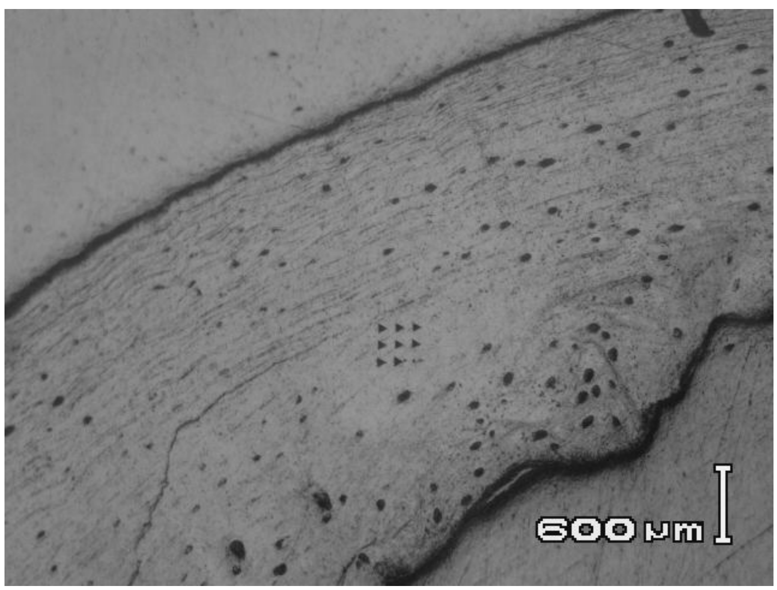


Bone 9238, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



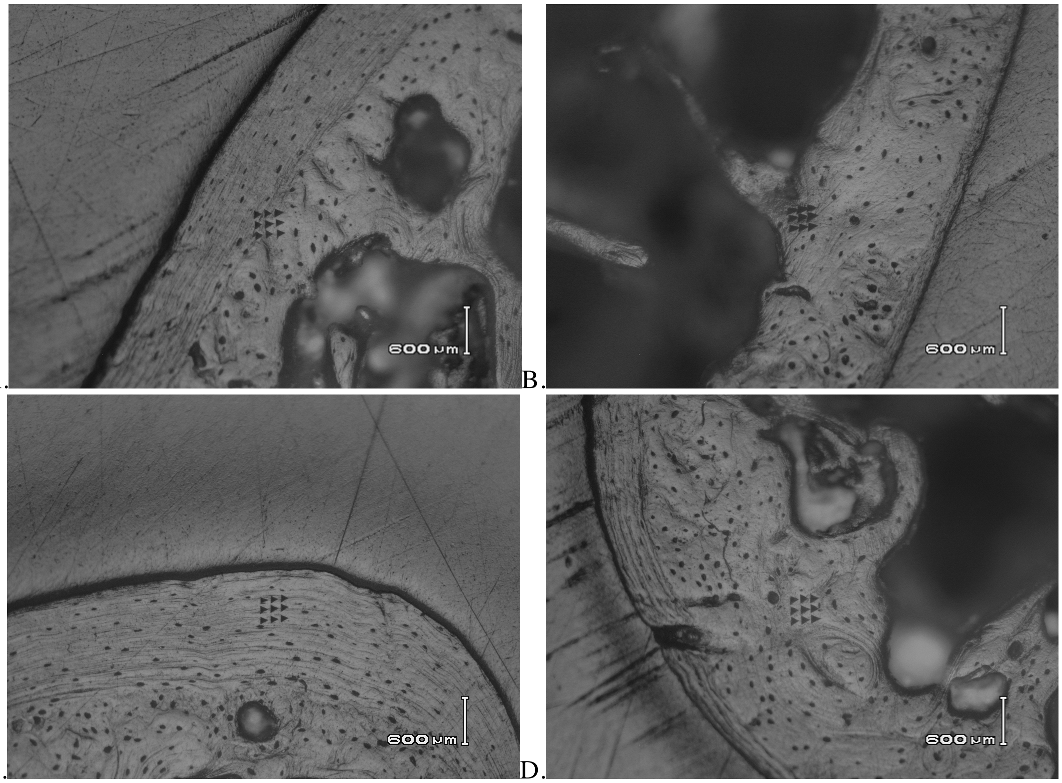
A.

Bone 7594, Left, A. Lateral, Partial Exposure

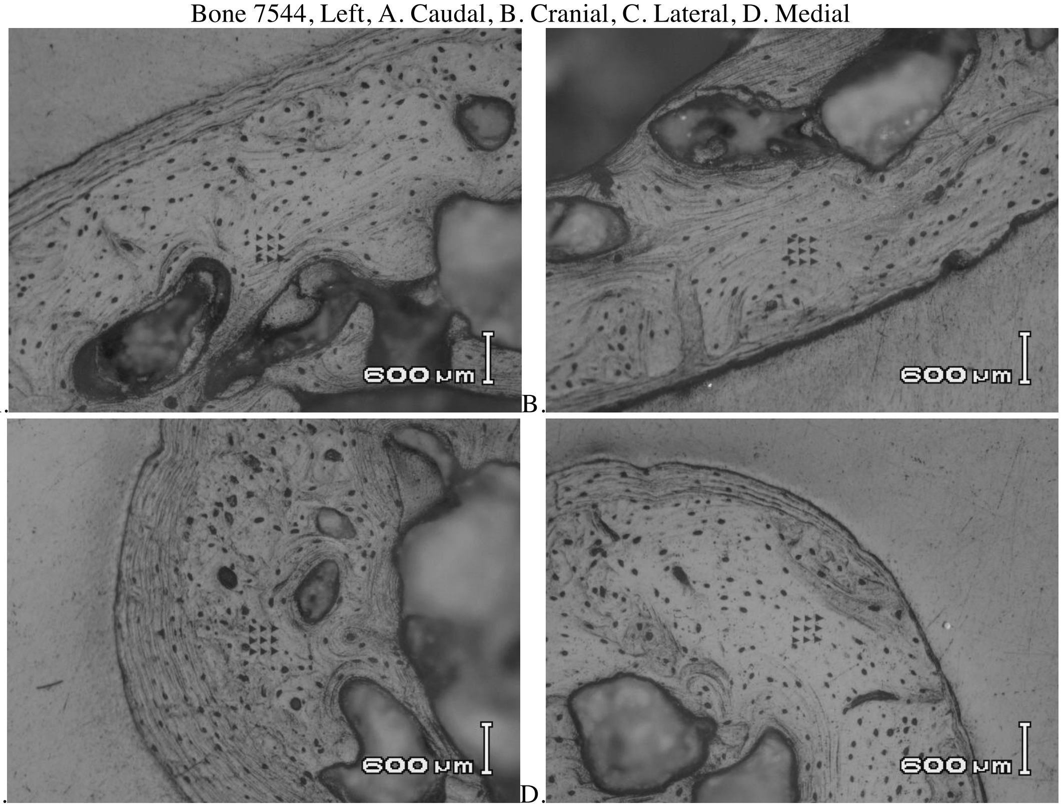


A.

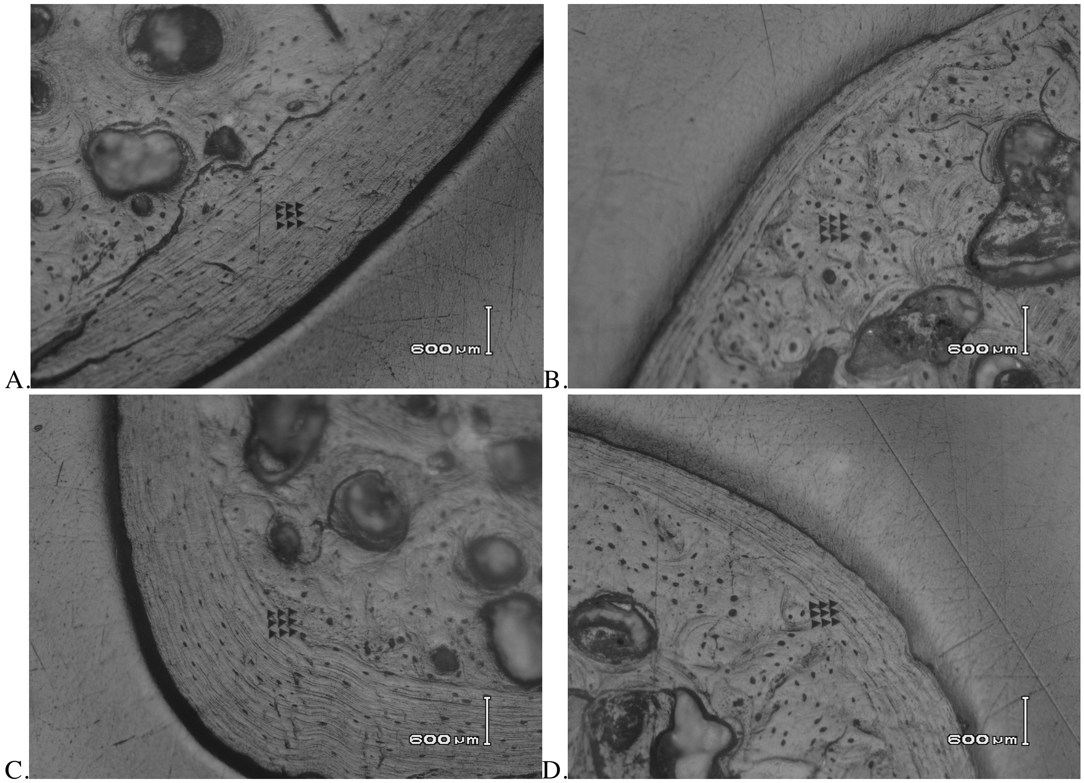
Bone 7594, Right, A. Cranial, Partial Exposure



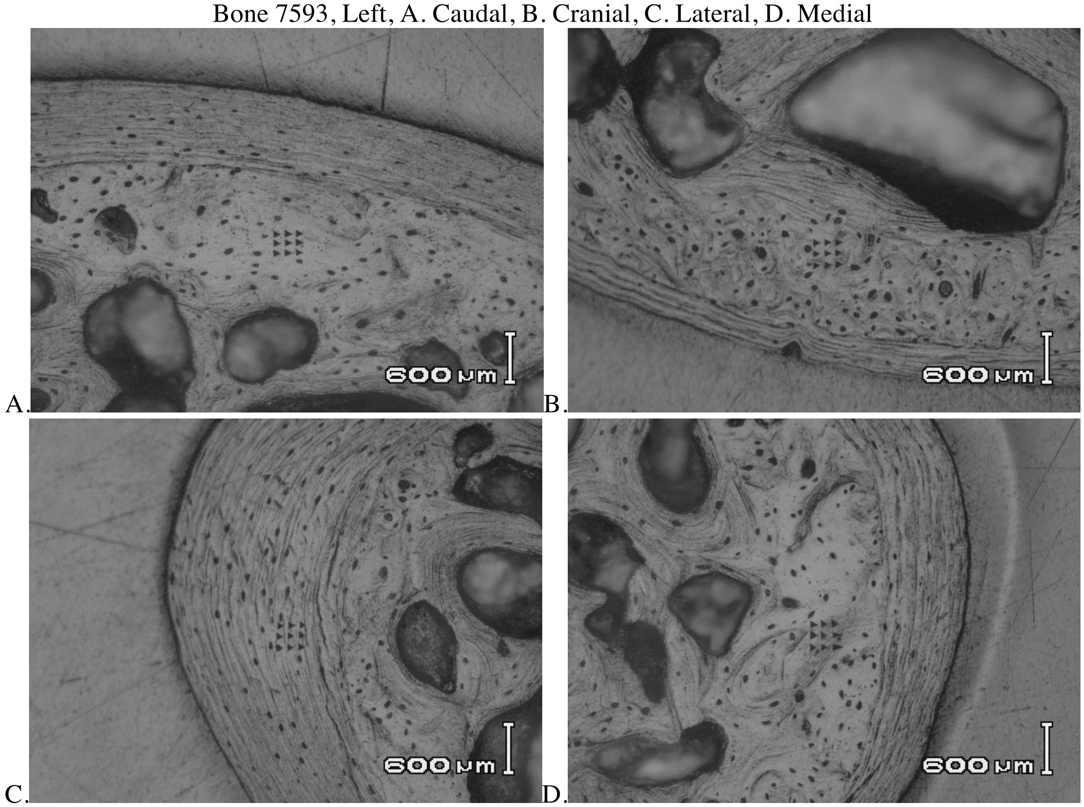
Bone 7544, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



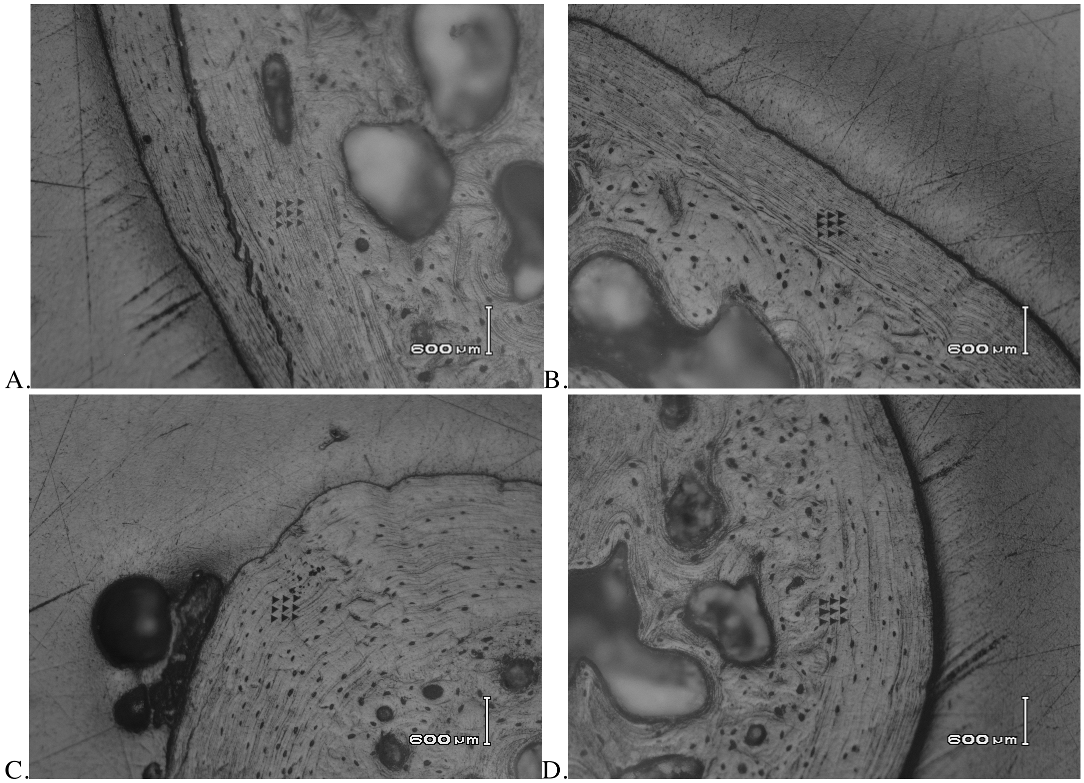
Bone 7544, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



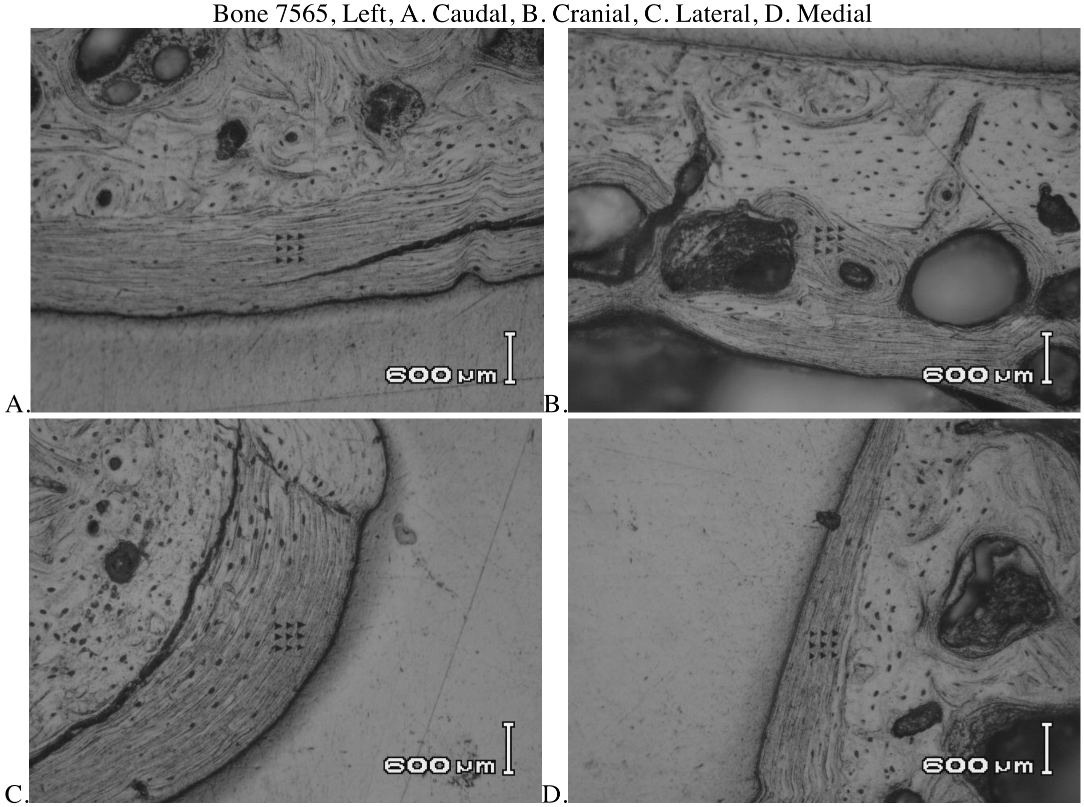
Bone 7593, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



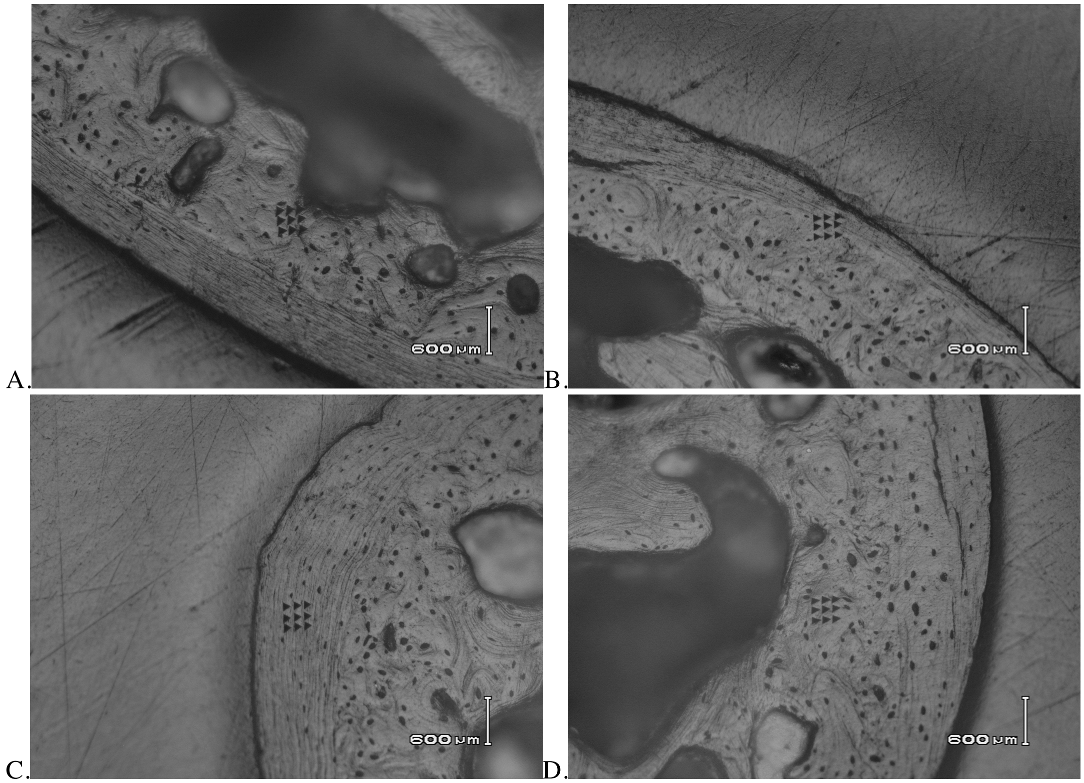
Bone 7593, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



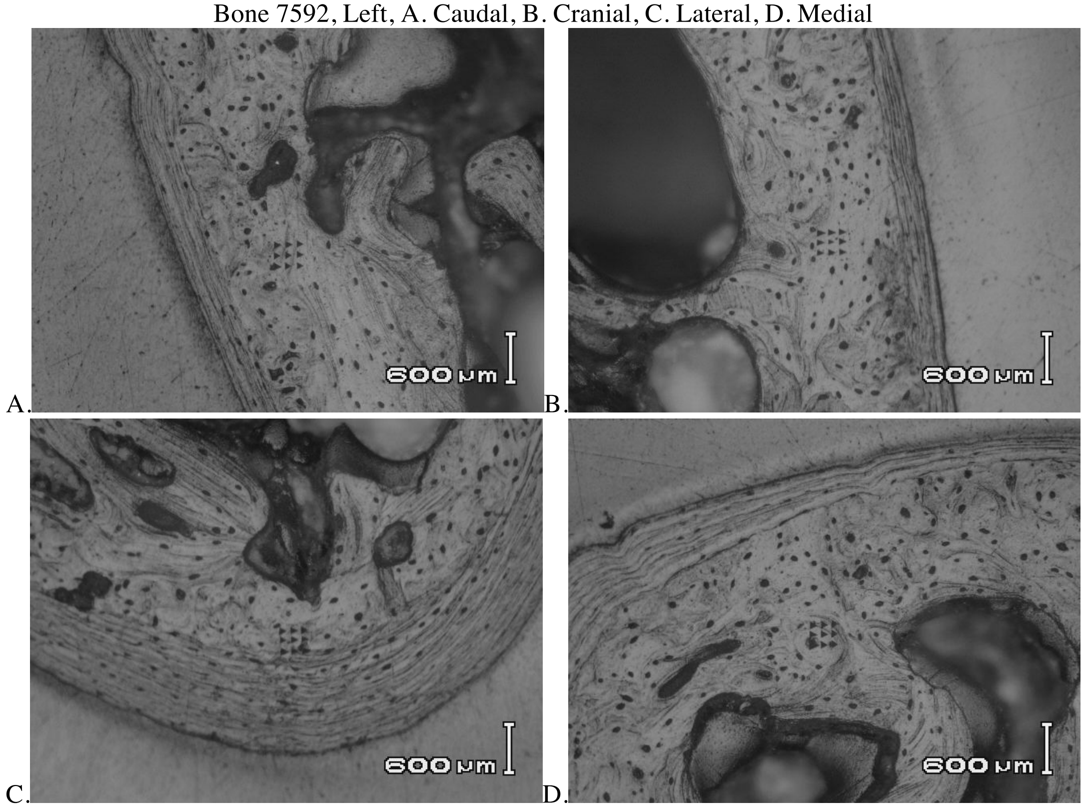
Bone 7565, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



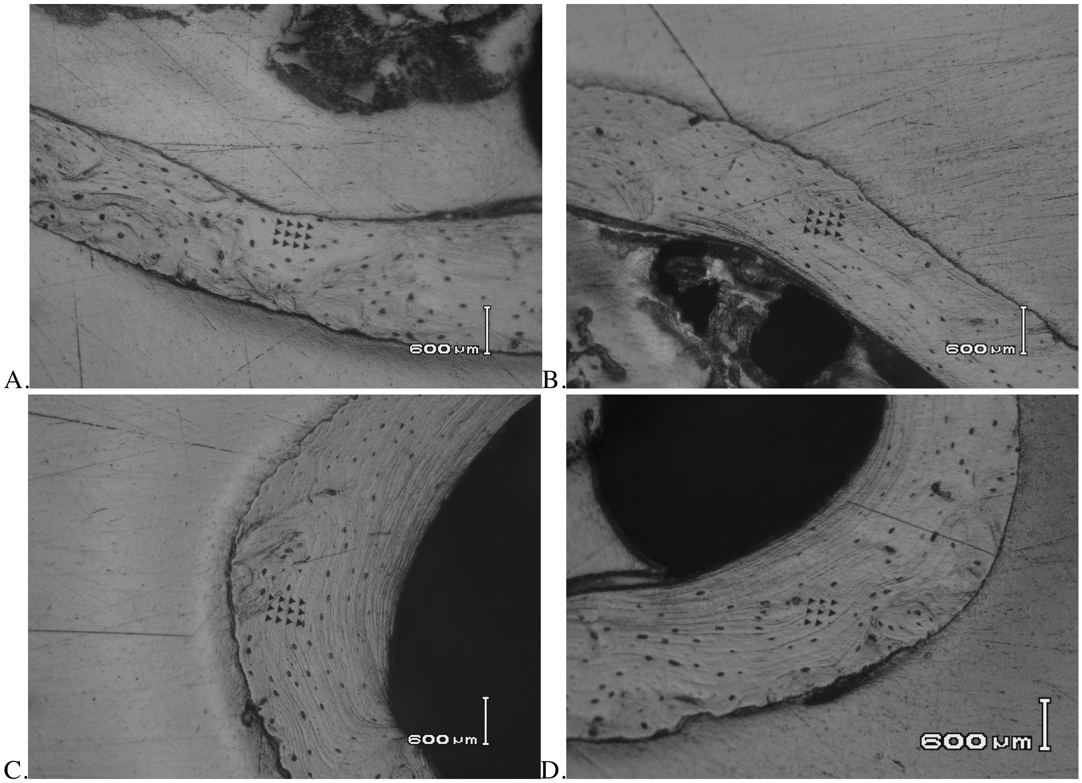
Bone 7565, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



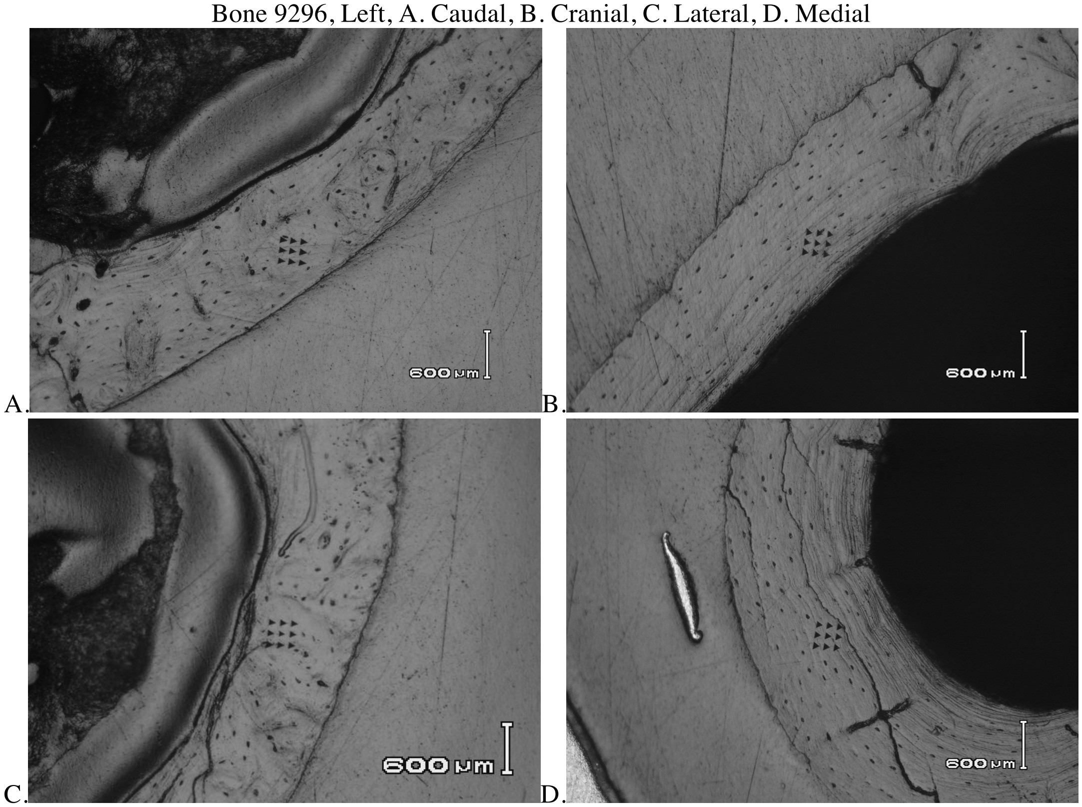
Bone 7592, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



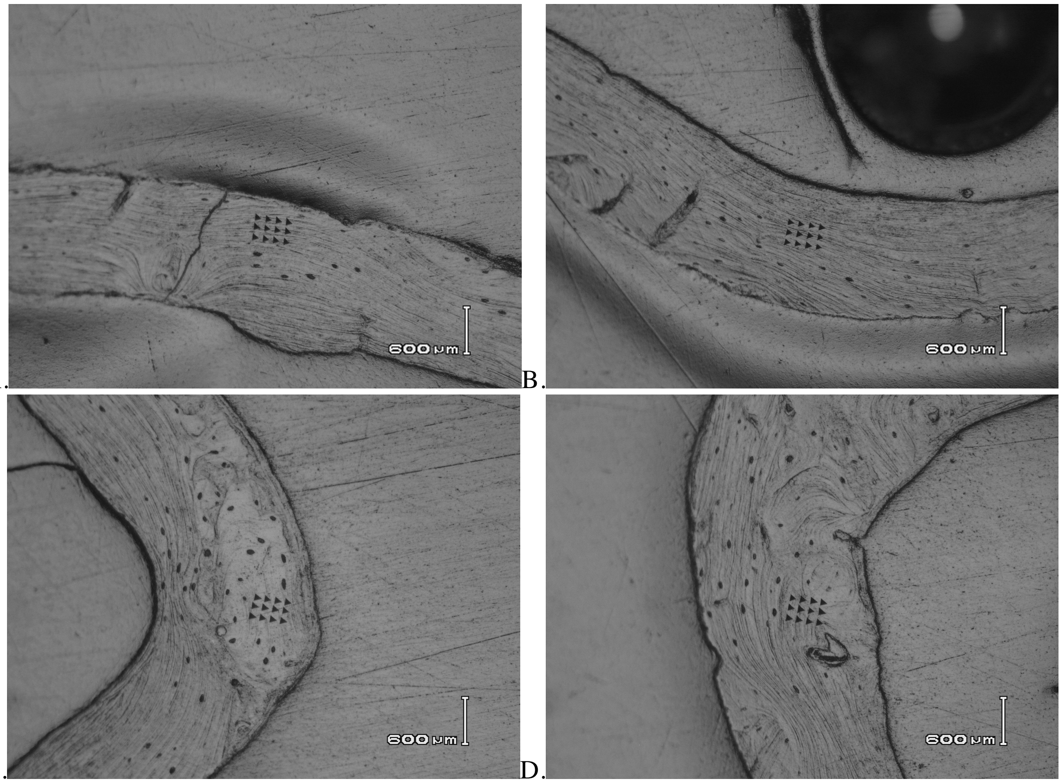
Bone 7592, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



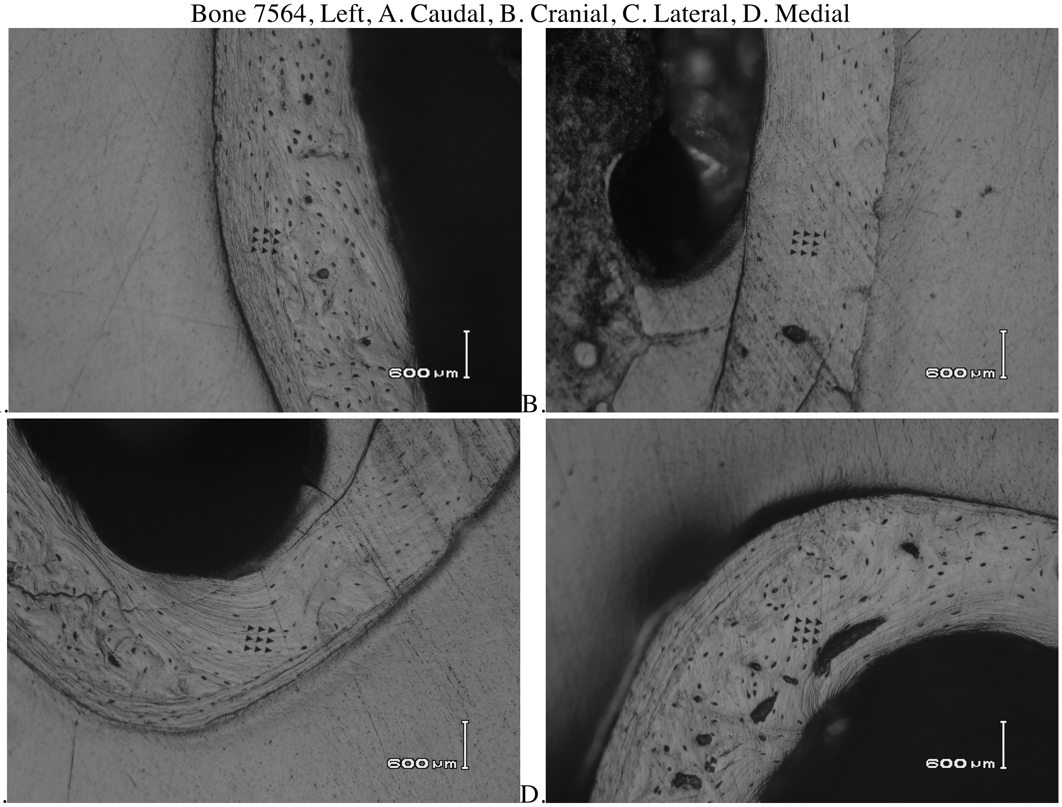
Bone 9296, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



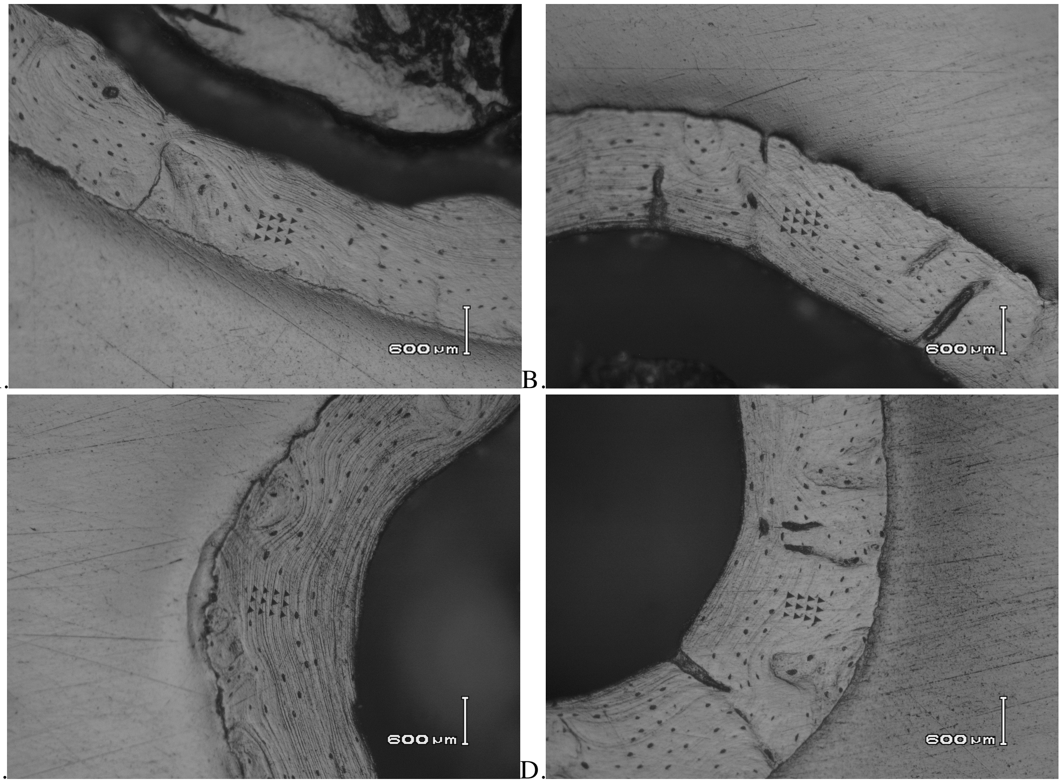
Bone 9296, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 7564, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

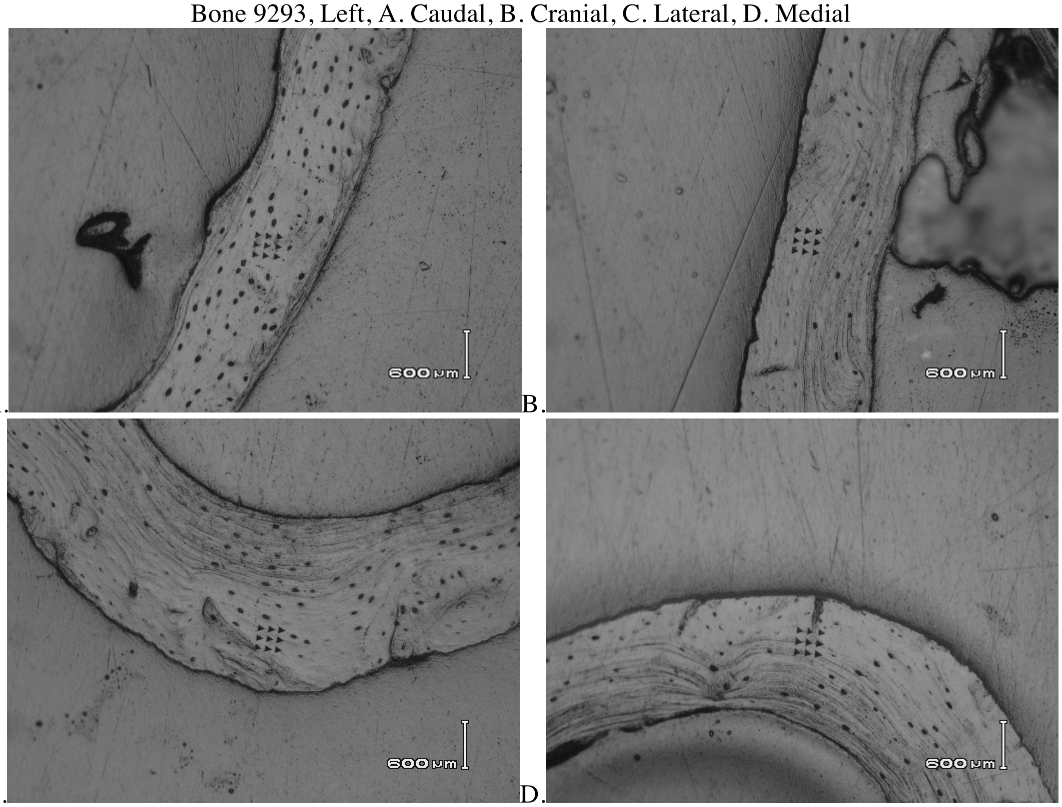


Bone 7564, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



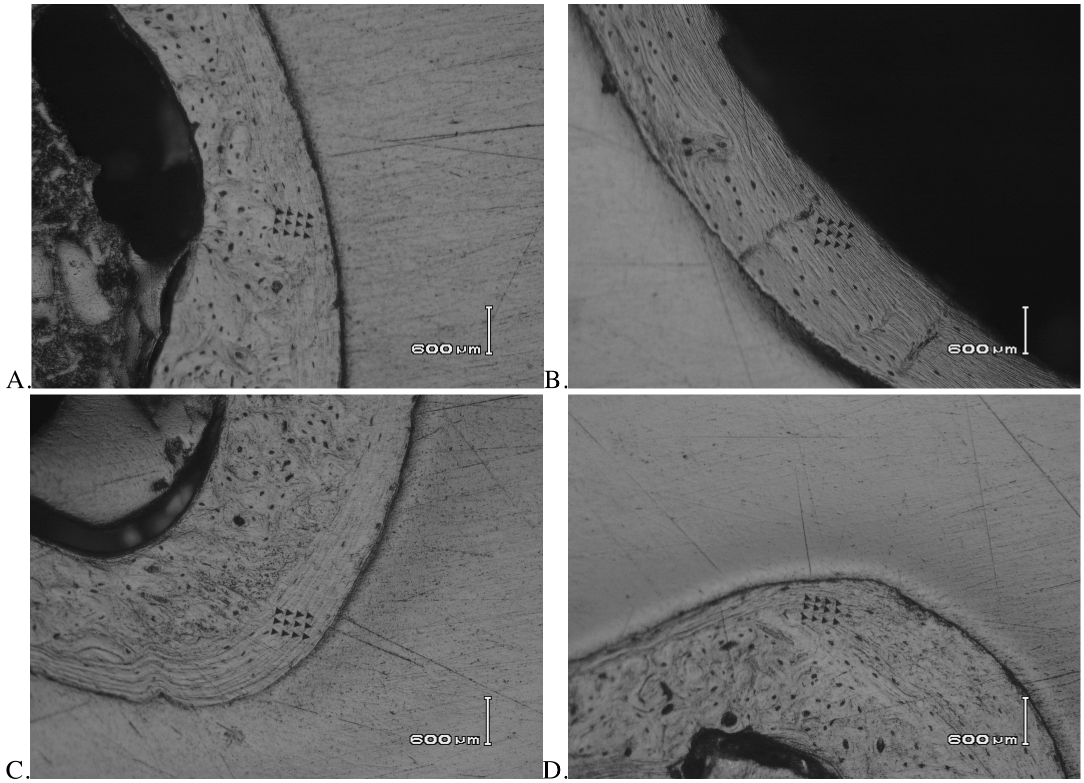
A. B. C. D.

Bone 9293, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

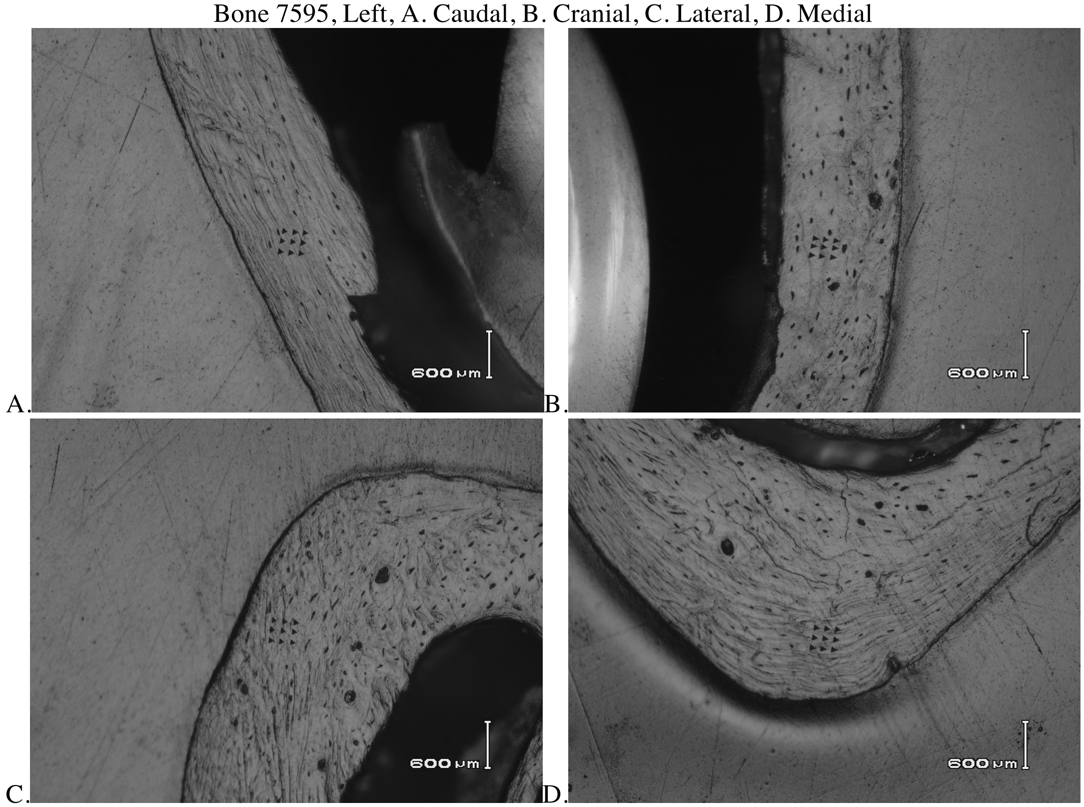


A. B. C. D.

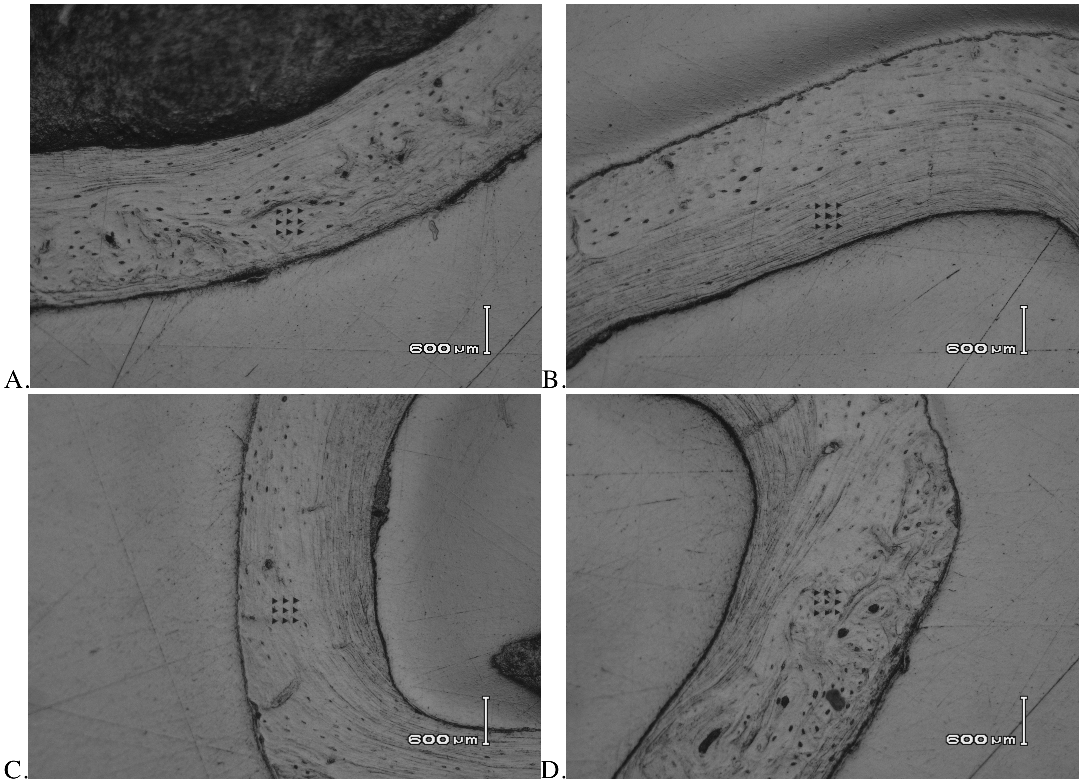
Bone 9293, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



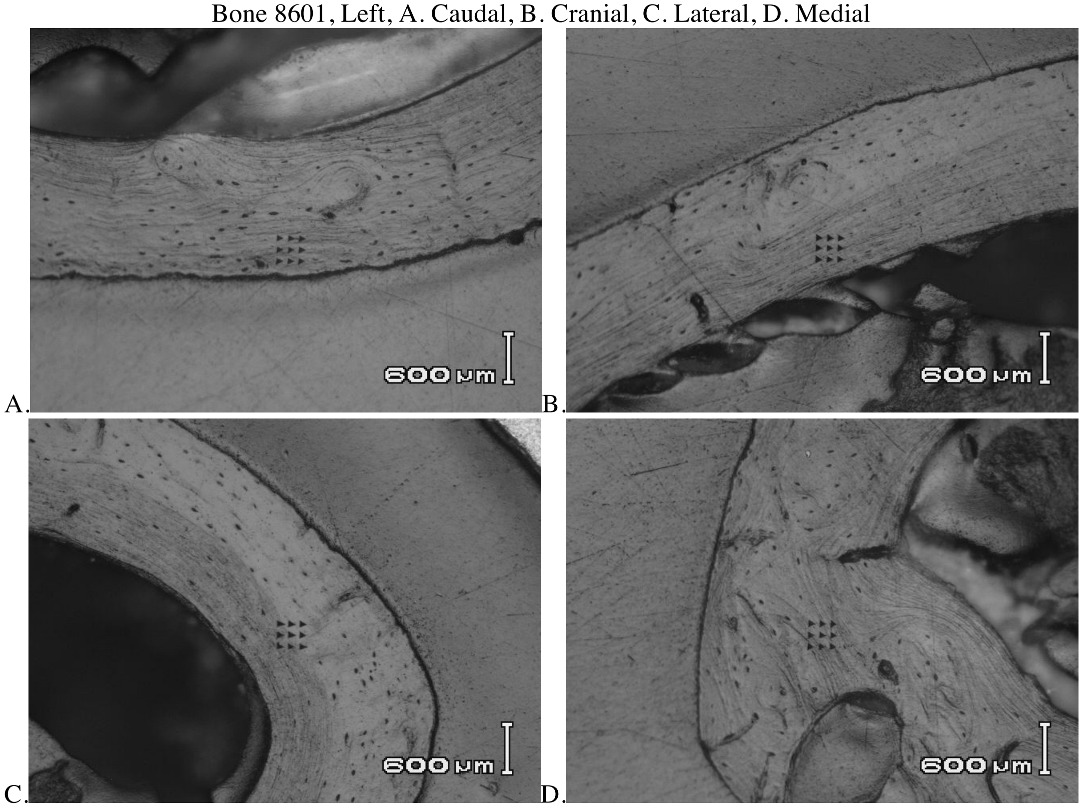
Bone 7595, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



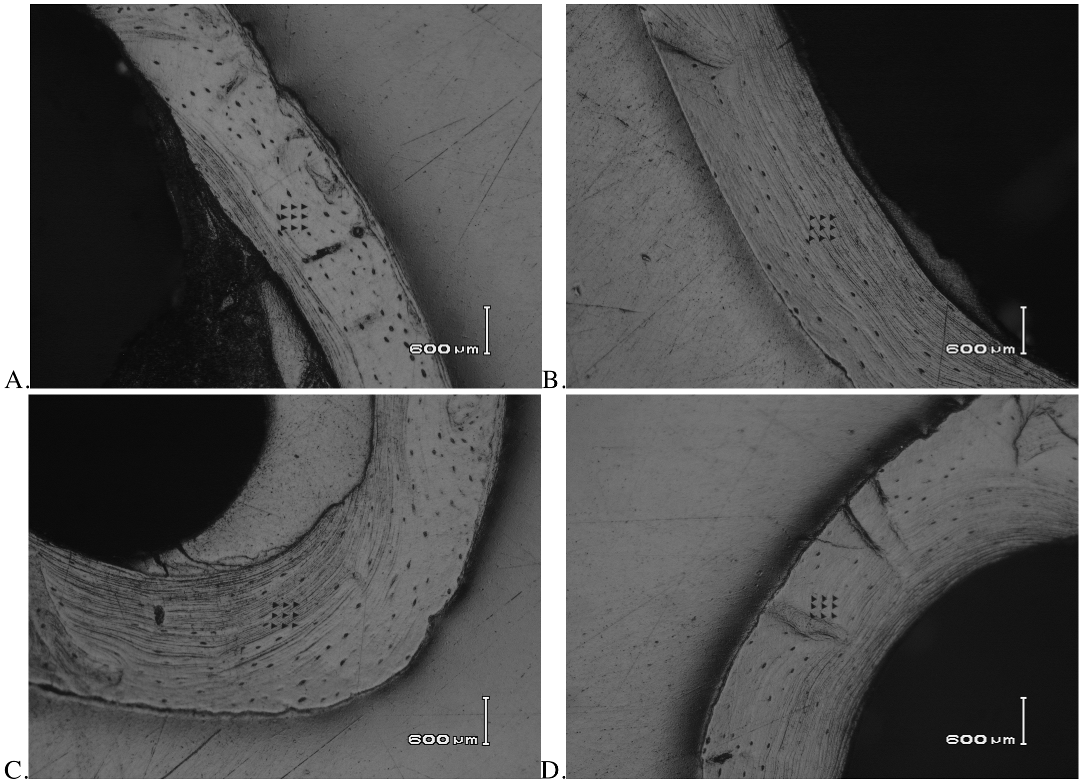
Bone 7595, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



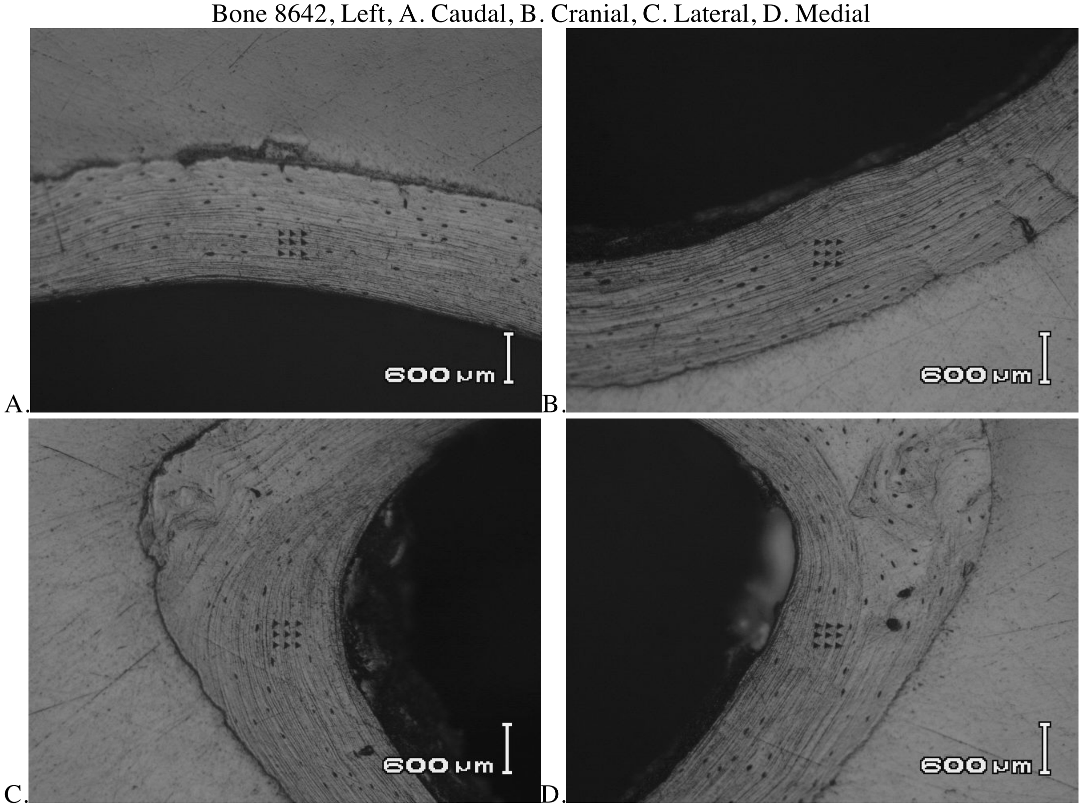
Bone 8601, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



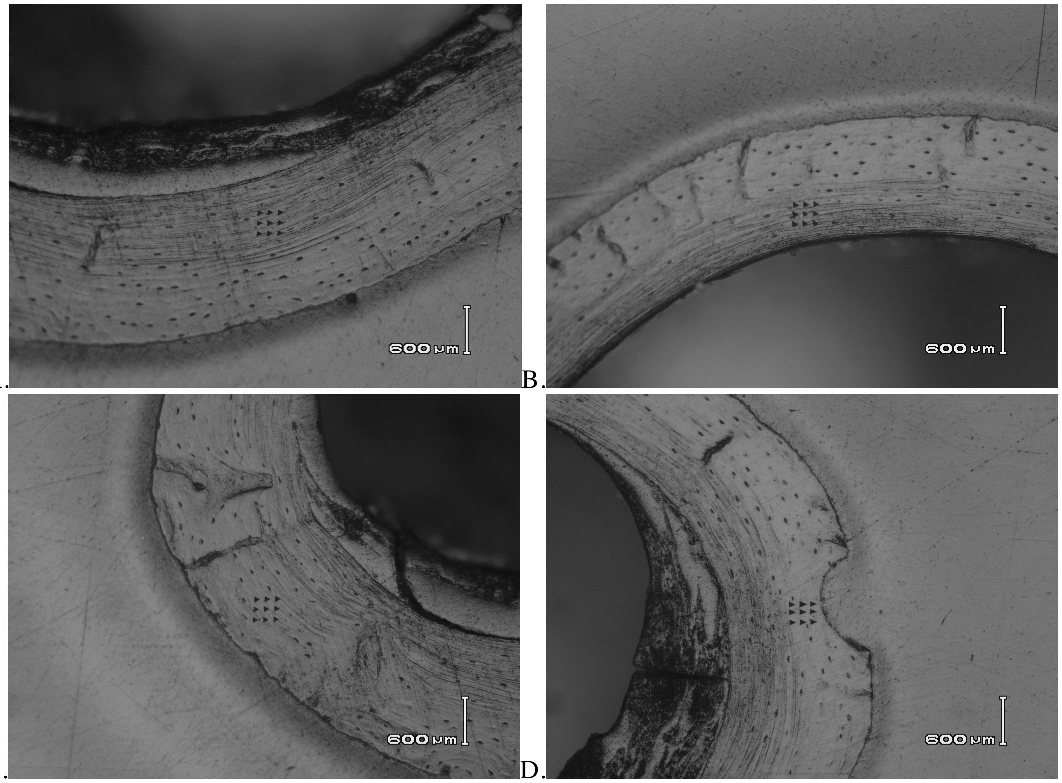
Bone 8601, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



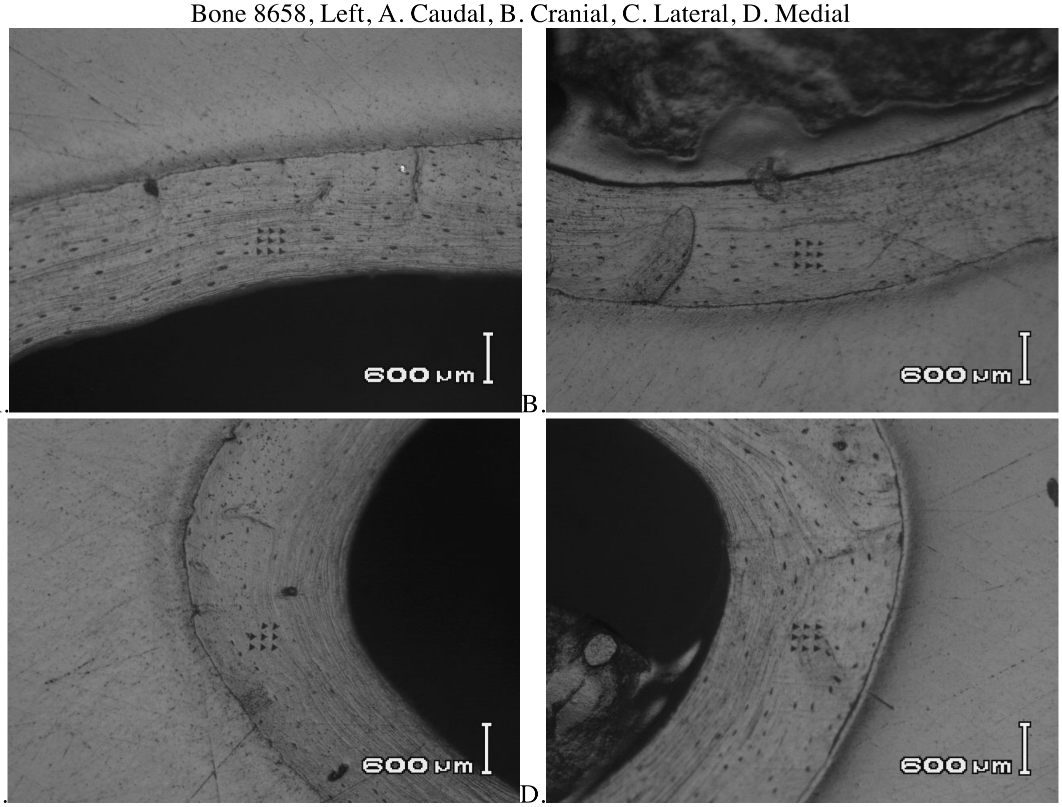
Bone 8642, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



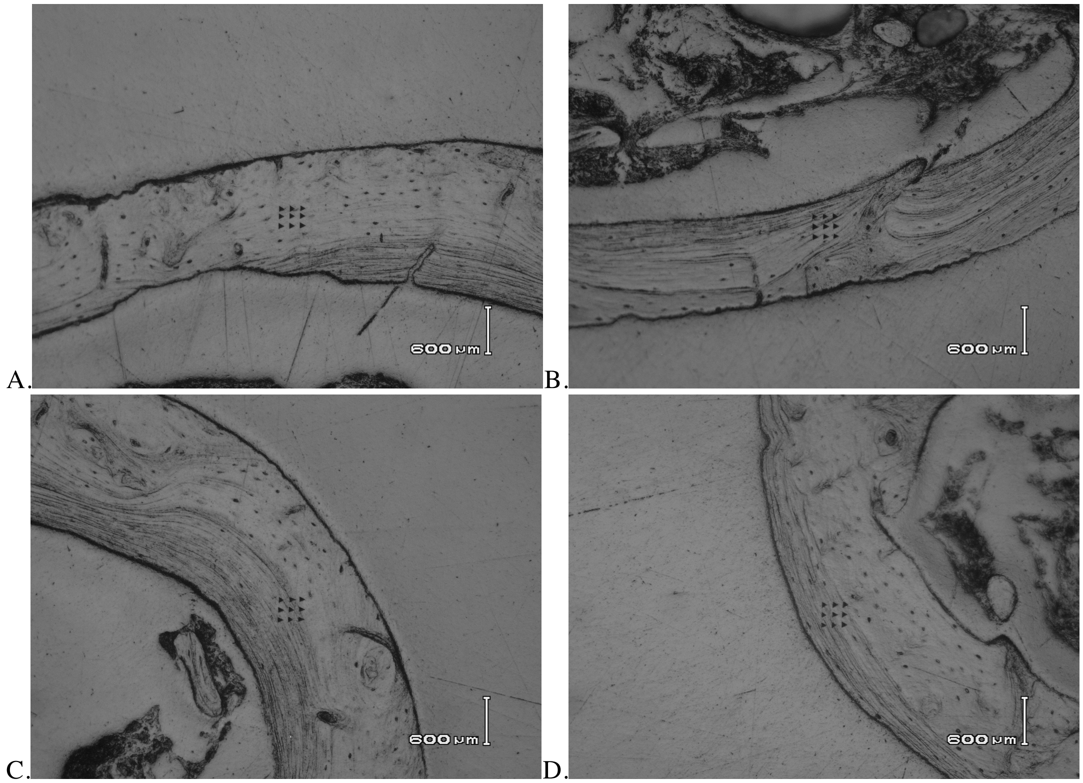
Bone 8642, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



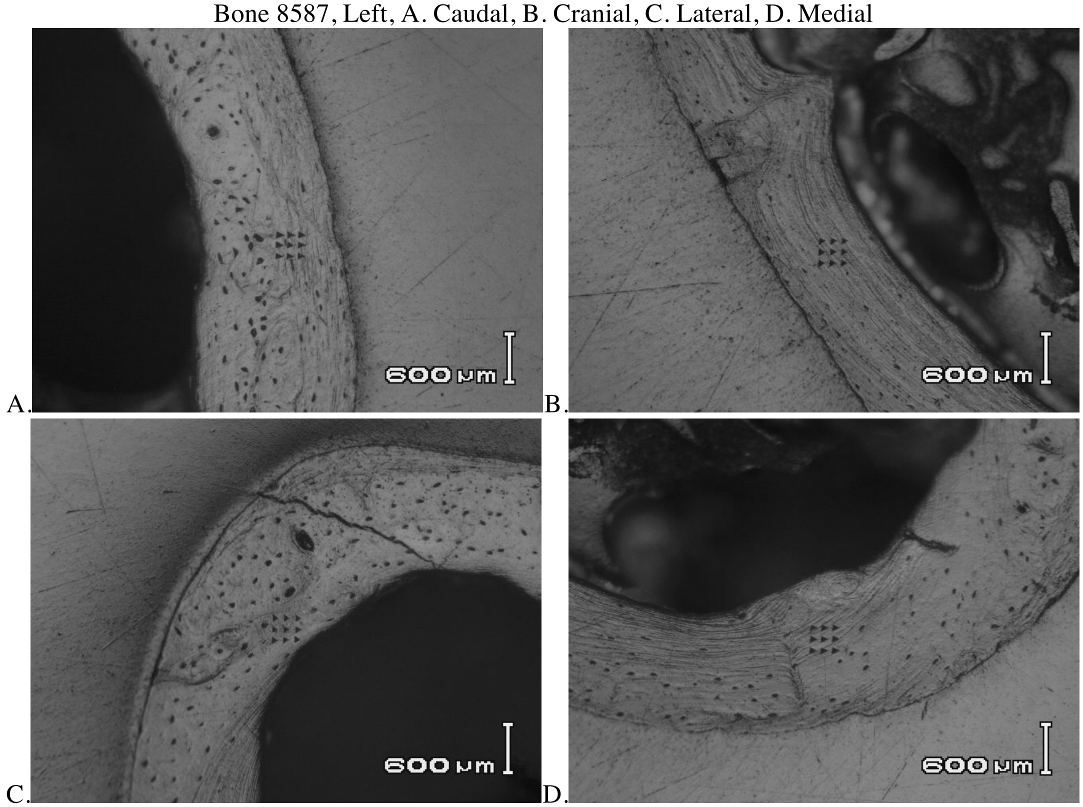
Bone 8658, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



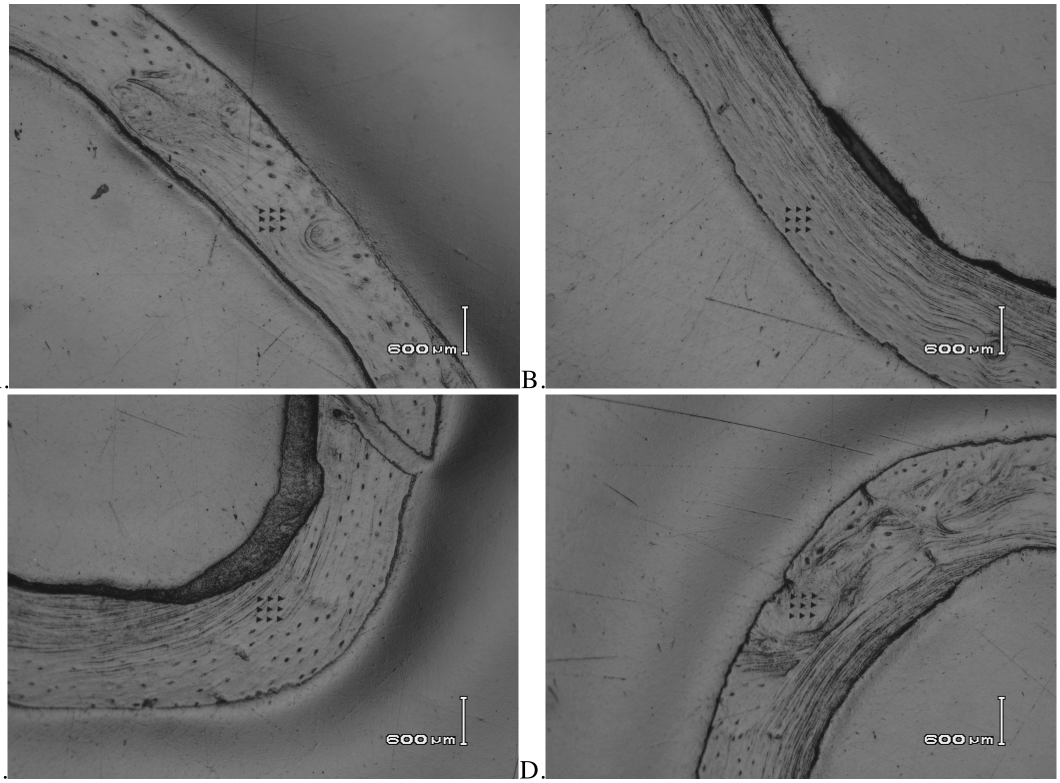
Bone 8658, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 8587, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

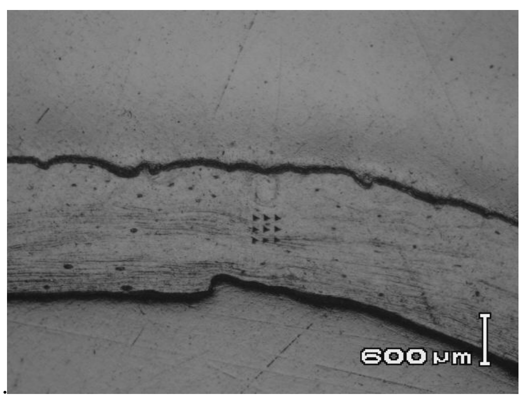


Bone 8587, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



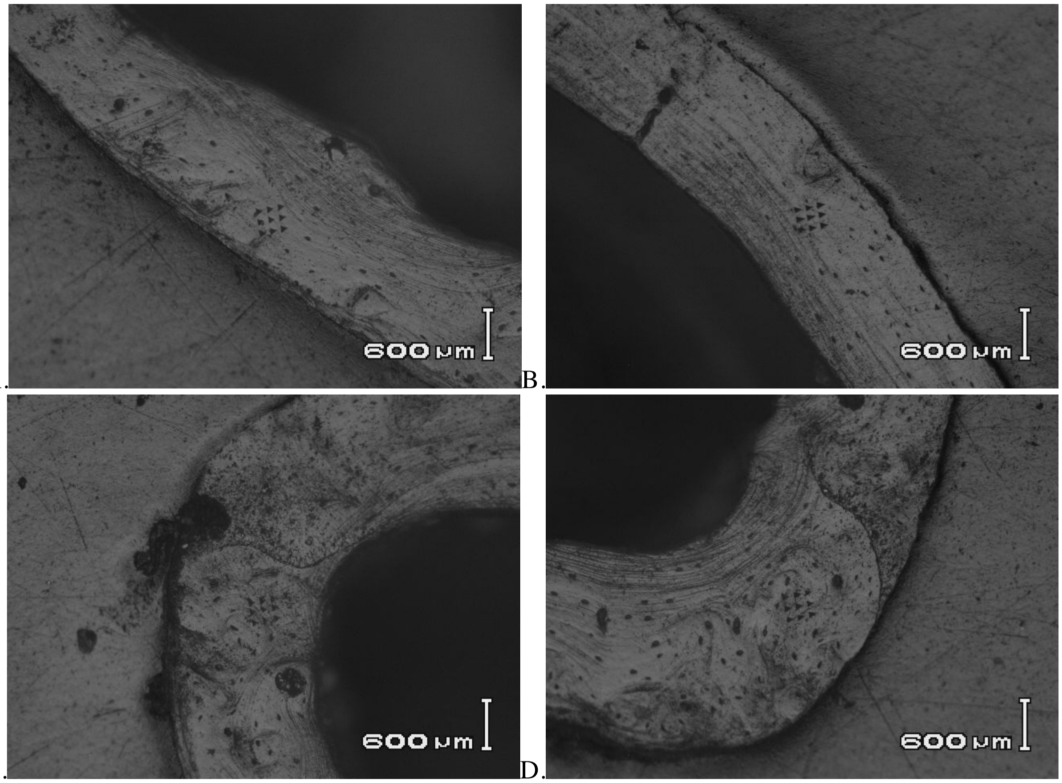
A. B. C. D.

Bone 8641, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

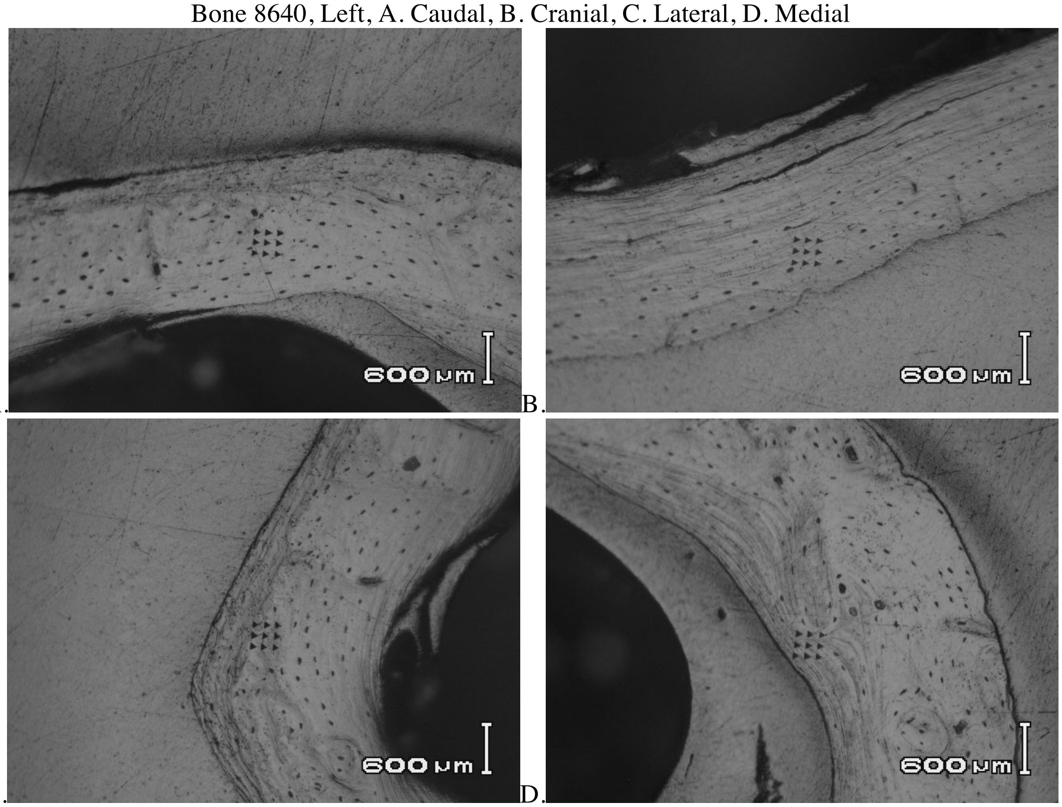


A.

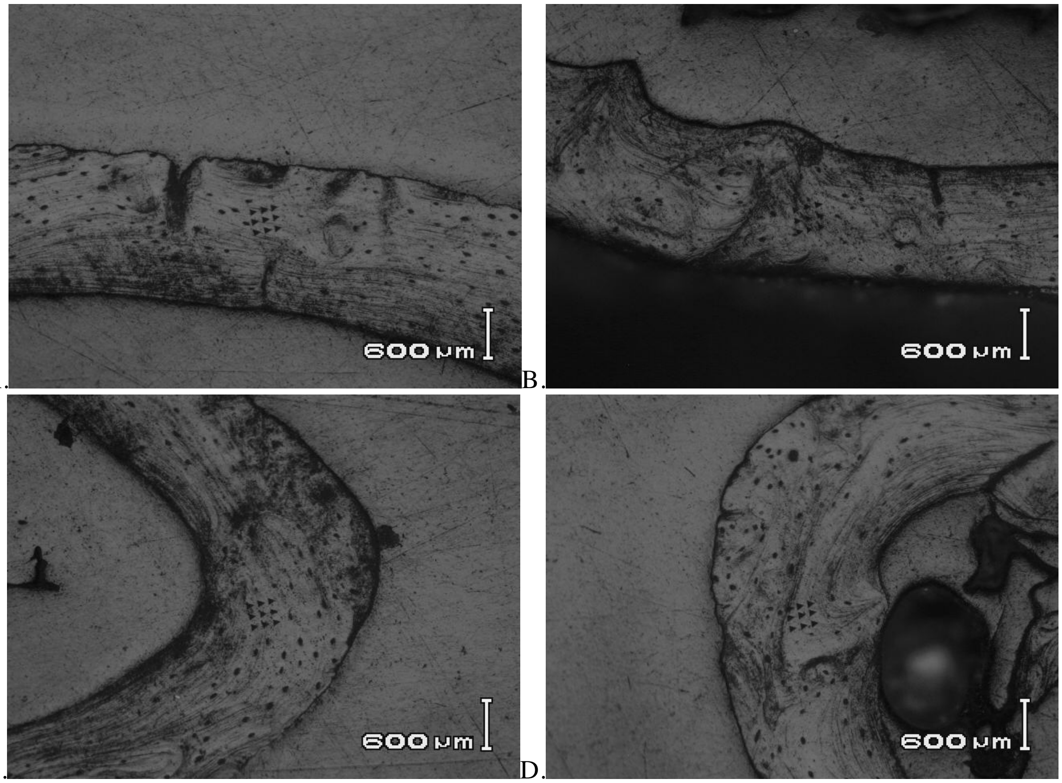
Bone 8641, Right, A. Caudal



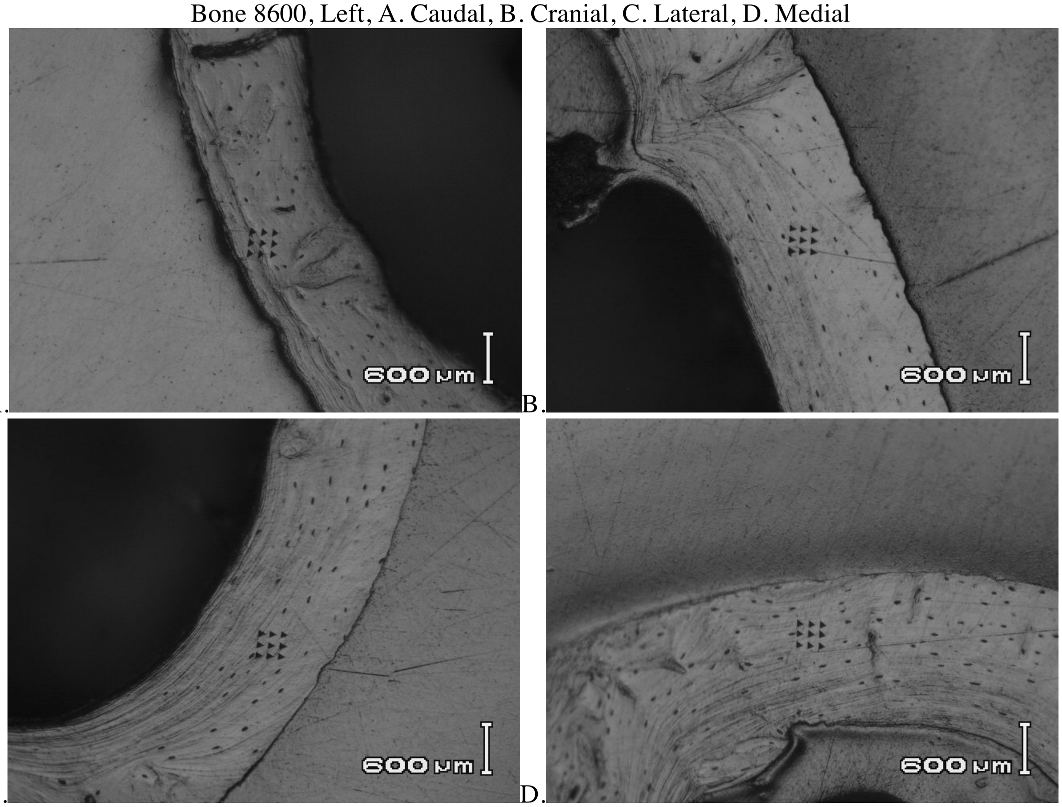
Bone 8640, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



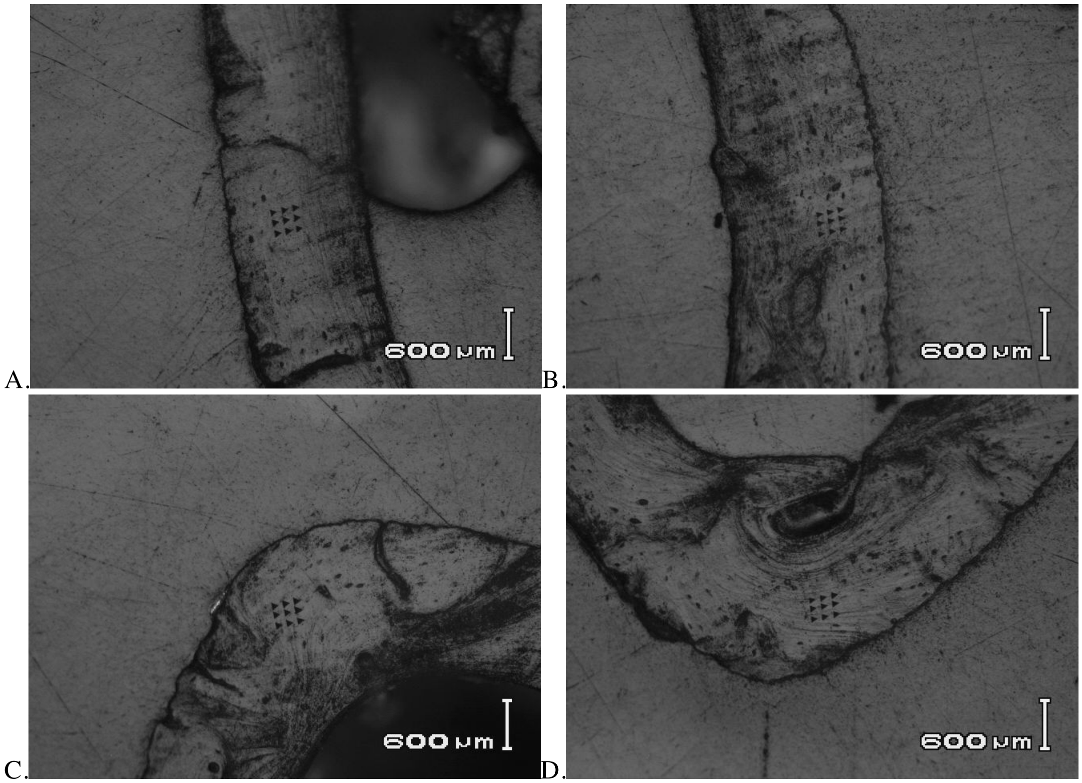
Bone 8640, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



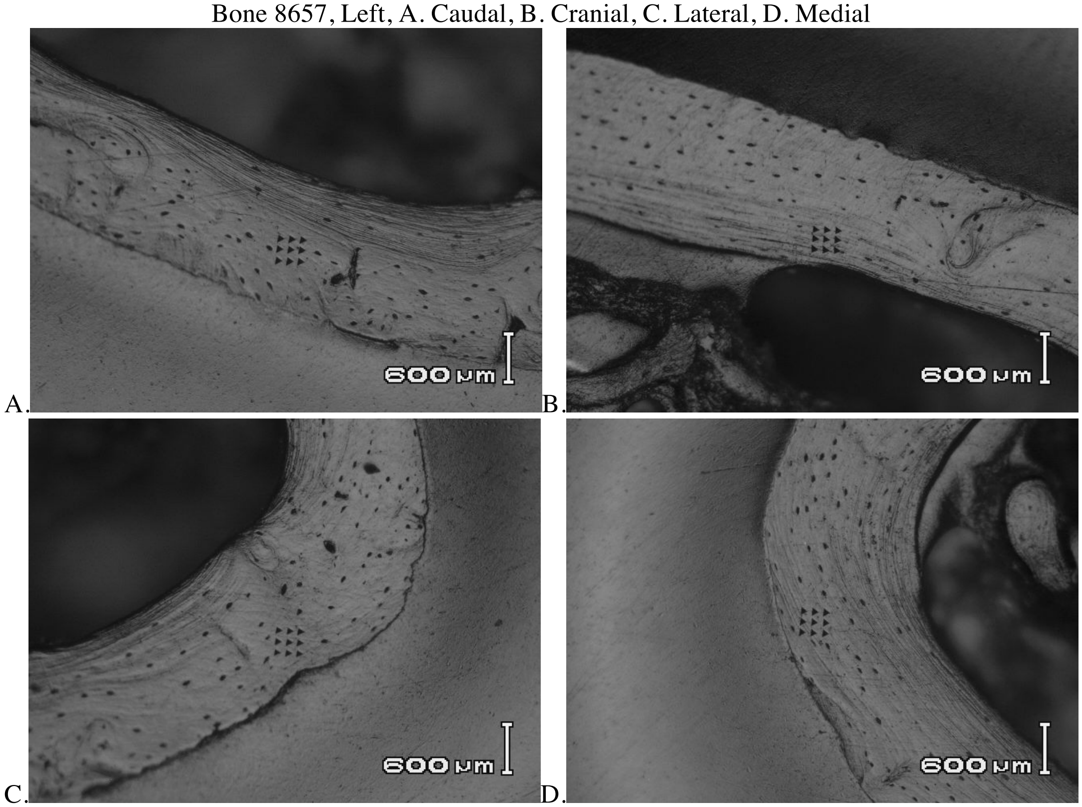
Bone 8600, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



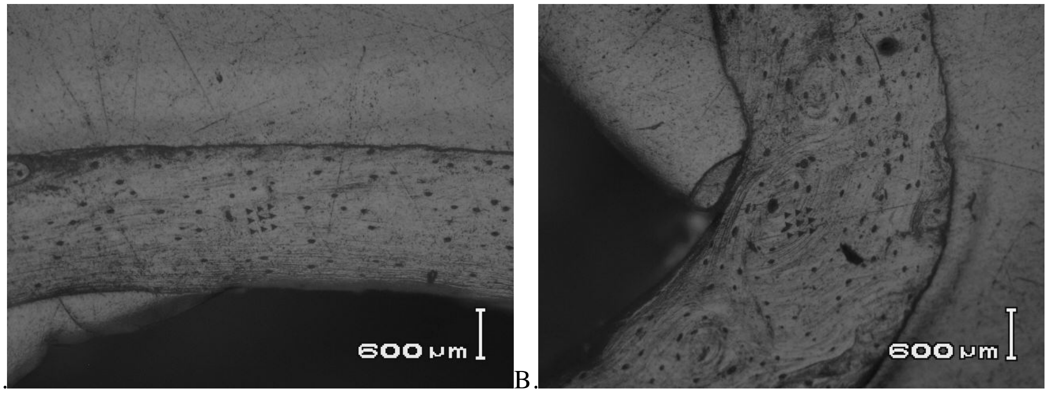
Bone 8600, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 8657, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

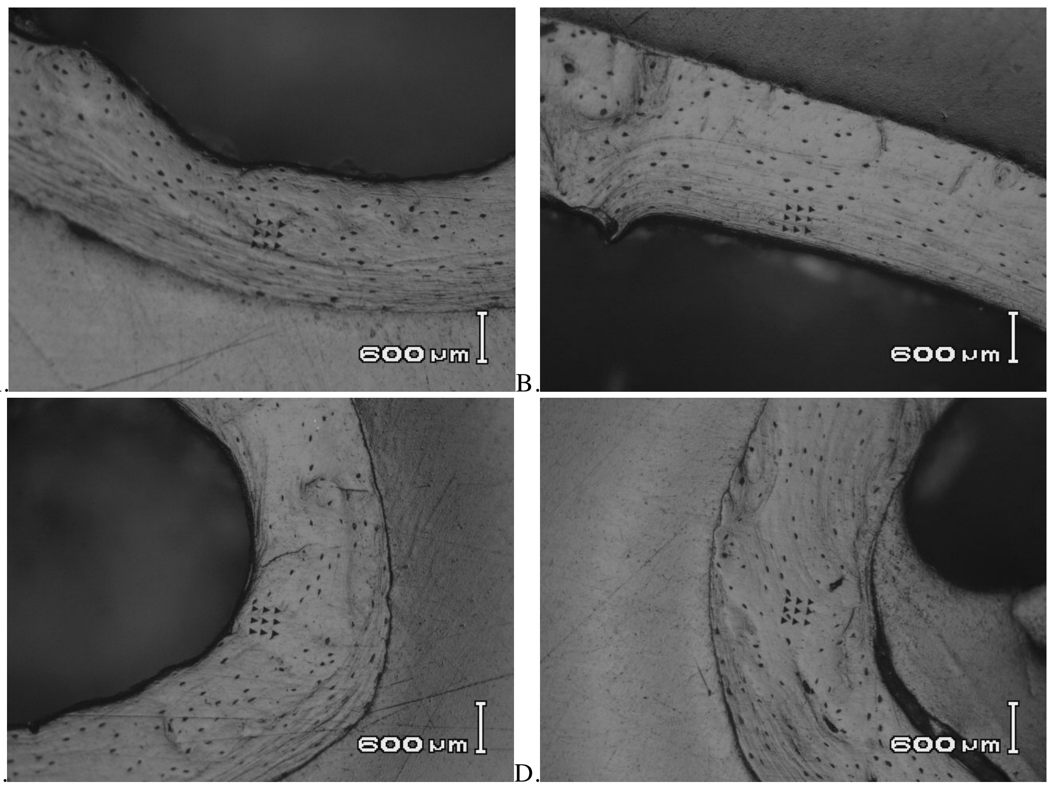


Bone 8657, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



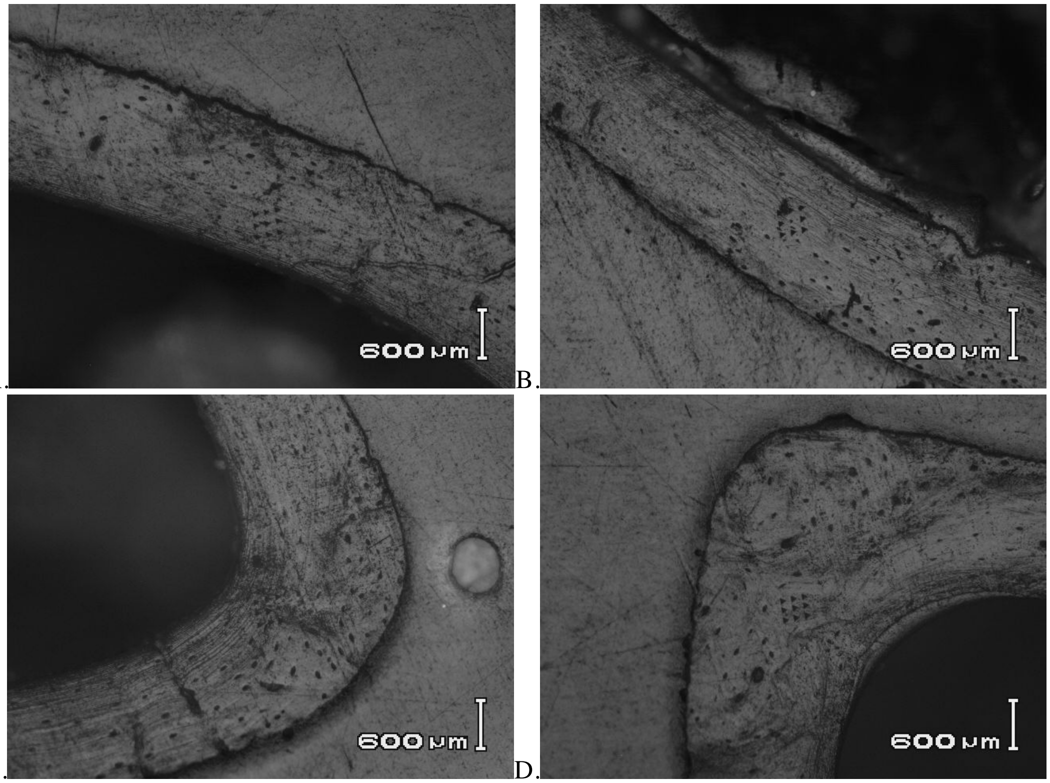
A. B.

Bone 8599, Left, A. Caudal, B. Cranial

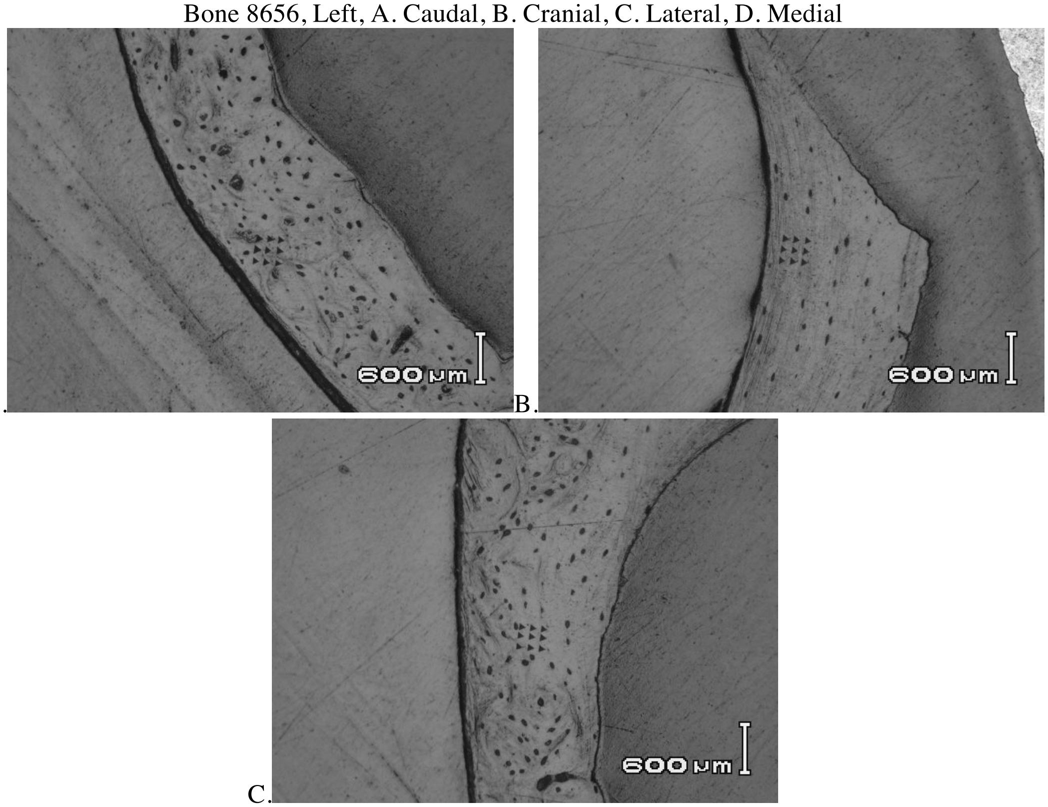


A. B. C. D.

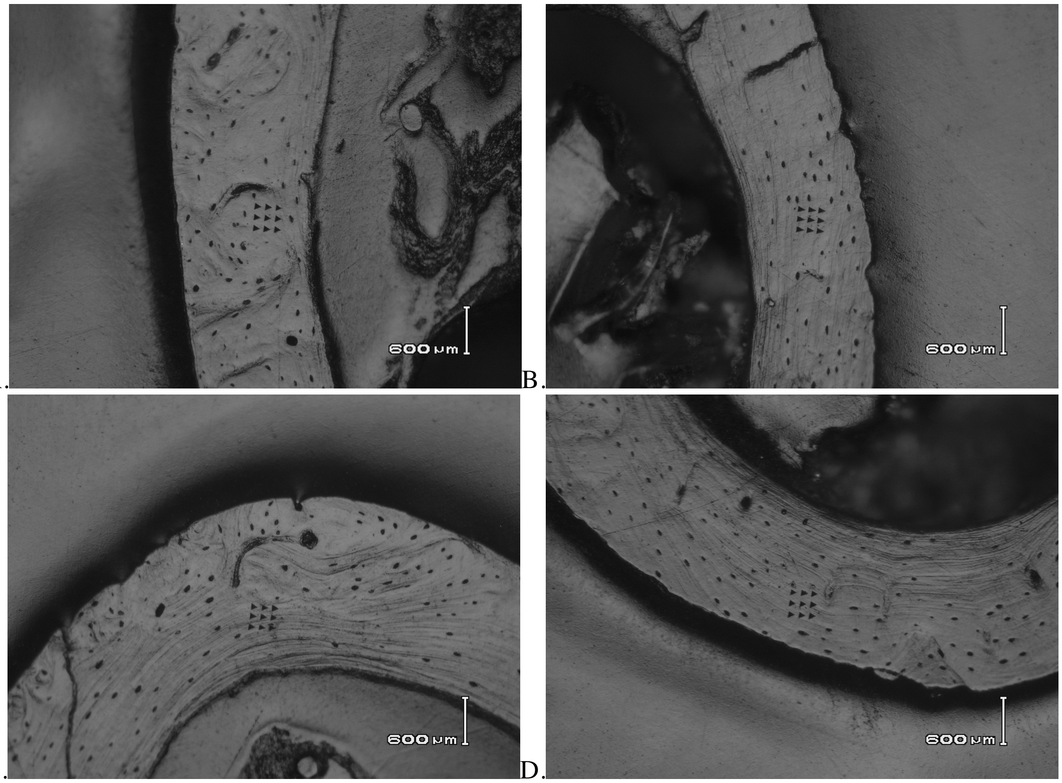
Bone 8599, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



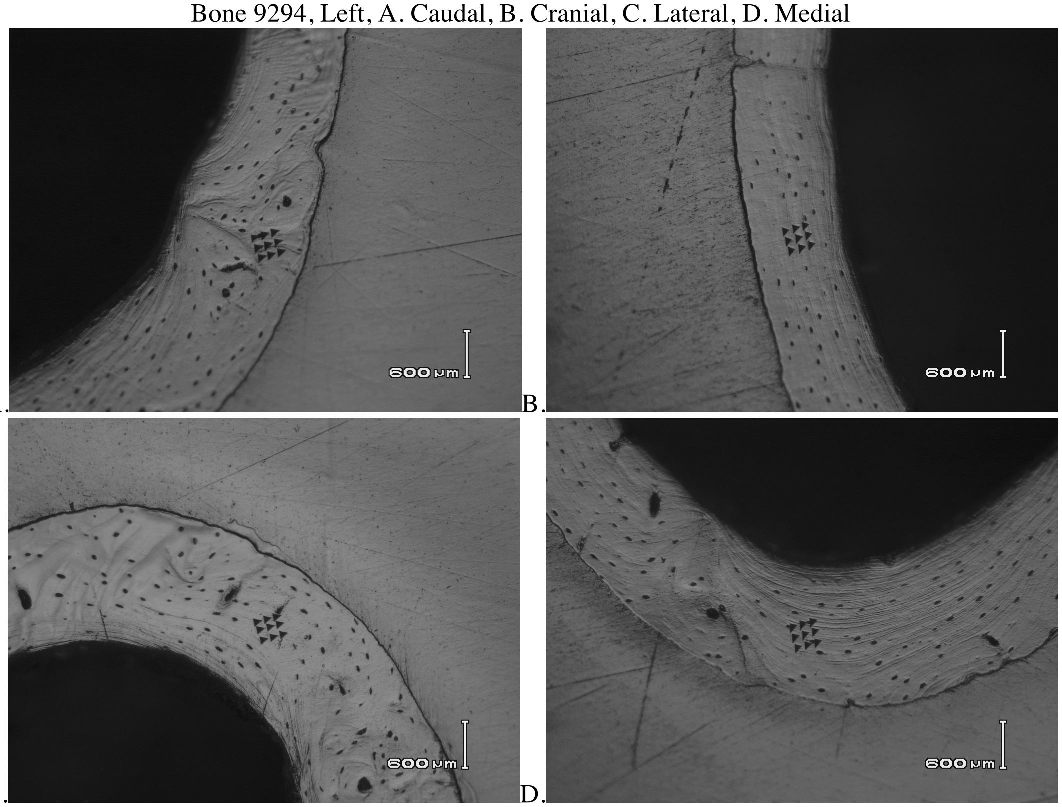
Bone 8656, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



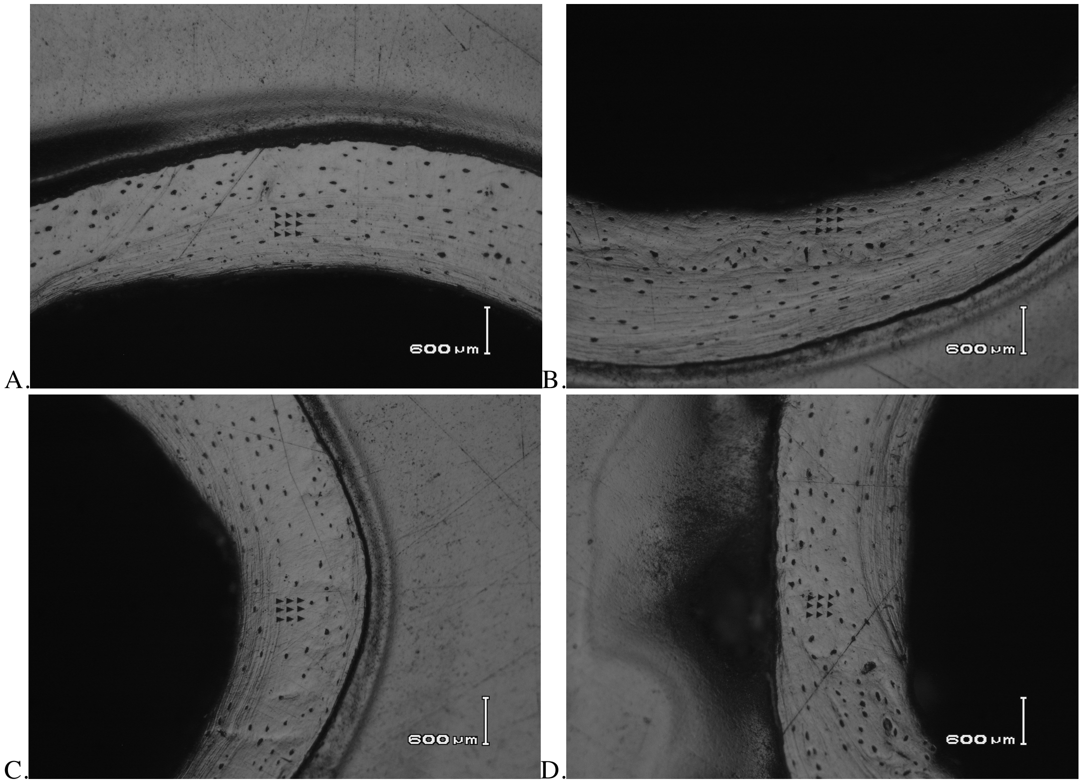
Bone 8656, Right, A. Caudal, B. Lateral, C. Medial



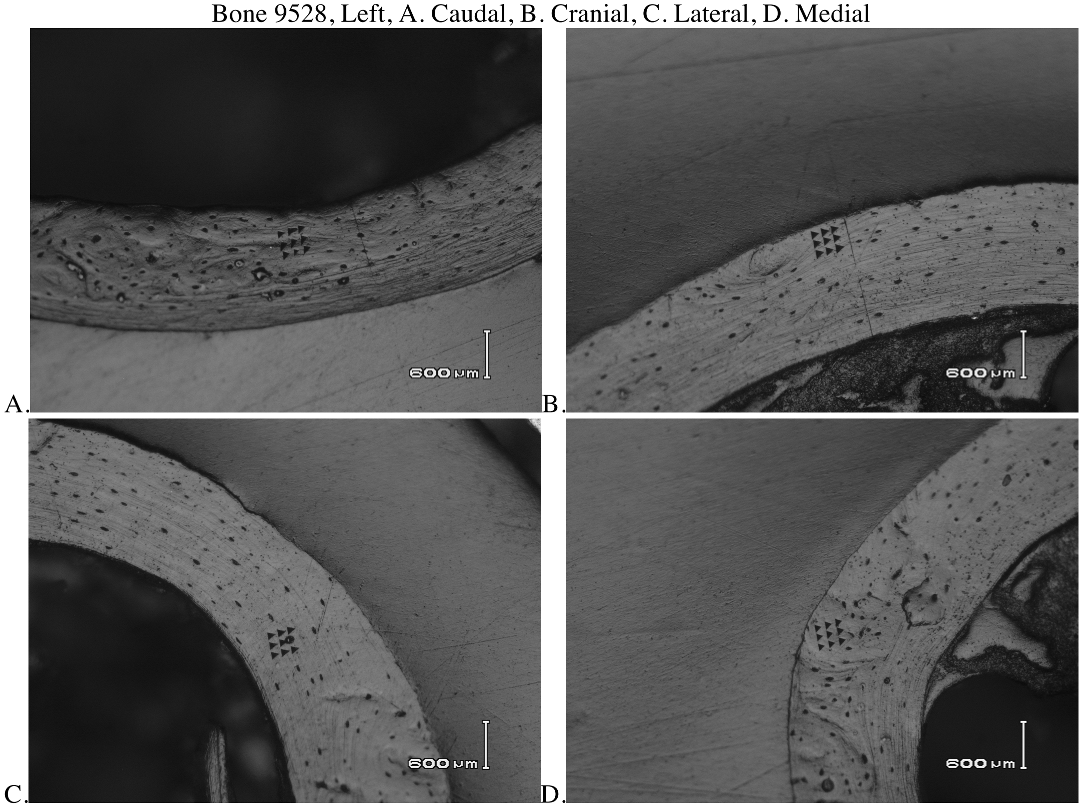
Bone 9294, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



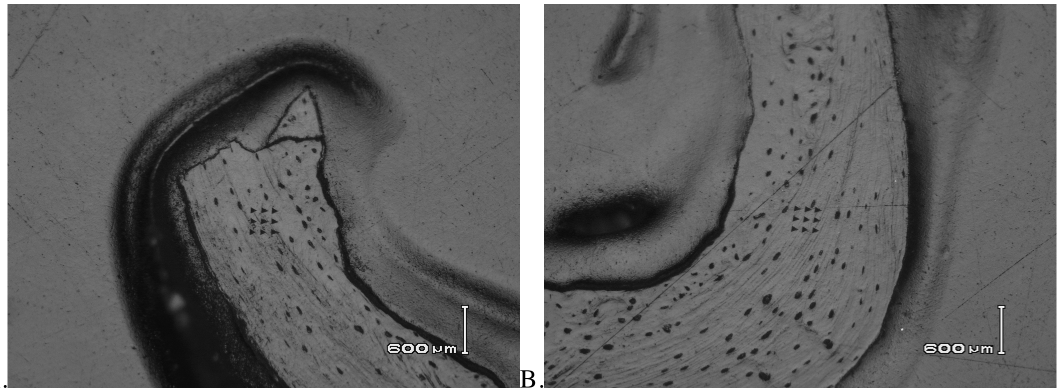
Bone 9294, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 9528, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

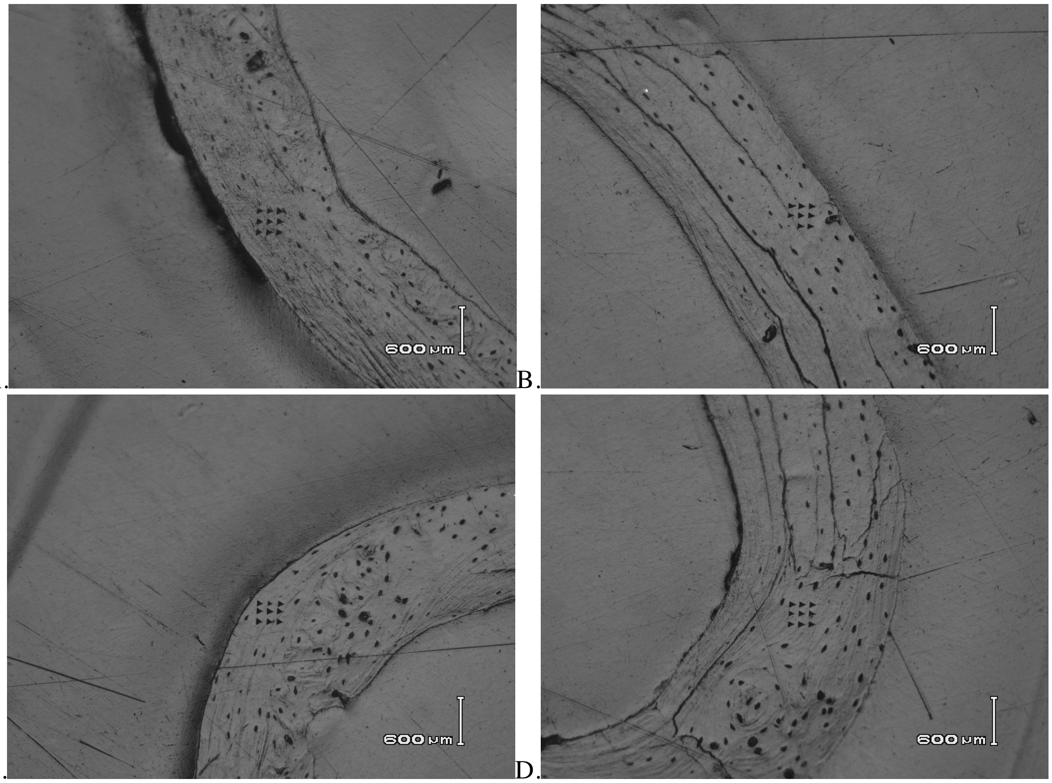


Bone 9528, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial

A. B.


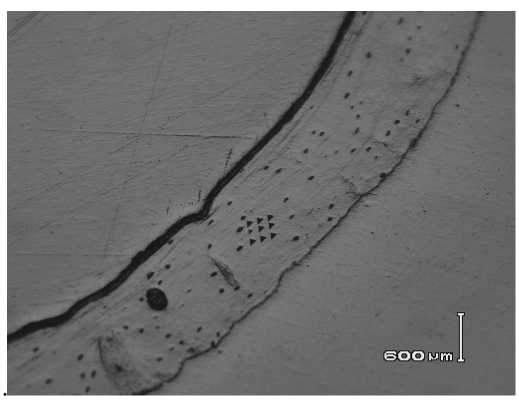
Bone 9530, Left, A. Caudal, B. Medial

*Bone 9530, Right, NO EXPOSURE



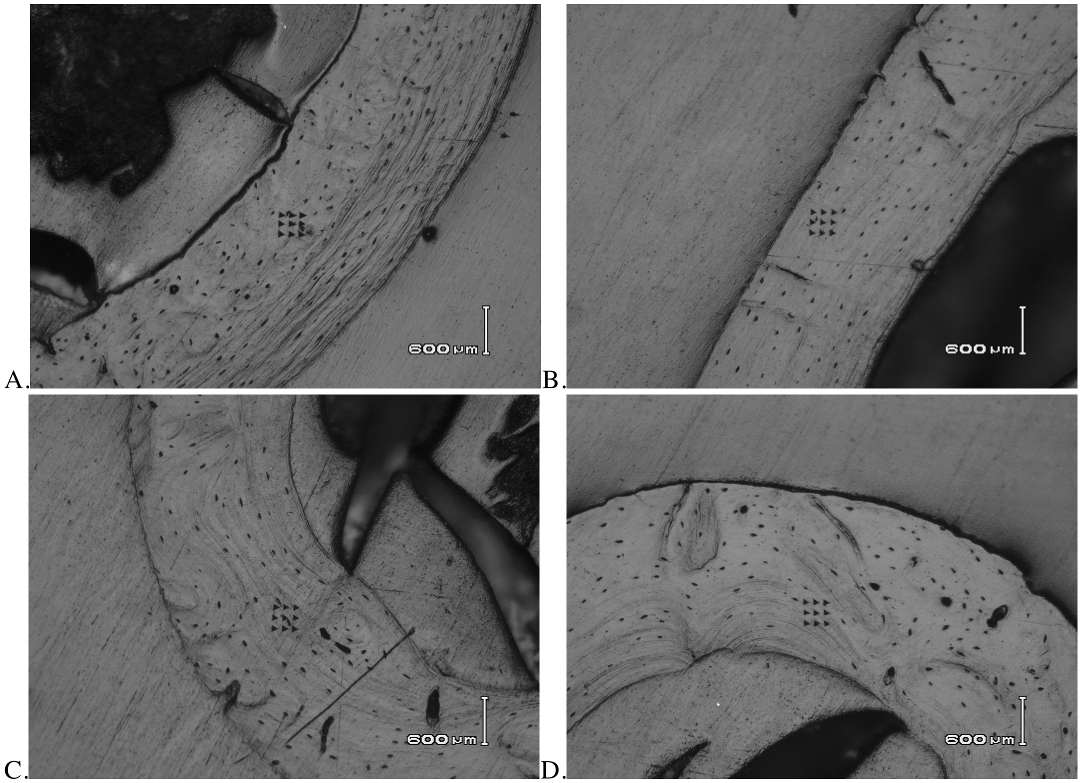
A. B. C. D.

Bone 9295, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

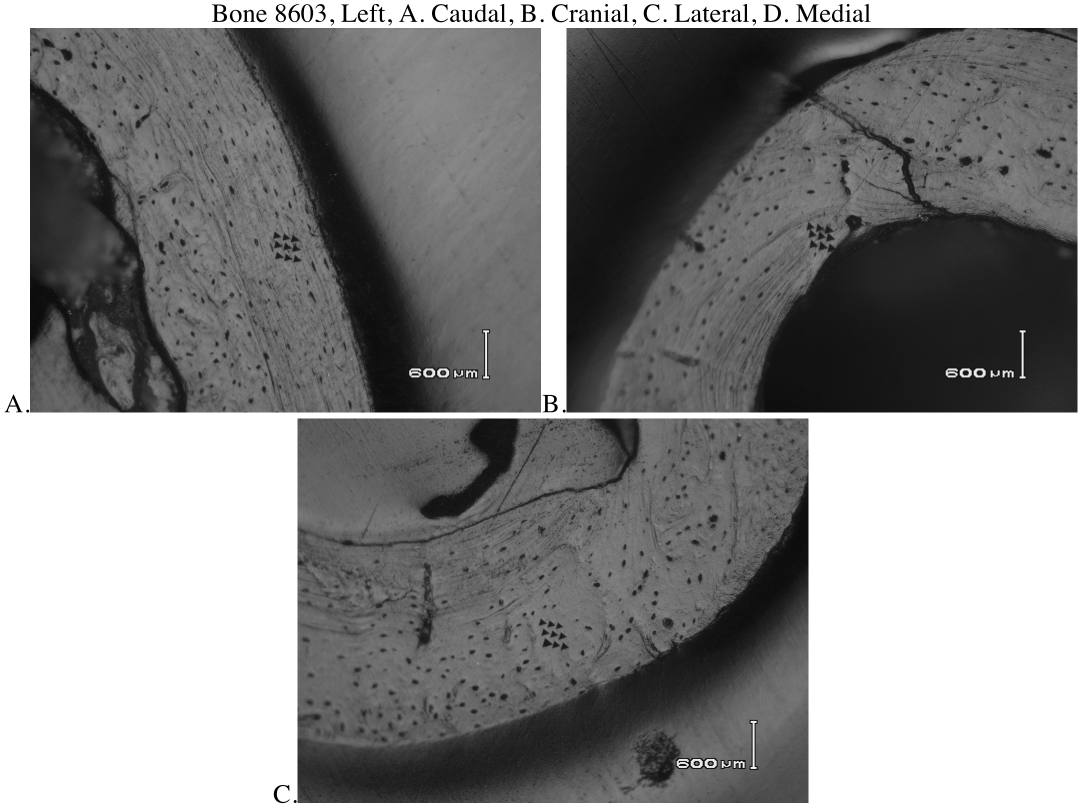


A.

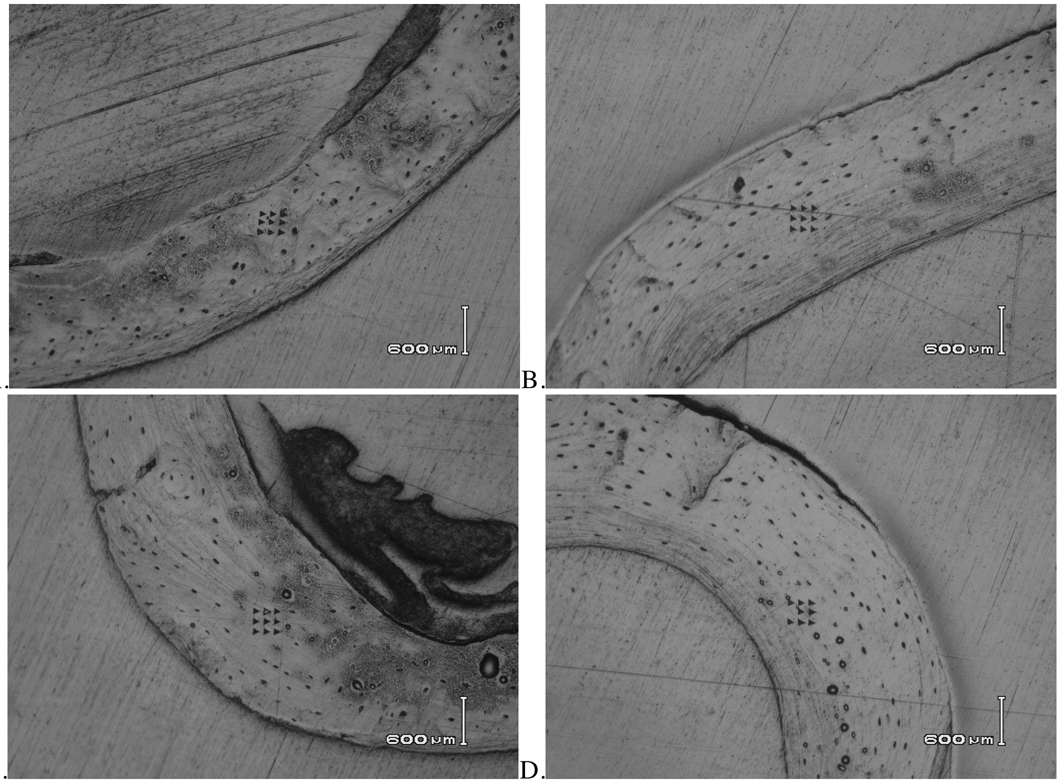
Bone 9295, Right, A. Cranial



Bone 8603, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

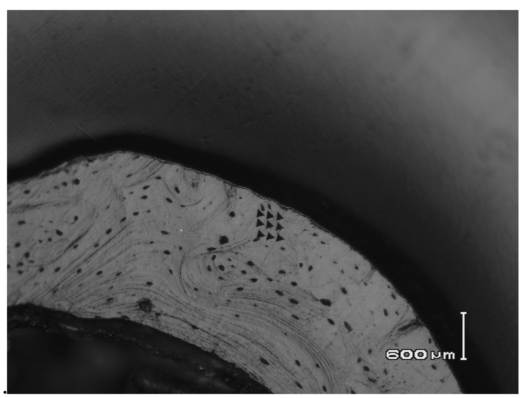


Bone 8603, Right, A. Caudal, B. Lateral, C. Medial



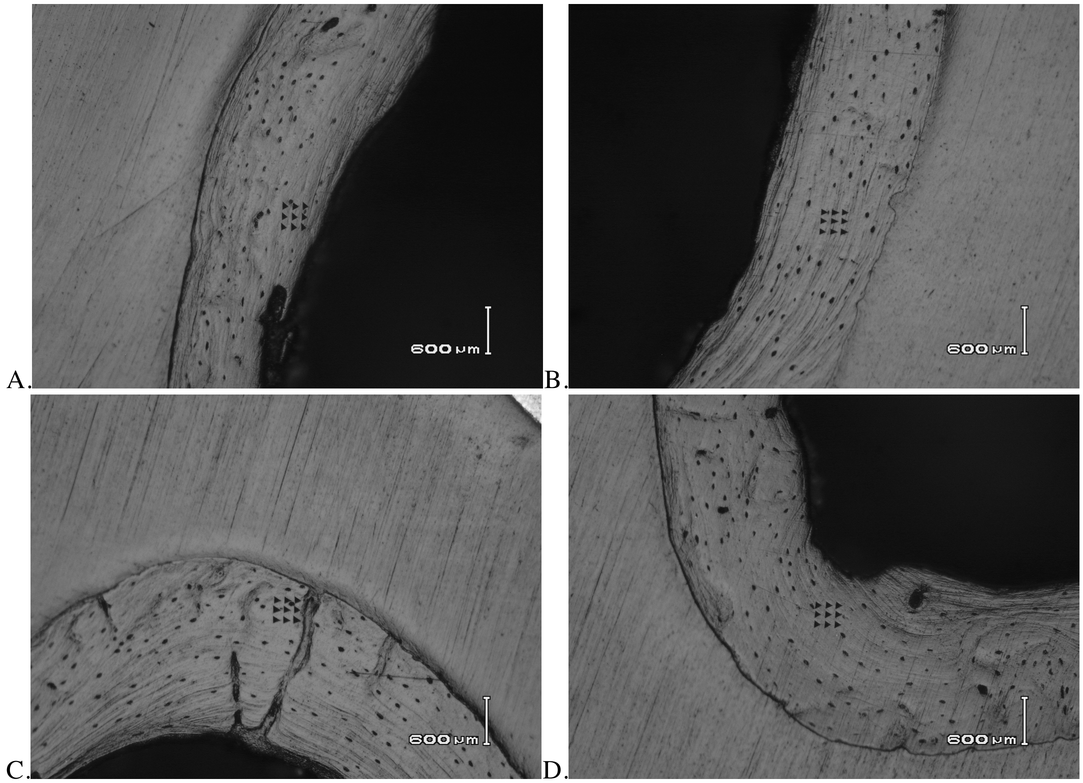
A. B. C. D.

Bone 8643, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

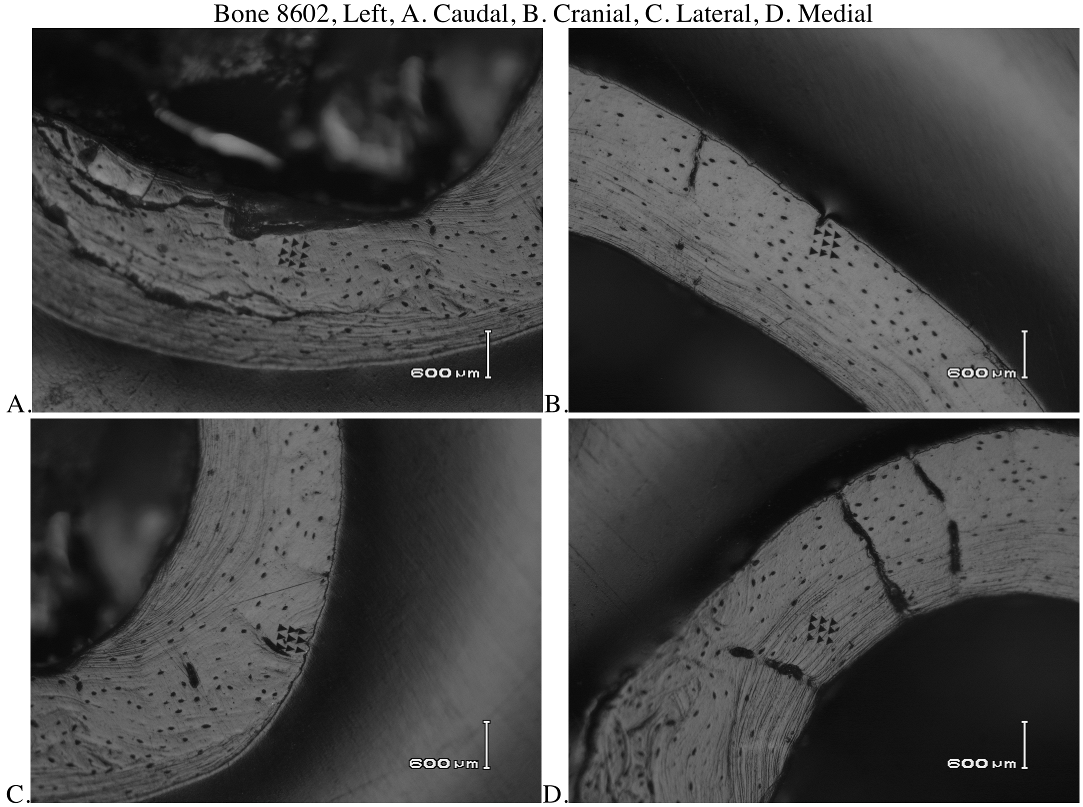


A.

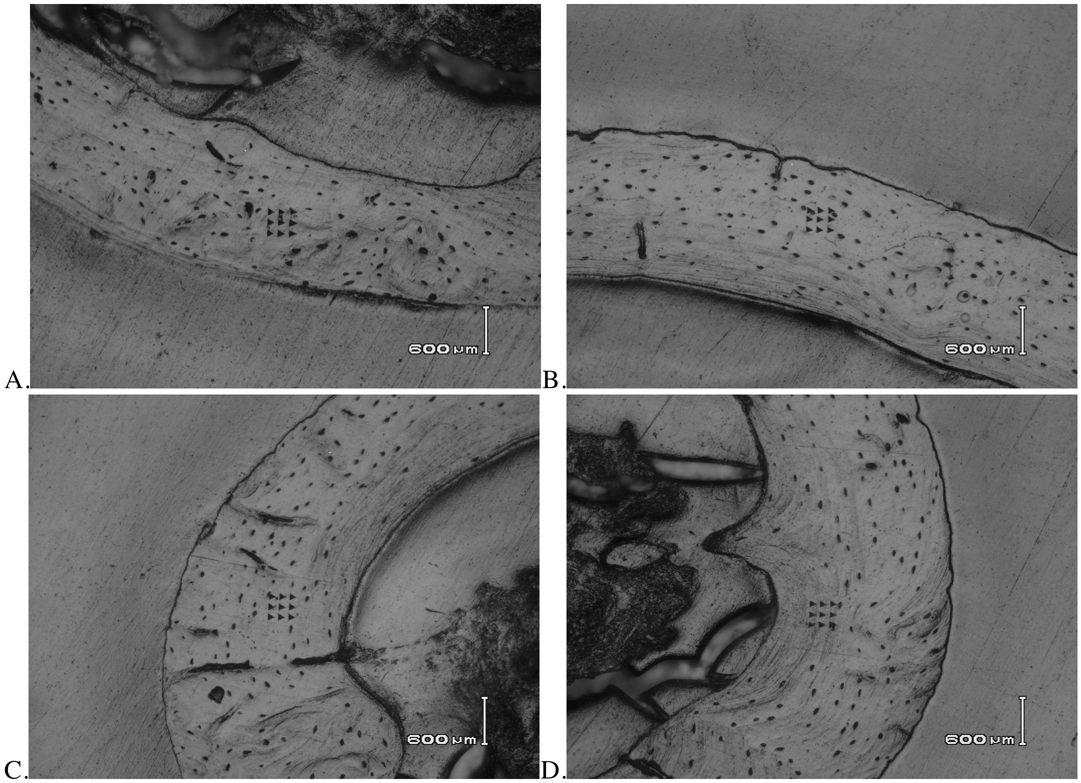
Bone 8643, Right, A. Medial



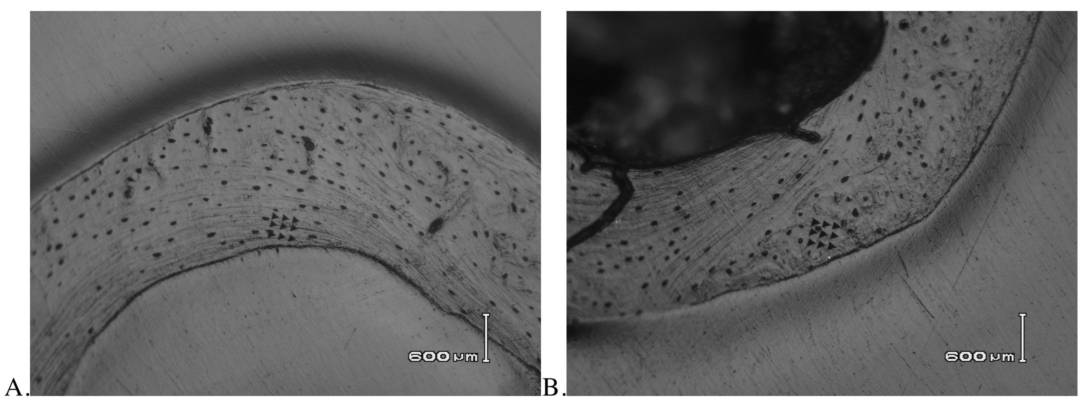
Bone 8602, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



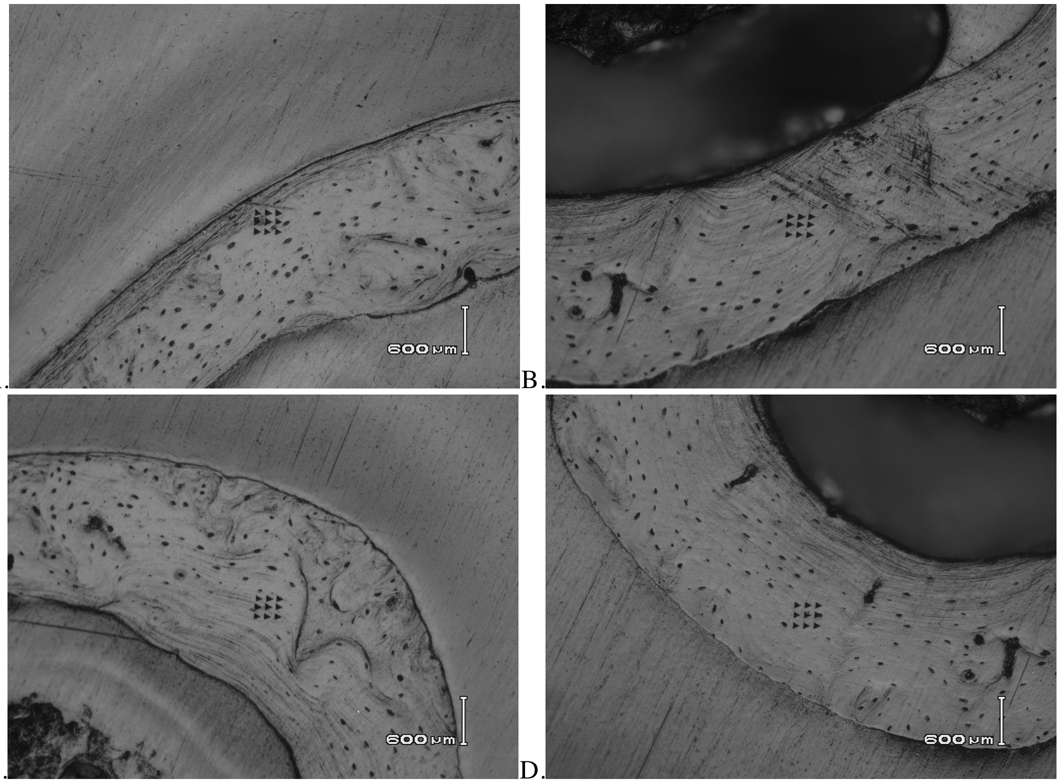
Bone 8602, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 9529, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

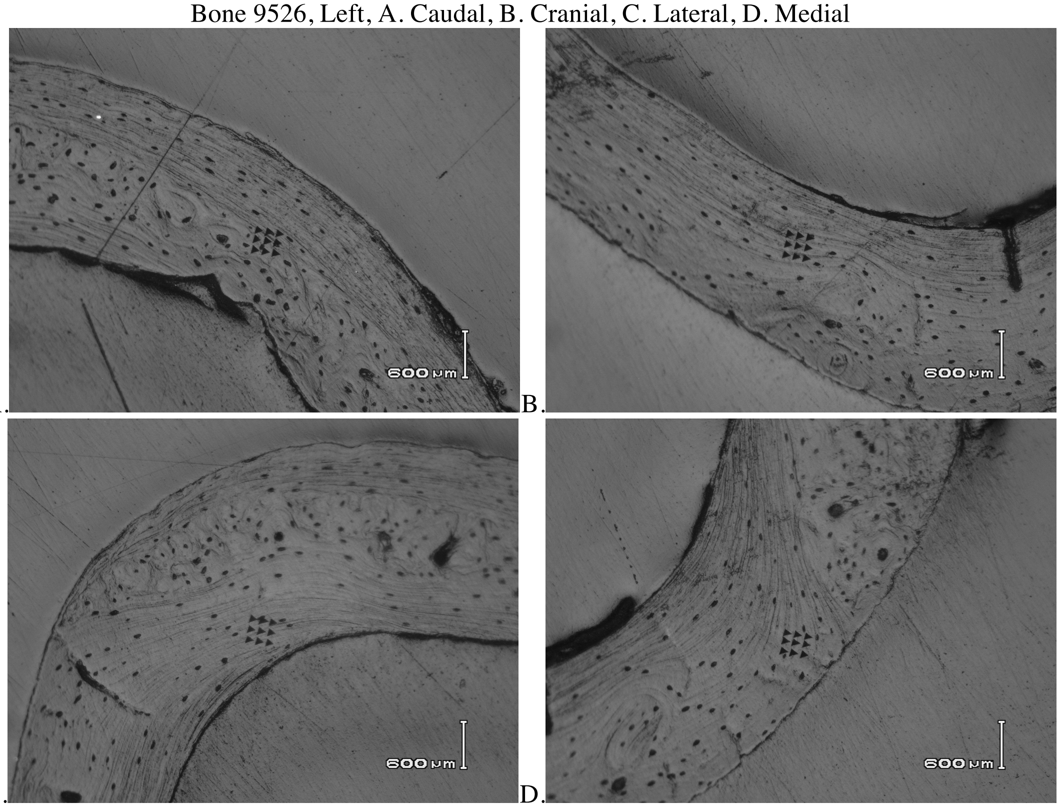


Bone 9529, Right, A. Lateral, B. Medial



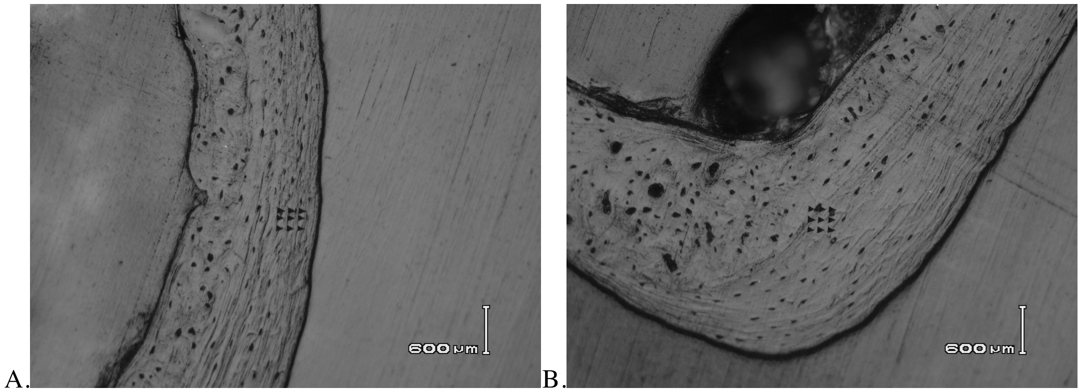
A. B. C. D.

Bone 9526, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

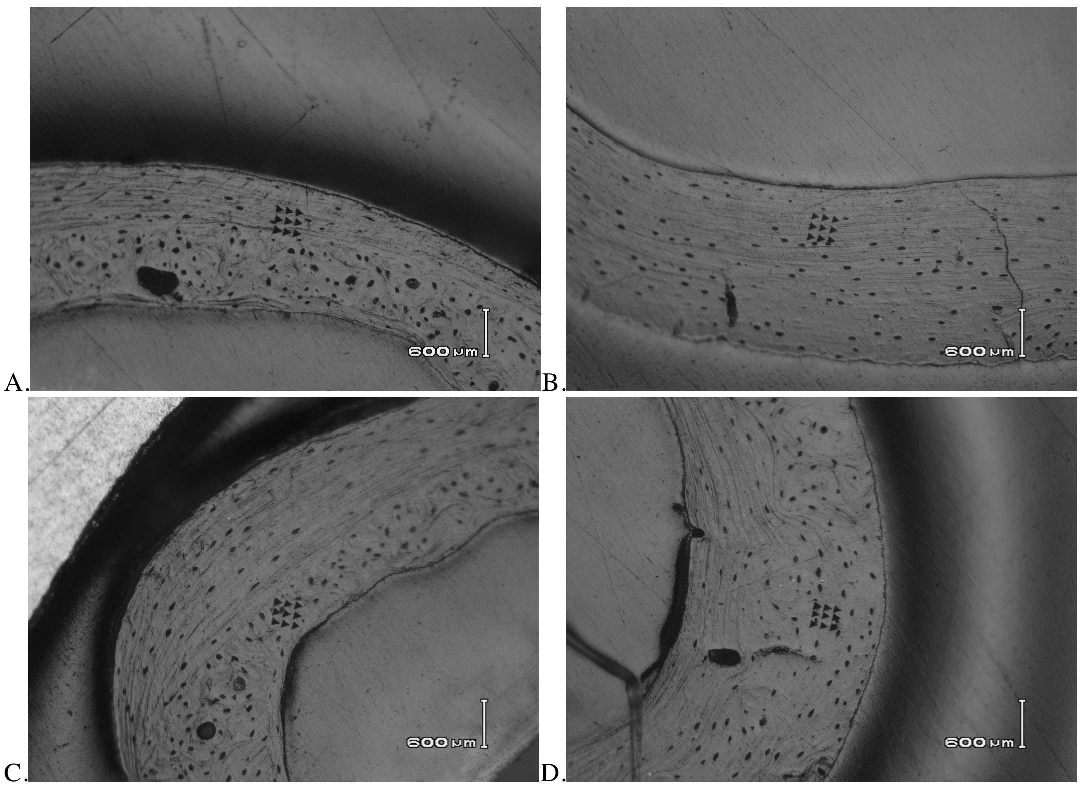


A. B. C. D.

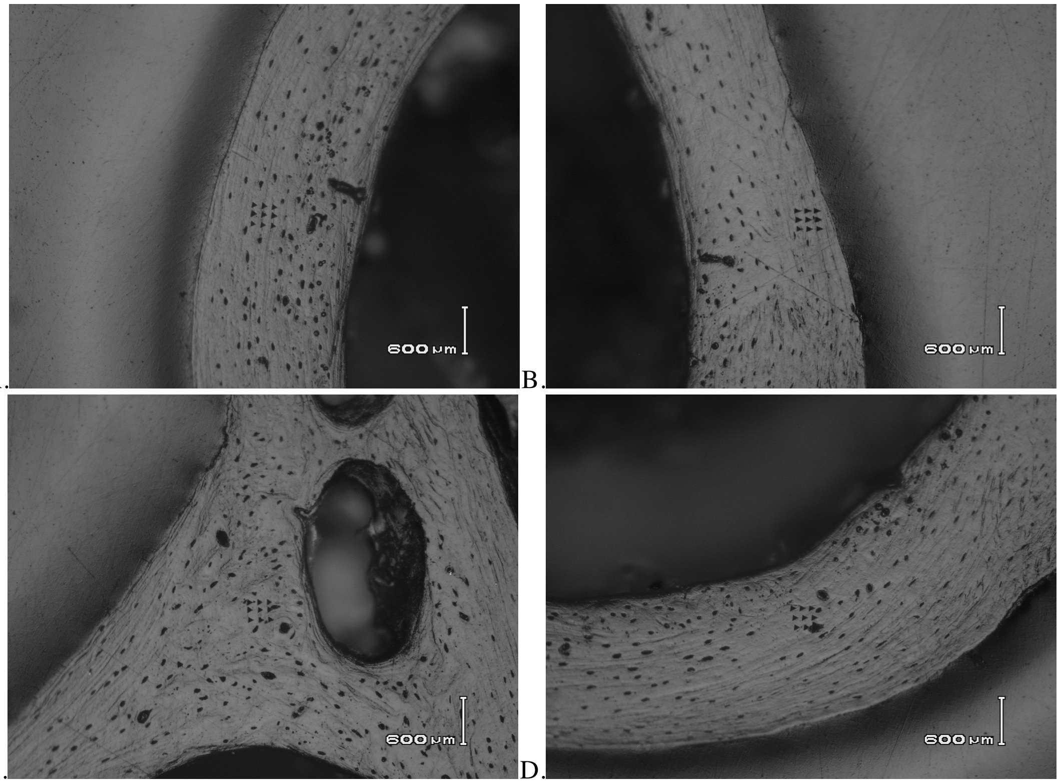
Bone 9526, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 9527, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

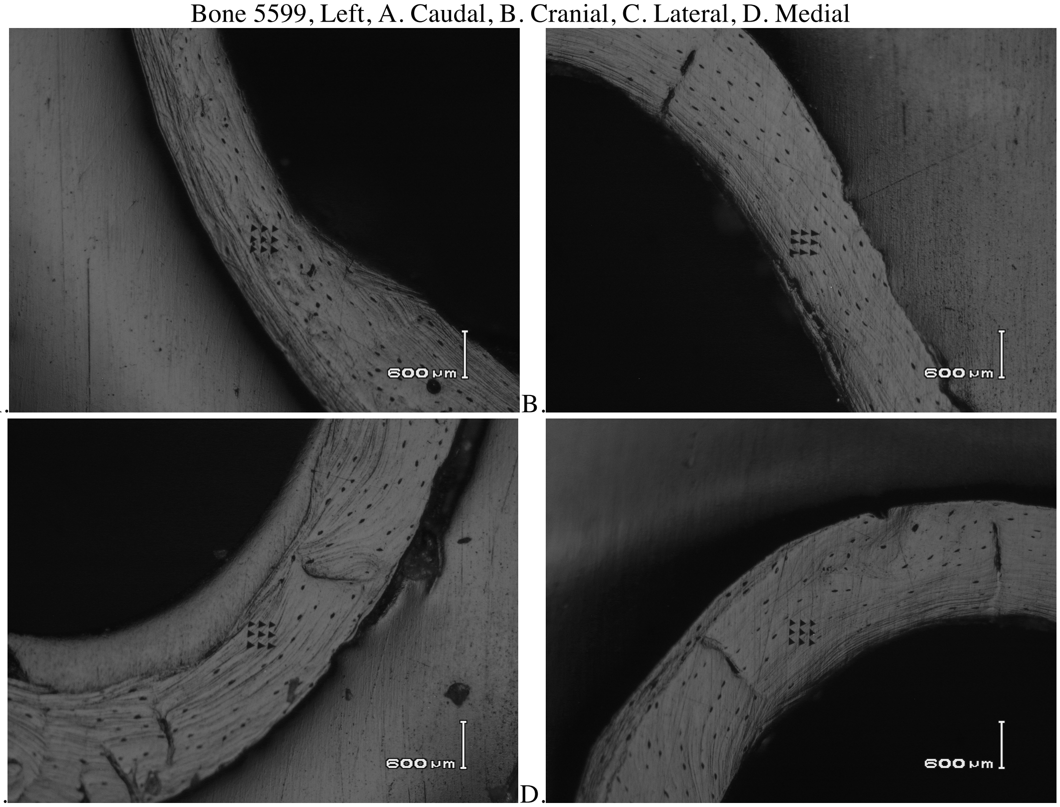


Bone 9527, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



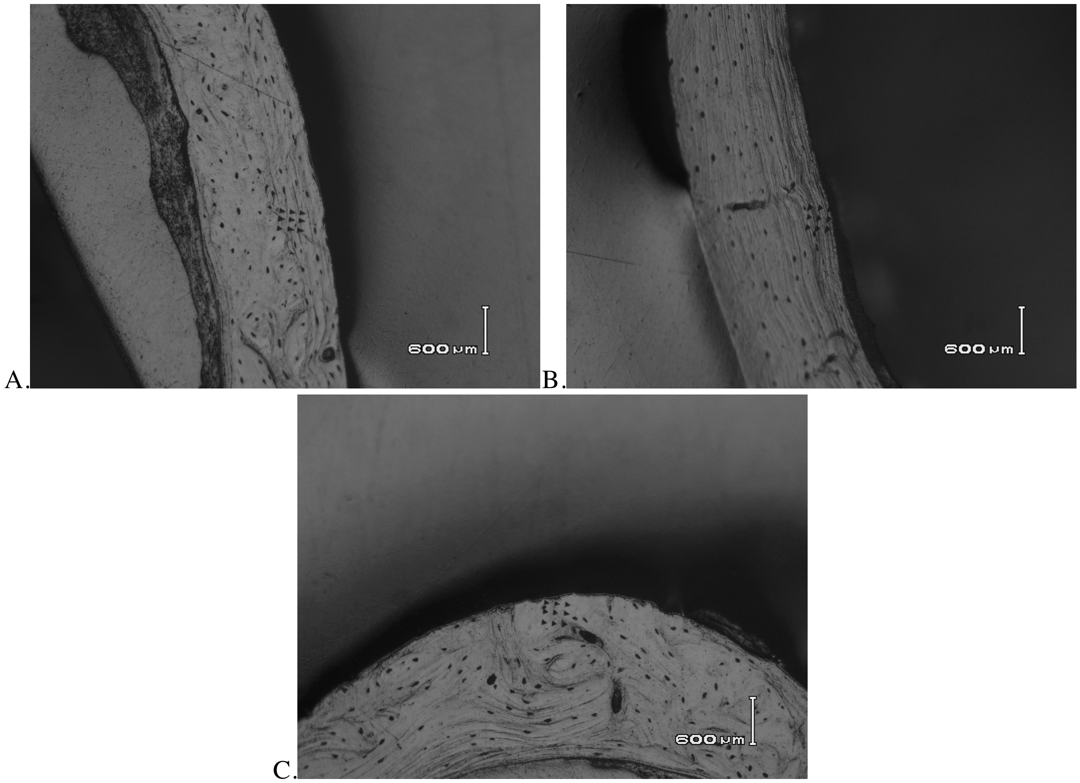
A. B. C. D.

Bone 5599, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

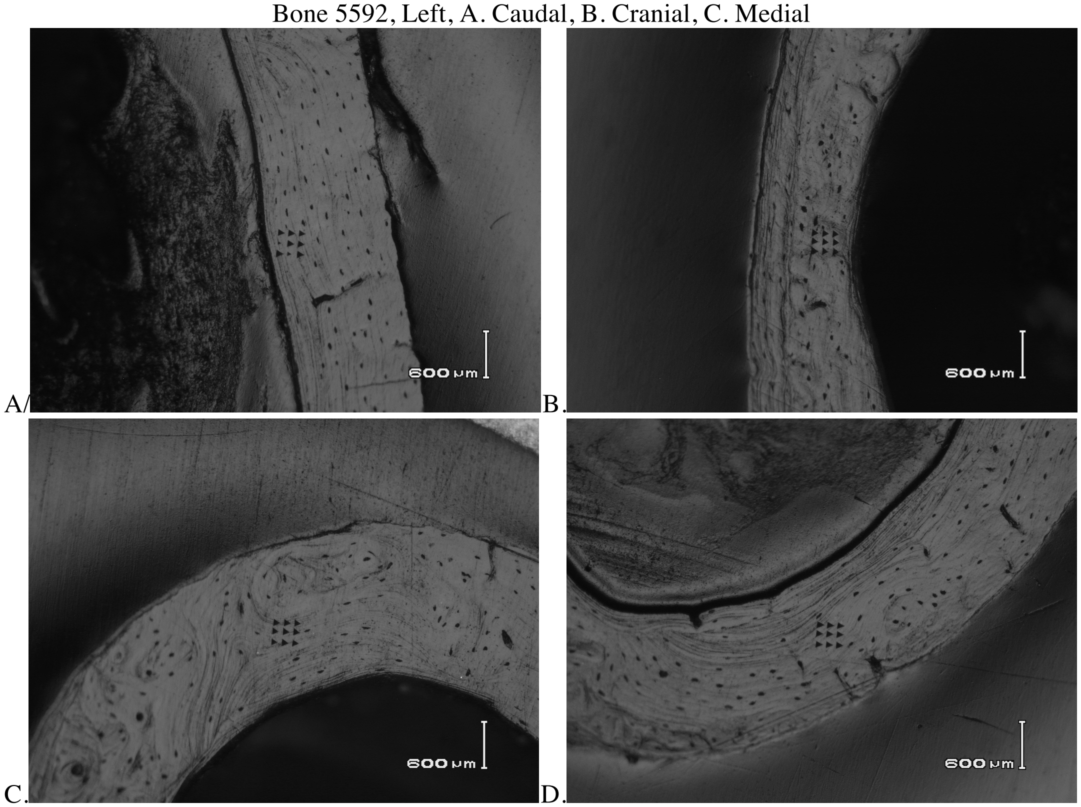


A. B. C. D.

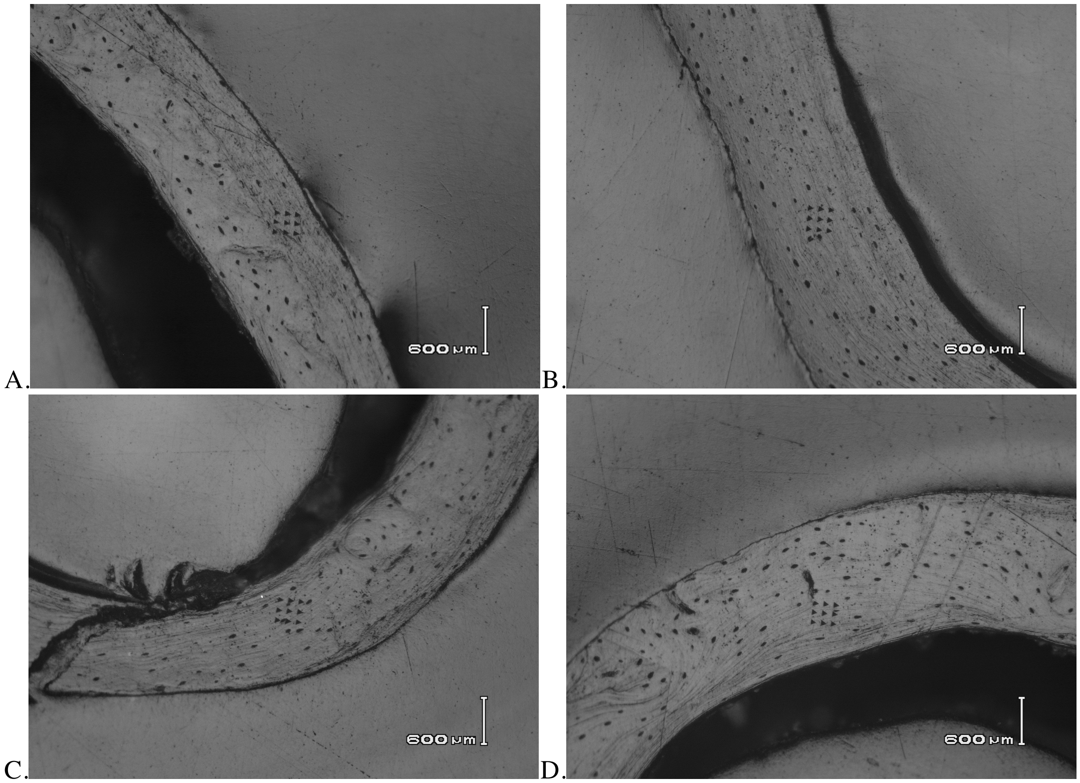
Bone 5599, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



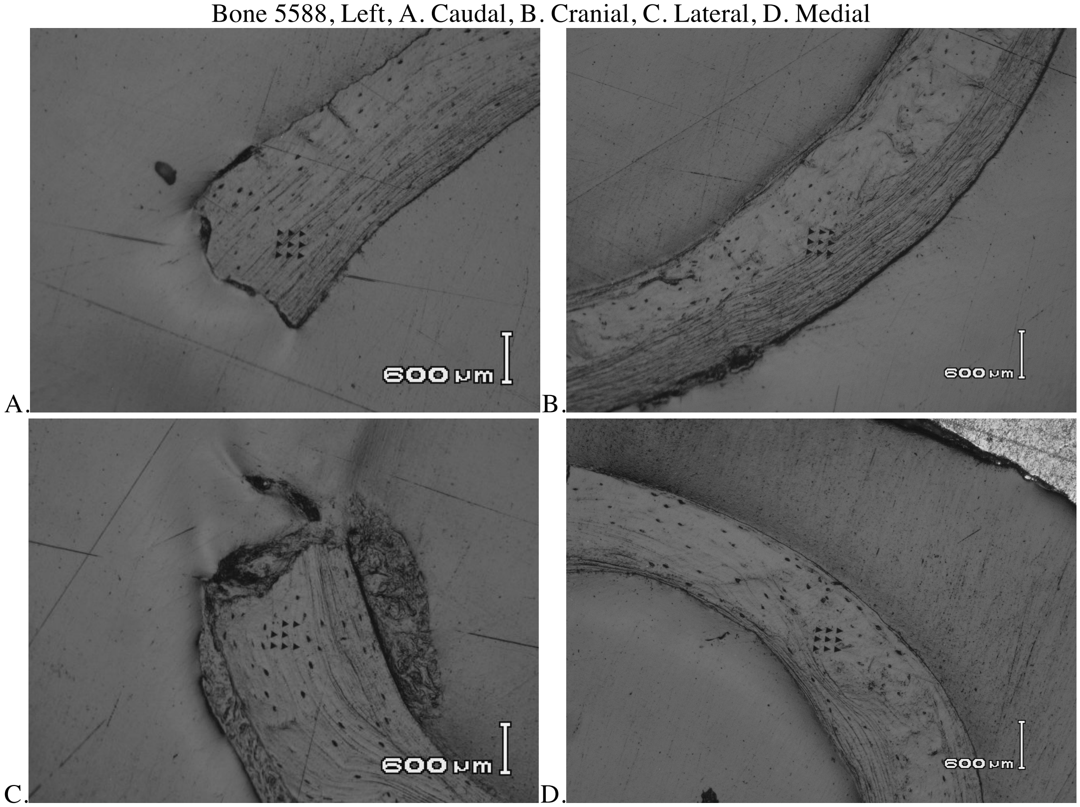
Bone 5592, Left, A. Caudal, B. Cranial, C. Medial



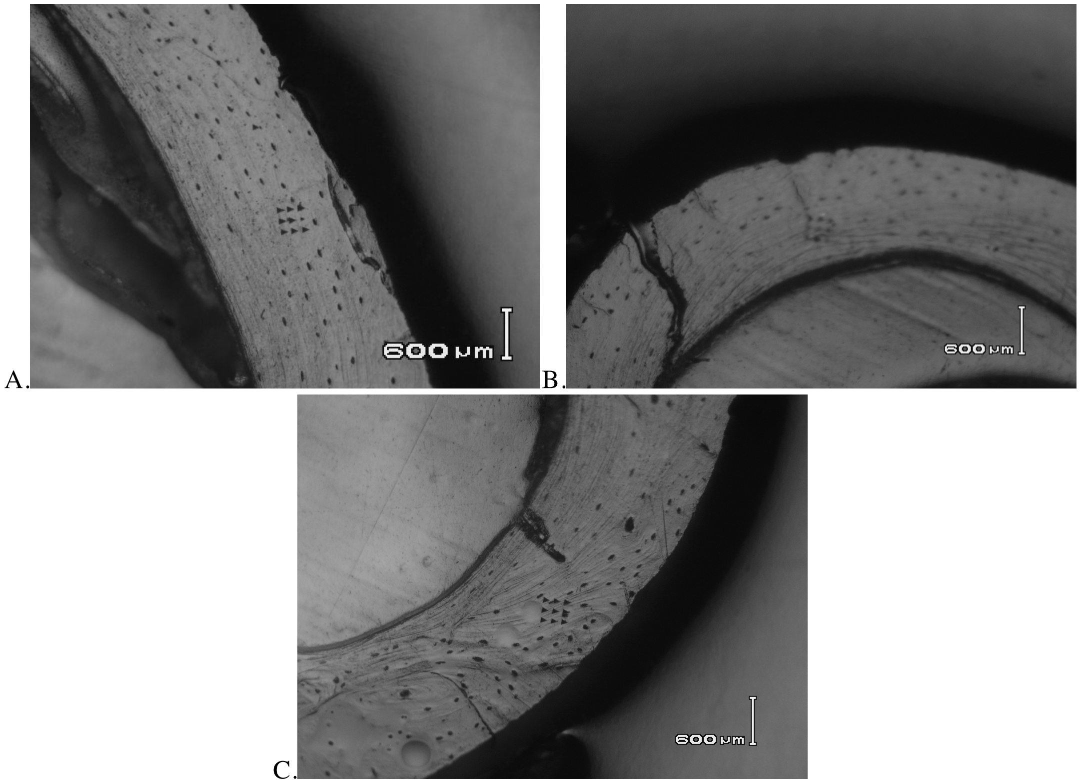
Bone 5592, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



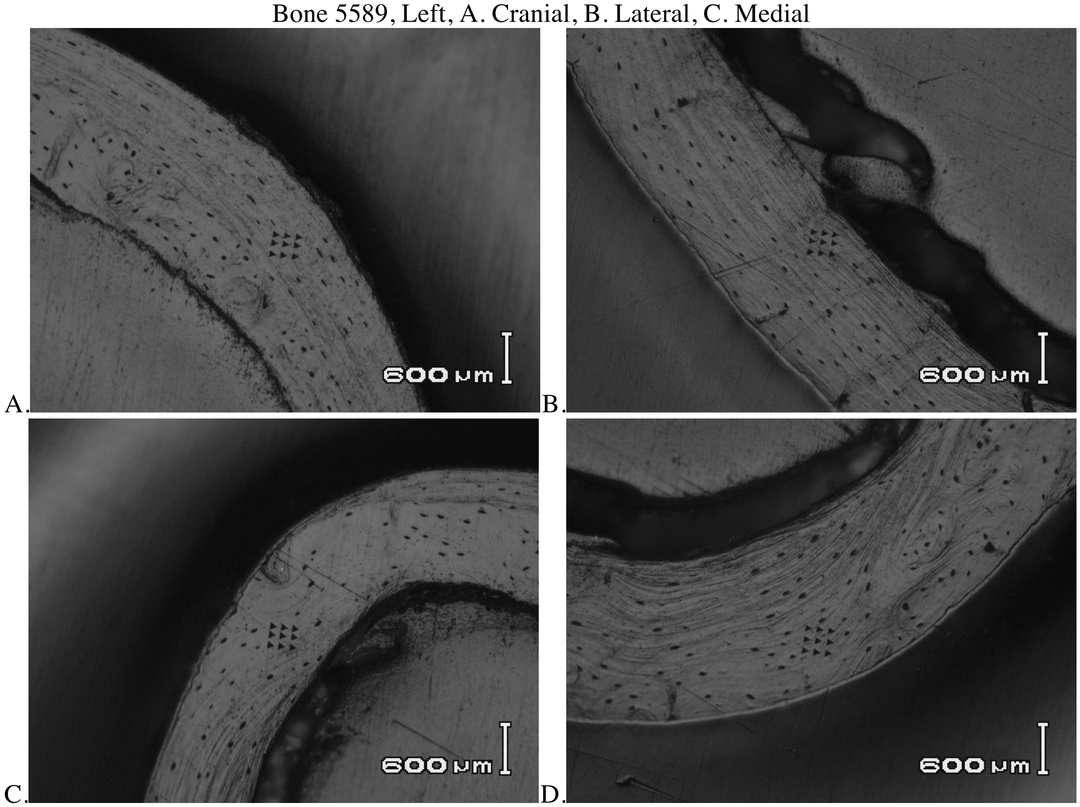
Bone 5588, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



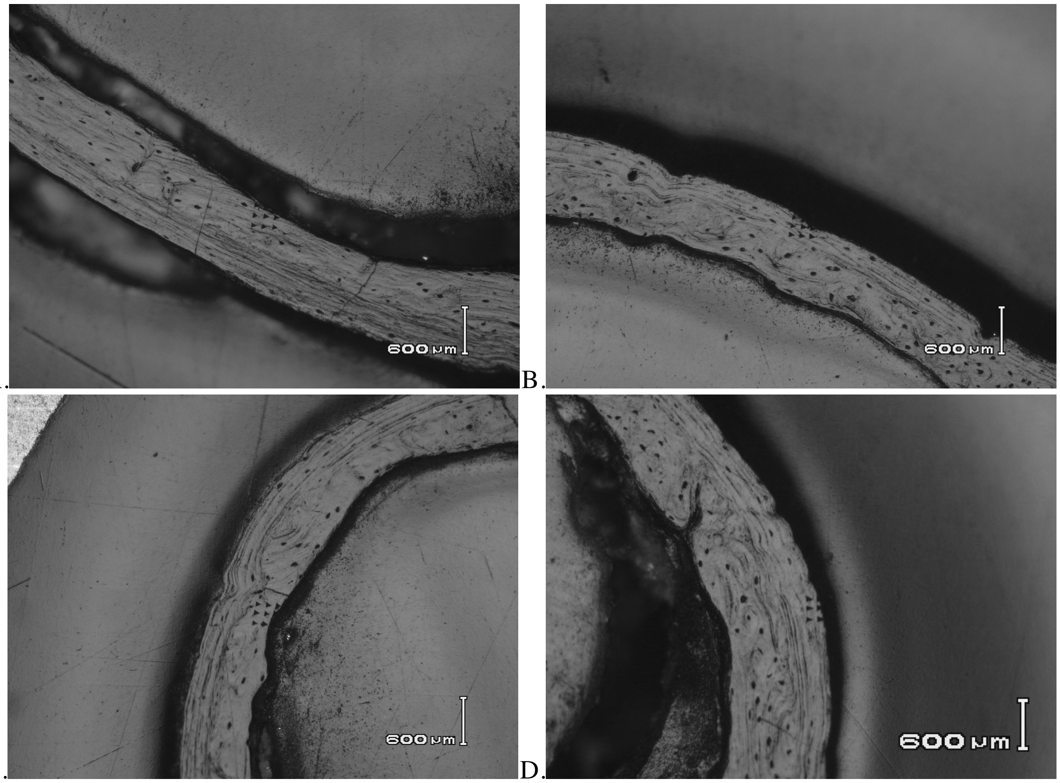
Bone 5588, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 5589, Left, A. Cranial, B. Lateral, C. Medial

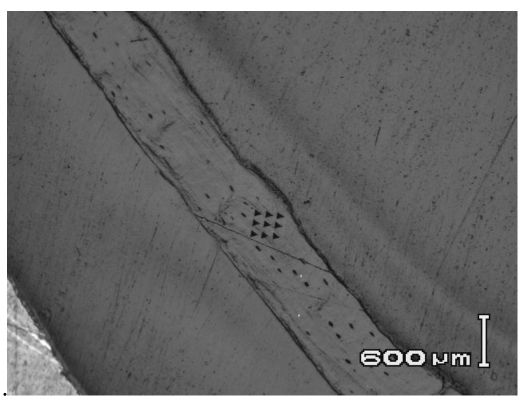


Bone 5589, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial



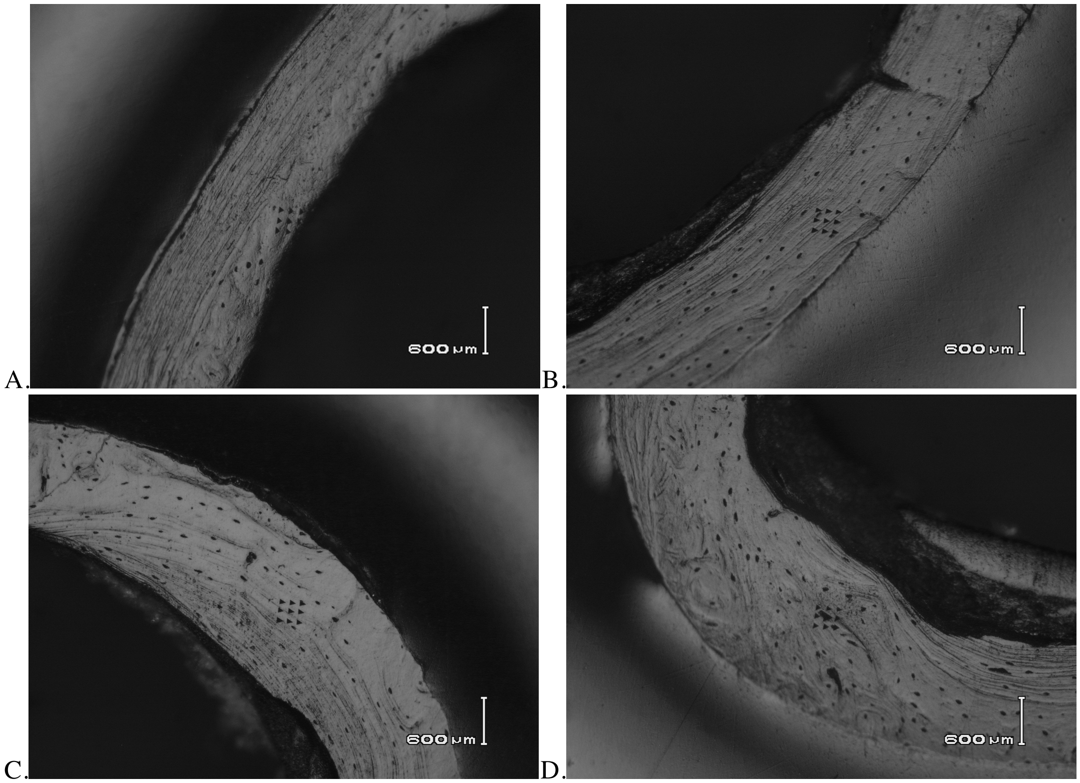
A. B. C. D.

Bone 8007, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

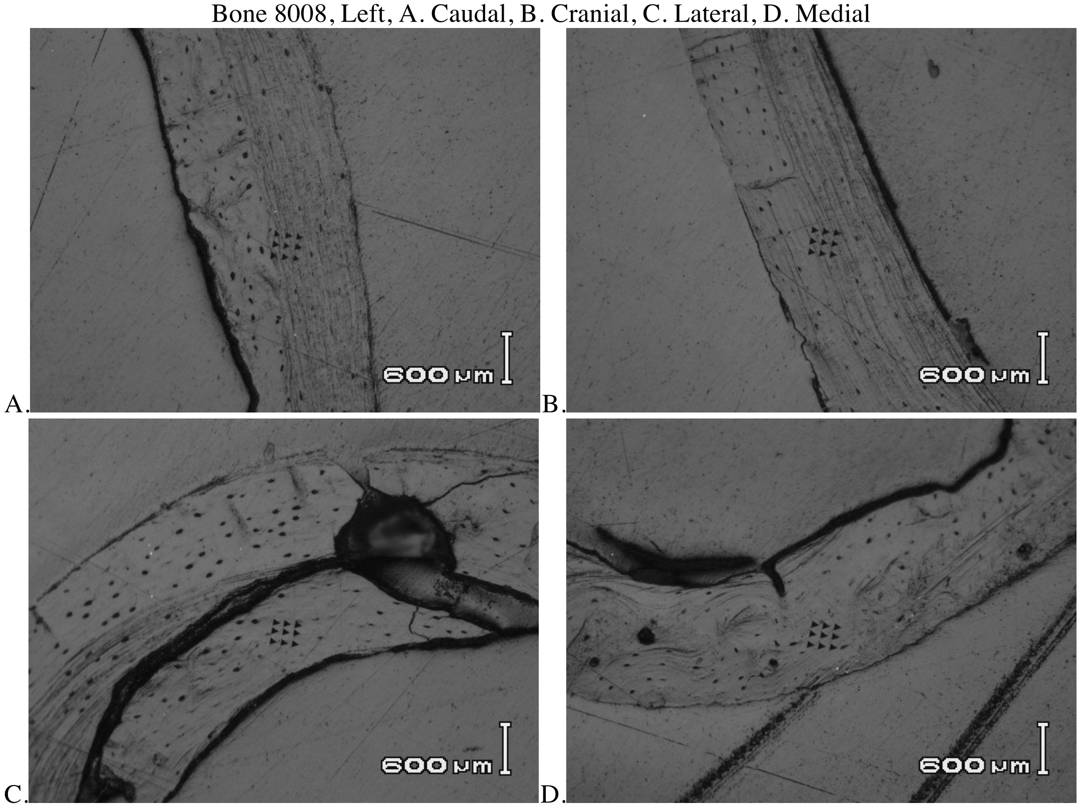


A.

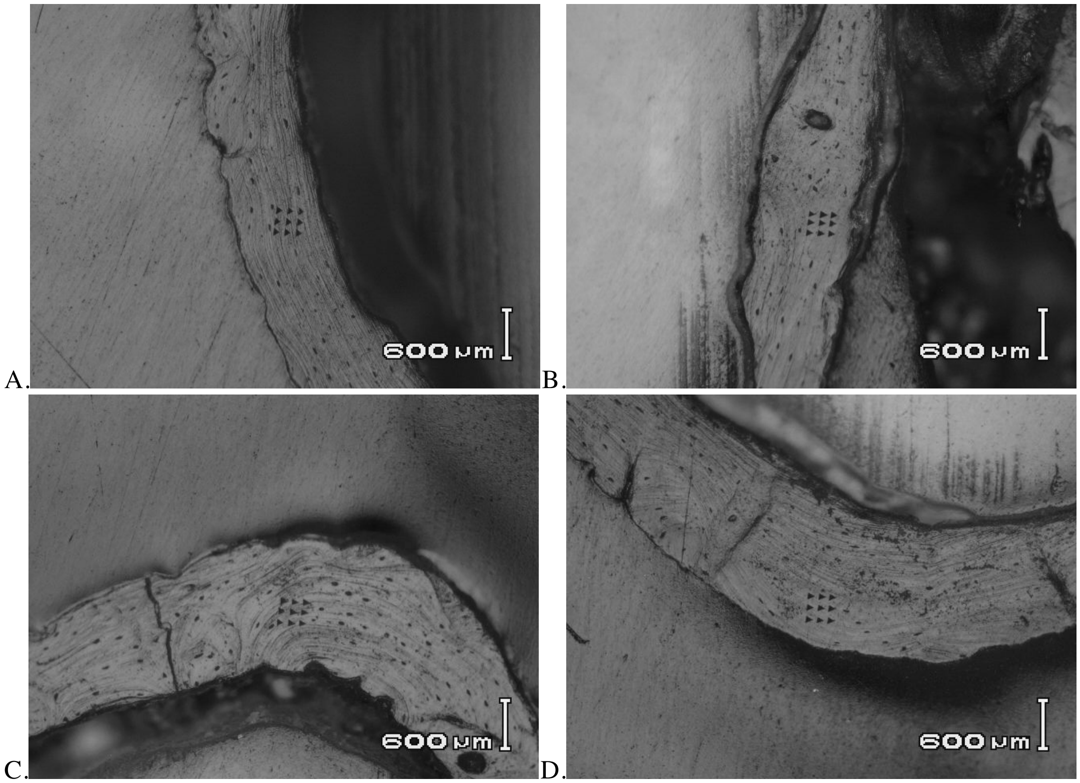
Bone 8007, Right, A. Cranial



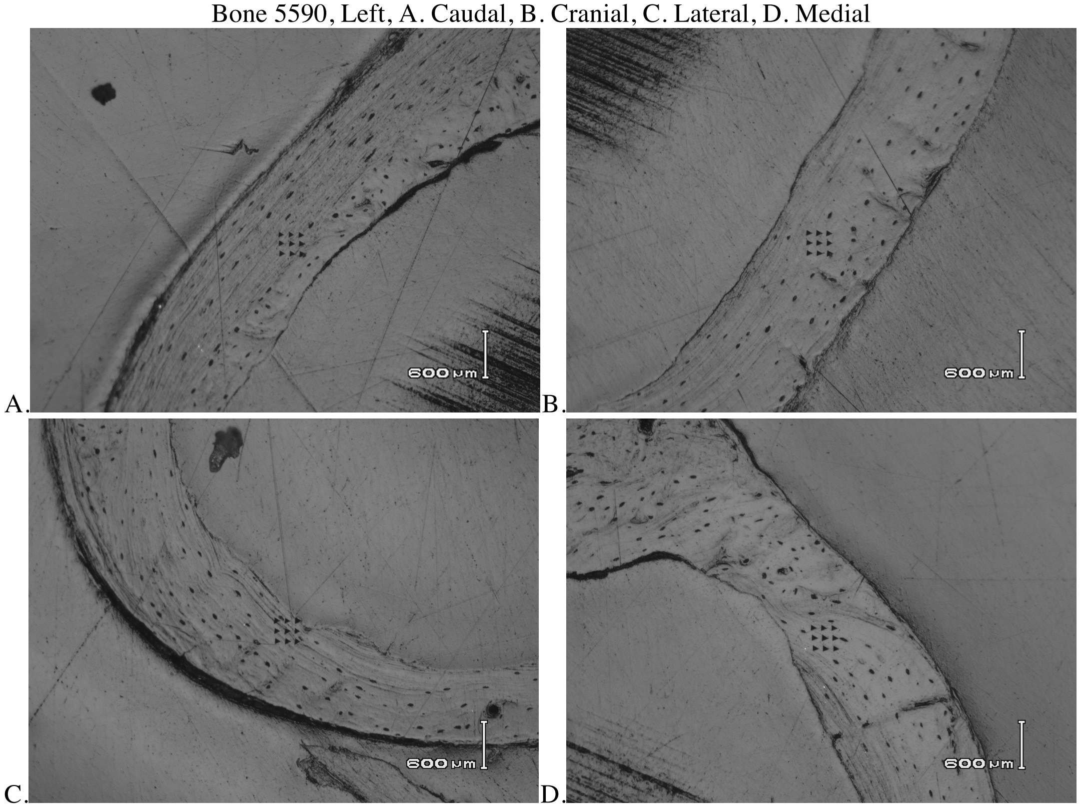
Bone 8008, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



Bone 8008, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial

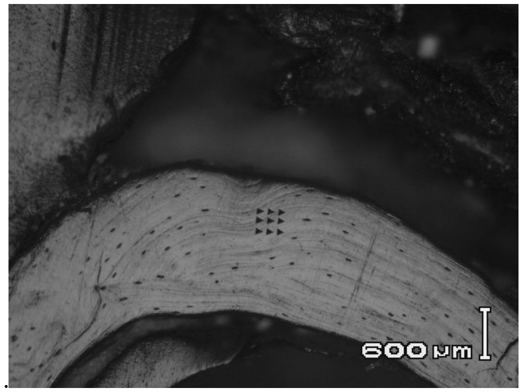


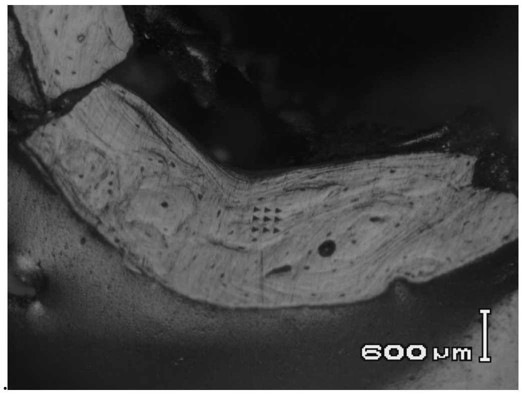
Bone 5590, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial



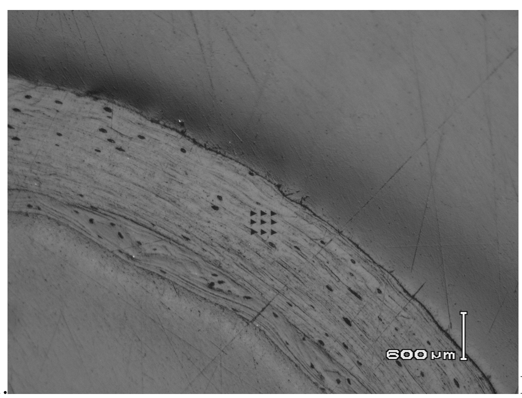
Bone 5590, Left, A. Caudal, B. Cranial, C. Lateral, D. Medial

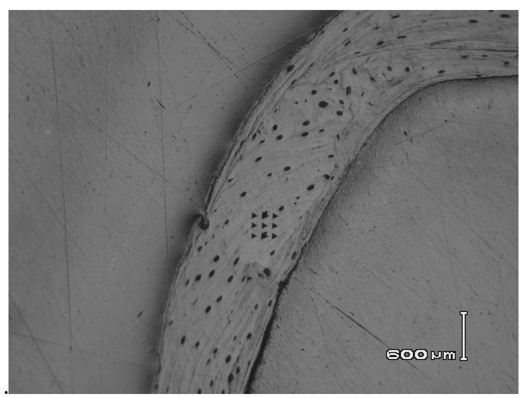
A. 

B. 

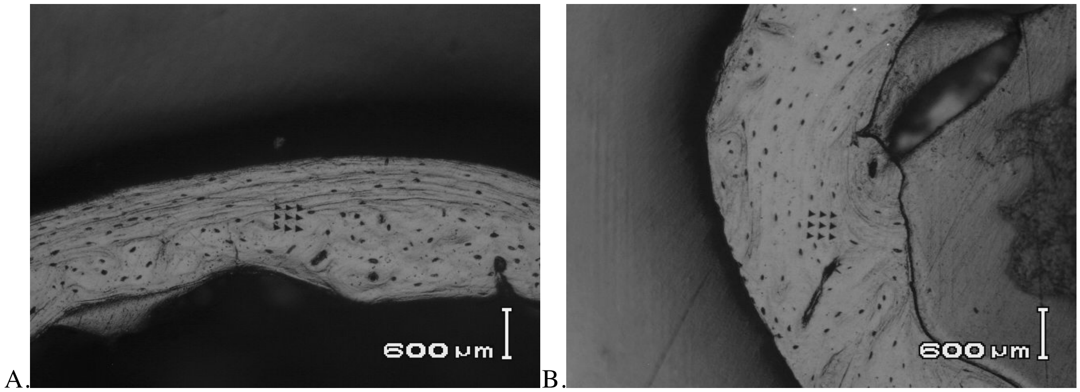
C. 

Bone 5598, Left, A. Caudal, B. Lateral, C. Medial

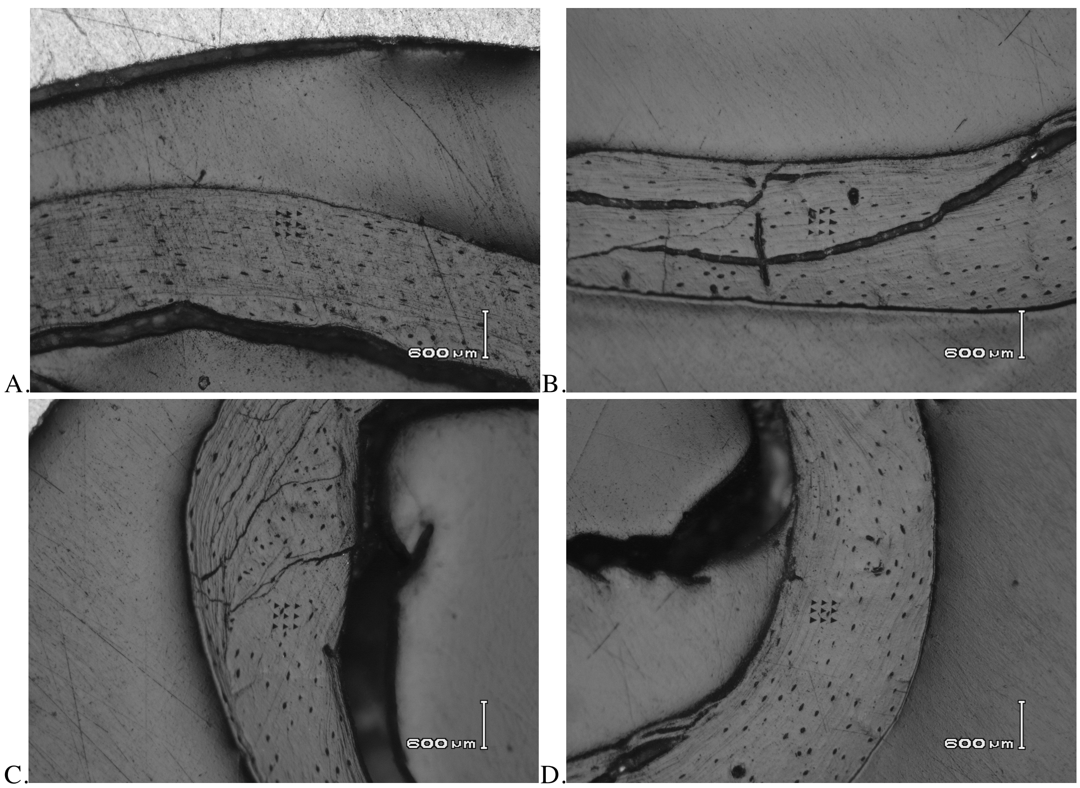
A. 

B. 

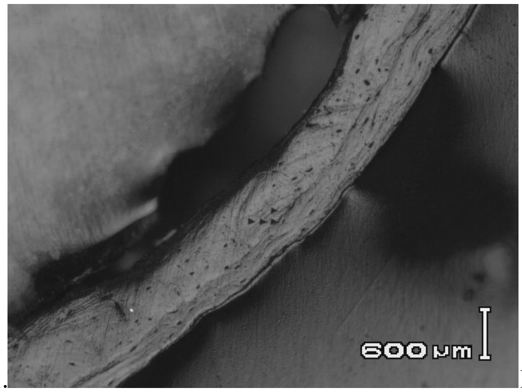
Bone 5598, Right, A. Caudal, B. Lateral

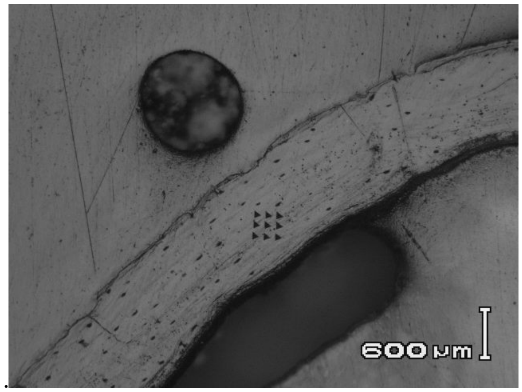


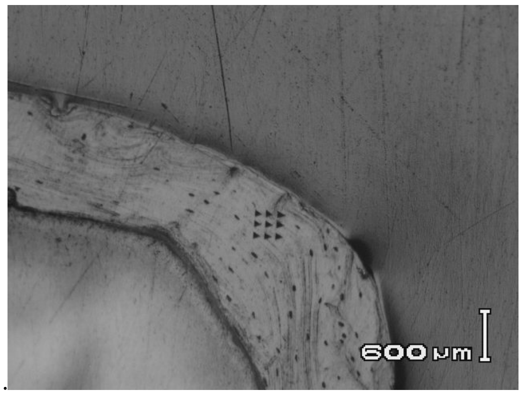
Bone 5593, Left, A. Caudal, B. Medial



Bone 5593, Right, A. Caudal, B. Cranial, C. Lateral, D. Medial

A. 

B. 

C. 

Bone 5597, Left, A. Caudal, B. Cranial, C. Medial

* Bone 5597, Right, NO EXPOSURE