

**Densitometric Comparison of Autogenous Cancellous Bone Graft and
Extracorporeal Shock Wave Therapy for Osteotomy Healing in the Tibial
Tuberosity Advancement Procedure in Dogs**

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ABSTRACT

Densitometric Comparison of Autogenous Cancellous Bone Graft and Extracorporeal Shock Wave Therapy for Osteotomy Healing in the Tibial Tuberosity Advancement Procedure in Dogs

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Objectives: To compare optical values in the osteotomy gap created after a Tibial Tuberosity Advancement (TTA) treated with autogenous cancellous bone graft (ACBG), extracorporeal shock wave therapy (ESWT), a combination of ACBG and ESWT, and absence of both ACBG and ESWT using densitometry.

Methods: Dogs presenting for surgical repair of a cranial cruciate ligament rupture were randomly assigned to one of four groups; TTA with ACBG (TTA-G), TTA with ACBG and ESWT (TTA-GS), TTA with ESWT (TTA-S), and TTA with no additional therapy (TTA-O). Mediolateral radiographs at 0, 4 and 8 weeks after surgery were evaluated to compare healing of the osteotomy gap via densitometry. An analysis of variance (ANOVA) statistical analysis was used to compare the densitometric values between groups.

Results: At 4 weeks after surgery, a significant difference in osteotomy gap density was noted between TTA-GS (8.4 millimeters of Aluminum equivalent [mmAeq]) and TTA-S (6.1mmAeq), and between TTA-GS (8.4 mmAeq) and TTA-O (6.4 mmAeq). There were no significant differences noted between groups at the 8 week recheck.

Clinical Significance: There were no significant differences in the osteotomy gap density at 8 weeks after surgery regardless of the treatment modality used. The combination of ACBG and ESWT may lead to increased density of the osteotomy gap in the first 4 weeks after surgery. Densitometry using an aluminum step wedge is a feasible method for comparison of bone healing after TTA in dogs.

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CHAPTER 1

Literature Review

1.1 Introduction to Extracorporeal Shock Wave Therapy (ESWT)

Extracorporeal Shock Wave Therapy is a treatment modality which utilizes high energy, high amplitude acoustic pressure waves ranging from 20-100 megapascals (MPa) [1]. These waves are characterized by very rapid buildup (5-10 nanoseconds) followed by an exponential decay to baseline, and a negative deflection. Each cycle takes approximately 300 nanoseconds to complete [2]. Acoustic pressure waves can be found in several places throughout nature (including the sound generated by an earthquake, applause, and thunder/lightening storms) and can permit a large transmission of energy [1, 3]. In order to utilize this therapy for medical purposes, there must be a controlled method for producing, transmitting, and focusing this energy.

Generation of shock waves involves the conversion of electrical energy to mechanical energy and can be accomplished through one of 3 methods: electrohydraulic, electromagnetic, or piezoelectric mechanisms [1, 3]. The electrohydraulic mechanism has been likened to the spark plug of a car and was the first method developed for the production of shock waves. Using this technique, a high voltage from a charged capacitor is applied across electrode tips. This creates a spark that heats and vaporizes surrounding liquid creating a gas bubble. Expansion and contraction of this bubble leads to the creation of a sonic pulse, or shock wave [3, 4]

The electromagnetic method involves use of an electromagnetic coil, opposing metal membrane, and a surrounding fluid medium. An electric current is passed through the coil creating a magnetic field. This, in turn, forces the opposing metal membrane

rapidly away from the coil thereby compressing the surrounding fluid and creating a shock wave. A lens is then used to focus the shock wave on an area of interest [3, 4]

In the final method, a large number of piezoelectric crystals are internally mounted on a sphere and surrounded by a fluid medium. A current is applied and causes rapid contraction and expansion of the crystals. This again causes compression and tension within the surrounding fluid and creation of a shock wave. The geometric orientation of the crystals focuses the shock wave on the treatment area of interest [3, 5].

The media in which a shock wave is released is very important. In air, the attenuation of shock waves is very high so energy is lost before an effect can be created. When produced within a liquid media, however, attenuation is very low and shock waves can be created and directed towards a point of interest [1].

1.2 Mechanism of Action of ESWT

Shock waves affect tissues through both direct and indirect actions. When shock waves travel through homogenous media (such as fluid or soft tissue structures), there is very little loss of energy until they encounter a hard acoustic interface (such as that between bone and soft tissue) [1]. When shock waves reach an interface, some of the waves are transmitted and some are reflected which leads to a large release in energy. This release in energy leads to the direct effect of shock waves which is microtrauma and microfractures at the interface surface.

Indirect effects of shock waves are facilitated by the creation of cavitation bubbles. As shock waves move through a liquid medium, they create tensile and compressive forces that eventually exceed the tensile force of the surrounding medium.

As a result, phase conversion occurs and a gas bubble (or cavitation bubble) forms.

These bubbles oscillate in diameter with subsequent shock waves and can absorb large amounts of energy as they expand. When the bubbles collapse (sometimes from 2mm to 5um), a large amount of energy is released in the form of a high energy, high temperature water jet. The jets are directed along the path of the shock waves and cause damage to acoustic interfaces in a similar fashion as the shock waves themselves (microtrauma and microfracture) [1, 3, 4].

1.3 Physical Parameters of ESWT

There are several measureable physical parameters of shock waves which can be used to guide therapy and make comparisons between different machines; These include pressure field, energy flux density, energy, and focal point [3]. A pressure field is measured in megapascals and is the pressure generated by a shock wave as a function of time and space. It is measured with a needle hydrophone on a polyvinylidene fluoride base. The energy flux density is a measure of energy per square area (mJ/mm^2) and is a common value used to make comparisons between different machines. It can also be defined as the maximal amount of acoustical energy that is transmitted through an area of 1mm^2 per pulse. Energy is the sum of all energy densities across the entire beam profile and is reported in millijoules (mJ). This is not the same value as energy flux density as it is the total acoustical energy per released shock wave, and is not influenced by time or space. Shock waves can generally be split into 3 energy levels; high energy ($0.6\text{mJ}/\text{mm}^2$), medium energy ($0.28\text{mJ}/\text{mm}^2$), and low energy ($0.08\text{mJ}/\text{mm}^2$). Lastly, the focal point is the area of interest being treated. This can be defined mathematically as the

area in millimeters within which the pressure of the shock waves is at least $\frac{1}{2}$ of its peak value. Alternatively, it can be defined as the volume of tissue defined in millimeters along the x, y, and z axes which the pressure exceeds 5MPa [3, 6].

The action of shock waves can be predicted based on several known factors of the treated material. For instance, if the acoustic impedance for a given substance is known, this can be used to determine what percentage of shock waves will be transmitted and what percentage will be reflected [3]. The acoustic impedance for a substance (Z), can be determined based on the following equation

$$Z = \rho c$$

Where ρ is the density of the medium and c is the sound velocity of the medium. When the acoustic impedance for 2 different substances are known, then amplitude of reflected shock waves (I_r) can be calculated using the following

$$I_r = I_o (Z_2 - Z_1) / (Z_2 + Z_1)$$

Where I_o is the amplitude of the initial sound wave, and Z_1 and Z_2 are the acoustic impedances of 2 opposing substances [3]. The large amount of energy released at an interface can be used to fracture or break the material making up that interface. To determine the maximum volume of an object than can be disintegrated (V), the following equation can be used

$$V = eEn$$

Where e is constant, E is the total energy of each shock wave pulse, and n is the number of applied shock wave pulses [3].

1.4 History of ESWT

The effect of shock waves on biological tissues was first noted during World War II when damage to lung tissue of submerged castaways was noted following underwater explosions [1]. Further research on the effects of shock waves on inanimate objects soon followed in Germany and included fracture of ceramic plates submerged in water. Application of shock waves for clinical medicine began in the 1970s when shock waves were used to treat a kidney stone in vivo using a process called lithotripsy. The first clinical patient with a kidney stone was treated with shock wave therapy in 1980 at Dornier Aerospace Engineering. Treatment using the first generation of machines (or lithotripters) required that the patient be fully submerged in a water bath [1]. Advances over the next decade included development of dry coupling (lithotripsy without the need for a water bath), treatment of additional diseases (such as gall bladder and salivary stones), and development of smaller and more portable machines [1, 3].

1.5 Orthopedic Applications of ESWT

Investigation into the effects of shock waves on orthopedic tissues began in the late 1980s due to concern for damage to the pelvis during treatment for urinary calculi [2]. It was found that the ilium of lithotripsy patients had focal areas of osteocyte death followed by a significant recruitment of osteoblasts within about 70 hours of treatment [4]. Although the exact mechanism by which shock waves effect orthopedic tissues is not completely understood, several effects have been noted and include enhanced healing of fractures, reduced inflammation/swelling, short term analgesia, improved vascularity, realignment of tendon fibers, and enhanced wound healing [7-9].

Shock waves have been used for the enhancement of bone healing after acute fractures as well as for treatment of delayed and non-unions [10-12]. In addition to faster healing after treatment with shock waves, treated bones have also shown greater biomechanical properties than untreated bones. In a study by Wang in 2001, shock waves were applied to a tibial gap model in dogs. Treated bones had significantly thicker and heavier cortical and woven bone as compared to the untreated limb [13]. In a study by Haupt in 1992, diaphyseal fractures in rats that were treated with shock waves had faster healing and 30% greater breaker strength than controls [14]. Animal studies have also showed greater bone stiffness, osteoblast activation, bone mineral density, and tensile load of treated bone compared to controls [12, 15, 16].

One mechanism by which shock waves may have this effect on healing bone is through activation, or up-regulation, of beneficial cytokines. In a study by Wang in 2003, the effects of extracorporeal shock wave therapy on the Achilles tendon insertion in New Zealand White rabbits was evaluated [17]. Biopsies of the treated area were taken 1, 4, 8, and 12 weeks after a single treatment. Elevations in endothelial nitric oxide, vascular endothelial growth factor, and proliferating cell nuclear antigen were noted within 1 week of treatment. These factors, which are involved in angiogenesis, then remained elevated until 8 weeks after treatment. Additionally, histopathologic analysis of the tissue showed an increase in the number of new blood vessels starting 4 weeks after treatment and lasting until 12 weeks after therapy. Increase in vascular density, as well as an increase in the aforementioned cytokines, may be one mechanism through which bone healing is enhanced after shock wave therapy [17]. Additional cytokines noted to be increased after shock wave therapy include Transforming Growth Factor Beta (involved in

differentiation of bone marrow stromal cells into osteoprogenitor cells) and Bone Morphogenic Protein (capable of inducing of new bone formation) [18].

The ability of shock waves to modulate pain sensation has also been investigated [19-23]. Force plate analysis of horses with lameness localized to the distal forelimb indicated improvement of force plate data (including peak vertical force and peak vertical impulse) within 8 hours after shock wave therapy [24]. Within 2 days of treatment, the force plate data was not significantly different from that recorded after peripheral nerve block. Analgesic effects were short lived, however, and generally diminished within several days of treatment.

More long-term relief of pain due to osteoarthritis has also been demonstrated in multiple studies. In 2007, Mueller showed that dogs with hip osteoarthritis treated with ESWT had improved peak vertical force, vertical impulse, and symmetry indices compared to control animals [22, 23]. Beneficial effects were documented from 1-3 months after treatment. When dogs with stifle arthritis were treated with shock wave therapy, improvement was documented in stifle range of motion and force plate data (although data was not significantly different from control animals) [21]. No benefit, however, was seen when dogs with elbow arthritis were treated with shock wave therapy [11].

The mechanism by which shock waves provide analgesia is not completely understood. However, one study evaluated the effects of shock wave therapy on a peripheral nerve (the palmar digital nerve in horses) in an attempt to elucidate the pain relief pathway [20]. This study showed a significantly slowed nerve conduction velocity 3 and 7 days after treatment. Additionally, microscopic analysis of the nerve showed

myelin sheath disruption with no evidence of damage to Schwann cell bodies or axons. It has also been hypothesized that the change in vascularization of treated tissue may, in part, be responsible for this beneficial effect [6].

In addition to skeletal structures, extracorporeal shock wave therapy has been used for treatment of soft tissues (including muscles, tendons, cartilage, and ligaments). In a study by Danova in 2003, dogs with supraspinatus calcifying tendinopathy were treated with shock wave therapy [25]. Treated dogs had improvement in activity levels at home, improvement in force plate data, and fragmentation of mineralized stones within the tendon. The junction between the patella and the patellar ligament in rabbits has also been treated with shock wave therapy after partial patellectomy [12]. Densitometric evaluation of the treated area showed almost 50% greater bone density 8 weeks after treatment and approximately 455% increase in bone mineral density 12 weeks after treatment. Histologic evaluation of the ligament showed better parallel alignment of collagen fibers in the treated group. Non-treated animals had poor alignment of the fibers along with extensive scar tissue formation and the bone ligament junction.

Studies have also investigated the effects of shock wave therapy on the open physis. When immature rabbit bone (including the physis) was treated with extracorporeal shock wave therapy in one study, no changes in macroscopic, radiographic, or histologic appearance occurred at 1, 3, 12, and 24 weeks after treatment indicating that it is likely safe to treat animals with open growth plates with shock wave therapy [26].

Currently, ESWT is used for treatment of a variety of orthopedic conditions in both human and veterinary medicine. It is approved by the Food and Drug

Administration (FDA) for treatment of plantar fasciitis in humans [27]. Additionally, in human medicine it has been used for treatment of lateral epicondylitis (tennis elbow), Achilles tendinopathy, delayed or non-union of fractures, and calcified tendonitis of the shoulder [7, 28, 29]. In veterinary medicine, it has been used for many different diseases including treatment of delayed or non-union of fractures, promotion of wound healing, navicular disease, and supraspinatus tendinopathy [10, 25, 30].

Several recent veterinary studies have emerged evaluating the effects of shock wave therapy on orthopedic tissues. In a study by Gallagher in 2012, dogs with patellar tendonitis after Tibial Plateau Leveling Osteotomy were treated with extracorporeal shock wave therapy [30]. Treated dogs had a significant decrease in patellar ligament thickness after treatment. In another study by Becker in 2015, dogs with instability, calcifying, or inflammatory conditions of the shoulder were treated with extracorporeal shock wave therapy [31]. Although conventional treatment methods had not been successful for these animals, treatment with extracorporeal shock wave therapy led to improvement in lameness scores for 65% of animals. In 2014, the effect of extracorporeal shock wave therapy on bone healing after tibial plateau leveling osteotomy was reported in an abstract by Duerr. Treatment using this modality led to increased healing scores 2 months after surgery compared to control animals [32].

1.6 Complications of ESWT

Extracorporeal Shock Wave Therapy for orthopedic disease has been associated with several minor complications. The most commonly reported complications include local petechiation and superficial pain at the treatment site [4, 33]. Additional reported

complications include dose dependent hemorrhage, leakage of macromolecules from treated muscle and dose dependent deep bone pain [34, 35]. Lung tissue appears to be susceptible to damage from shock waves which precludes treatment of surrounding thoracic structures (such as rib or clavicular fractures) [7, 36].

1.7 Bone Graft

Bone graft is a material commonly used to enhance bone healing in a variety of surgical procedures including treatment of fracture non-union, arthrodesis, septic osteomyelitis, tumor resection, replacement of joint surfaces, periprosthetic augmentation, and spinal fusion [37, 38]. Bone graft can either be an autograft (transferred from one site to another on the same individual), allograft (transferred from one individual to another of the same species), or a xenograft (transferred from one individual to another of a different species) [39].

There are 4 properties of bone graft which have been described; these include osteogenesis, osteoinduction, osteoconduction, and osteopromotion [40]. Osteogenesis is the supply and support of bone forming cells. It is a function performed either by donor or host cells [39, 41]. Osteoinduction is the capacity to induce bone formation when placed into a site where no bone formation would occur otherwise. This is accomplished through recruitment of bone forming cells, chemoattraction, and migration. Examples of substances which provide this feature include demineralized bone matrix, bone morphogenic proteins (BMPs), tumor necrosis factor, and prostoglandin E2 [39]. Osteoconduction is the supply of scaffolding for mesenchymal cell proliferation and vascular ingrowth. This scaffolding has surface characteristic that allow for adherence of

cells and has an interconnecting porosity for cellular ingrowth, fibrovascular invasion, and bone ingrowth. Osteoconductive substances may share in the load bearing of the structure and include autogenous cancellous bone graft, hydroxyapatite, and certain synthetic materials (including ceramics, bioactive glass, and tricalcium phosphate) [42]. Osteopromotion is the enhancement of regenerating bone without cells or a scaffold. These materials cannot induce bone formation alone. One example of an osteopromotive substance is platelet rich plasma [40].

1.8 Autogenous Cancellous Bone Graft (ACBG)

Autogenous Cancellous bone graft uses all of the above strategies for bone regeneration. Use of ACBG not only provides transplanted osteoblasts, but also induces host mesenchymal cells to differentiate along the bone forming cell line [39]. Cancellous graft is composed of highly porous trabeculae which provide scaffolding for new bone formation. Additionally, it provides mesenchymal stem cells, osteoclasts, and living bone cells [39]. Common sites for collection of ACBG include the distal humerus, wing of the ilium, proximal tibia, and proximal humerus [40, 41]

The benefits of using ACBG include an increased osteogenic potential compared to cortical graft, no issues with compatibility compared to allograft or xenograft, and decreased risk of disease transmission compared to allograft or xenograft [37, 43]. Potential complications associated with the use of ACBG include the need for a separate surgical site, increased operative time, increased cost associated with graft harvest, donor site morbidity (including pain or iatrogenic bone fracture), and limited quantity [41].

1.9 Mechanism of Action of ACBG

Bone graft incorporation is the interaction between graft and host tissues which results in new bone formation and healing across a bony defect [44]. Phases of incorporation include inflammation, revascularization, osteoinduction, osteoconduction, and finally, remodeling [42]. During the first week after placement of the bone graft, there is a large invasion of blood vessels and increase in vascularity to the grafted tissue. A hematoma forms which is rich in growth factors and cytokines. Transplanted osteoblasts have been noted to survive for up to 1 week after implantation [45]. Soon after, migration and proliferation of mesenchymal stem cells occurs. Granulation tissue, which is rich in fibrous tissue and blood vessels, begins to invade the graft site and contributes to the survival of graft cells. Granulation tissue formation and vascular ingrowth is generally complete by about 2 weeks after placement. Also, by this time, the central graft region changes from a predominantly fibrous tissue composition to one with marrow composition. Osteoprogenitor cells then begin to differentiate into bone forming cells (osteoblasts), and poorly mineralized bone is laid down [39]. Cortices at the edge of the defect may show increased porosity during this period. Six weeks after bone graft placement, blood vessels can be noted penetrating the cortex from the marrow and periosteum and a mature cortical rim forms around the grafted site [45]. Eight weeks after placement, bone marrow sinusoids become visible and cortical bone formation proceeds. Twelve weeks after placement of the bone graft, a solid bony union is generally seen and the radiographic appearance of the grafted area is similar to mature cancellous bone [46].

Histologic and histochemical analysis of bone graft fusion has been previously performed to evaluate the contribution of grafted cells to healed bone [45]. One week

after placement of the bone graft, newly formed woven bone and cartilage was apparent. Most of the graft fragments, however, contained empty lacunae suggesting absence of viable osteocytes. Two weeks after placement of the bone graft, islands of endochondral ossification were noted but fewer graft-derived cells were present compared to the first week. Six weeks after placement of the bone graft, graft-derived osteocytes were present within the cortical rim. Histochemical analysis of the tissue suggested that these cells represented de novo osteocytes derived from graft precursors rather than survival of transplanted osteoblasts.

1.10 Primary Bone Healing

Bone healing can typically be divided into either primary or secondary healing [47]. Primary bone healing occurs whenever the biological environments provides less than 1mm interfragmentary gap and less than 2% interfragmentary strain. Strain is defined as a ratio between the change in fracture gap length relative to the original size of the fracture gap. Primary bone healing can additionally be separated into gap primary bone healing, and contact primary bone healing. Contact primary bone healing occurs when the fracture edges are in direct contact with one another (no fracture gap) and where there is zero interfragmentary strain. Bony bridging across the fracture gap occurs without any callous tissue intermediate. Haversian remodeling occurs across the fracture line to slowly bridge the fracture with longitudinally oriented osteons [48].

Gap primary bone healing occurs when interfragmentary gap and strain are still present (although the gap is still less than 1mm and the strain is still less than 2%) [49]. During this type of healing, granulation tissue forms first within the fracture gap and

lamellar bone then forms at the fragment ends. This lamellar bone is initially oriented transversely to the long axis of the bone until remodeling occurs. Eventually Haversian remodeling proceeds as osteons cross the fracture gap and mature bone is laid down.

1.11 Secondary Bone Healing

Secondary bone healing occurs when the interfragmentary gap is greater than 1mm and the interfragmentary strain is greater than 2% [47]. During this method of healing, intermediate stages of tissue are formed with increasing strength and stiffness until bony union is achieved. The inflammatory phase occurs first and begins immediately after trauma to the bone and soft tissues. This phase is characterized by hematoma formation, ischemic bone necrosis, and formation of a fibrin mesh at the fracture line [50]. The hematoma is osteoinductive and has the capability of inducing bone formation even at ectopic sites. Although it does not provide any mechanical support to the healing bone, it does provide a scaffolding into which the callous and fibrous tissue can form. Necrosis and hypoxia of the bone edges can occur and is characterized by presence of empty lacunae.

Bone resorption at the edges of the fracture gap can occur starting within 1 week after trauma and is noted radiographically by widening of the fracture gap. Mononuclear cell invasion proceeds and results in the removal of necrotic tissue and formation of a callous. Neovascularization is also initiated through production of vascular endothelial growth factor and local acidity in the environment. Blood supply to the healing fracture is obtained by a transient extraosseous blood supply from the surrounding tissues. By the

end of the 1st week, the hematoma is resorbed. The end of the inflammatory period is usually associated with a decrease in pain and swelling at the fracture site [47].

The next phase in secondary bone healing is the reparative phase. During this period, mesenchymal stem cells differentiate into fibroblasts, chondroblasts, and osteoblasts. Granulation tissue forms within several days and provides enhanced biomechanical properties to the fracture site as it can typically withstand tension up to 0.1Nm/mm^2 [47]. Maturation of granulation tissue follows with the deposition of collagen (type I predominates as the maturation process proceeds). This mature tissue has greater strength compared to the previously noted granulation tissue ($1\text{-}60\text{Nm/mm}^2$). Within a few weeks, a soft callous begins to form giving the healing bone even greater strength ($4\text{-}19\text{Nm/mm}^2$). Callous formation provides an enlarged cross-sectional area which significantly increases the fracture's resistance to bending forces and decreases the local strain. Additionally, callous has a good resistance to compression. Bone Morphogenetic Protein (BMP) and Transforming Growth Factor Beta ($\text{TGF-}\beta$) trigger differentiation of stem cells into chondroblasts and osteoblasts [47].

Radiographically, this callus may not be fully visualized until mineralization completes. Mineralization occurs as calcium granules are accumulated within the mitochondria in chondrocytes. Macrophages then degrade the non-mineralized matrix, and osteoprogenitor cells form new trabeculae. Mineralization first occurs near the periosteum and is visible on radiographs as a collar around the fracture site.

Mineralization then proceeds from the fracture ends across the fracture gap. At the end of the reparative phase, radiographic disappearance of the fracture line occurs [49, 51].

During the remodeling phase, biomechanical properties of the healing fracture begin to return towards that of the original bone. Unneeded bone is resorbed and newly formed bone is converted to lamellar bone through Haversian remodeling. Due to its piezoelectric nature, bone formation is dictated by the applied forces. Compressive forces result in osteoblastic activity whereas tensile forces stimulate osteoclast activity and bone resorption. The remodeling phase is the longest phase of fracture healing and may last several years [49].

1.12 Healing of Metaphyseal Bone

Healing in the metaphysis of bone has been investigated previously through creation of incomplete distal femoral osteotomies in rabbits, rats, and dogs [52]. Intertrabecular cellular proliferation was visible within 3 days. Similar to secondary bone healing, the hematoma at the fracture gap was replaced by loose connective tissue by 7 days. Bridging of trabeculae by woven or lamellar bone was documented prior to bridging of the cortical shell. An absolute increase in bone density was seen after healing was complete [52].

1.13 Assessment of Bone Healing in Human Medicine

Assessment of bone healing is important after fracture fixation and elective orthopedic surgeries. Multiple different methods exist for assessment of healing and include measurement of bone mineral density and radiopacity. In human medicine, these methods include radiogrammetry, densitometry, dual and single photon absorptiometry, neutron activation analysis, quantitative CT, and dual energy quantitative CT [53].

Radiogrammetry is the simplest method and involves qualitative assessment of a plain roentgenogram to determine the thickness of cortical bone, the shape of the inner surface of the cortical bone, and the changes of opacity within the bone.

Densitometry was the first quantitative method for evaluation of bone healing and compares bone absorption of ionizing radiation by comparing it to a metal step wedge. Single-photon absorptiometry is similar to densitometry, but instead of x-rays, it utilizes emitted photons from a ^{125}I source. The area of interest is placed within a water bath, and a scintillation detector is used for assessment of bone density [53]. One limitation of this method is that it can only be used for assessment of the distal extremity due to the need for submersion in a water bath.

Bone mineral density throughout the whole body can be assessed with either dual photon absorptiometry or neutron activation analysis. Dual photon absorptiometry uses ^{153}Gd to emit 2 distinct photons. Simultaneous equations can then be used to calculate the density of bone by subtracting the effects of soft tissues in the surrounding field. This method can be influenced and altered based on the presence of bony degenerative changes (such as osteophytes), fracture compression, or extraskeletal mineralization [54]. Neutron activation analysis is a method for determination of the amount of calcium within bone. Neutrons are directed at the area of interest and are able to convert body ^{48}Ca to ^{49}Ca . As ^{49}Ca decays, gamma photons are released and measured. Availability of this technique is limited to specialized facilities [53].

Dual energy radiography is an additional method used to measure bone density with greater precision and accuracy compared to dual photon absorptiometry. This method uses an x-ray source rather than a radioisotope and has very high resolution of

images. It has a shorter scan time, lower radiation dose, lower cost, and better bone edge detection than dual photon absorptiometry [55].

Computed Tomography (CT) can also be used for estimation of bone mineral density. Quantitative CT can measure the density of trabecular bone with a very high sensitivity by converting the measured Hounsfield Units (HU) to volume of bone based on a standard calibration solution of potassium monohydrate phosphate. Dual energy quantitative CT can also be used to decrease the errors encountered with quantitative CT introduced by varying degrees of soft tissue and fat in the surrounding bone [53].

1.14 Assessment of Bone Healing in Veterinary Medicine

In veterinary medicine, bone healing can be qualitatively assessed based on the radiographic appearance of the healing bone using a variety of factors. Several grading scales exist based on the degree of bone resorption, remodeling of fracture edges, degree of new bone proliferation, and size of the fracture gap [38, 56, 57]. Additional scales of bone healing rely on subjective measures of callus formation, periosteal reaction, or the number of bridging cortices [58-60].

1.15 Densitometry

Densitometry is another method for evaluation of radiographic bone healing in veterinary medicine which allows for quantitative assessment of a fracture gap.

Densitometry facilitates conversion of the optical density of healing bone to an equivalent thickness of metal standard [61]. The optical density is a logarithmic measure of the ratio of transmitted to incident light through a film image. Multiple factors exist which

prevent direct comparison of optical density between images (these include exposure parameters, film characteristics, and processing conditions). Conversion of optical density to an equivalent thickness of metal allows for standardized comparisons across films.

To perform this technique, a metal step wedge is radiographed adjacent to the area of interest. Aluminum is typically used for production of step wedges with a recommended purity of 99.5% according to the International Organization for Standardization [62]. However, use of aluminum step wedges of slightly lower purity levels has been investigated. Acceptable step wedges include those composed of at least 98% aluminum by weight, less than 0.05% copper, and less than 1% iron [61].

In order to use densitometry, a linear relationship between steps of the step wedge must be confirmed by creation of a calibration curve of optical density (OD) versus step height (d). Although the resulting graph is usually non-linear, there is a linear relationship between the logarithm of the step height and the optical density [61].

Imaging software can be used to determine the optical density of each step of the step wedge. The following equation can then be used to aid in the conversion of optical density to millimeters of aluminum equivalent.

$$OD = m \times \log (d) + C$$

Where OD is the optical density, M is the gradient (or slope), d is the height of the step, and C is the intercept on the optical density axis [61, 63]. An alternative equation is the following:

$$\text{Log } (d_i) = (C - OD_i) / (-m)$$

Where OD_i measures the optical density for any material i on the film, $\text{Log}(d_i)$ is the radiopacity aluminum equivalent of that specimen thickness, and C is the intercept on the optical density axis, and m is the gradient (or slope).

In human and veterinary medicine, densitometry is currently used for comparison of radiographs to digital images, evaluation of dental radiographs, and assessment of bone density and bone healing [53, 57, 61, 64]. Radiographic assessment of bone healing is especially important after veterinary orthopedic procedures since return to normal activity cannot be permitted until bony union is achieved. Having an objective evaluation of bony union aids in the decision making prior to allowing an animal to return to pre-surgical activity levels

1.16 Cranial Cruciate Ligament Anatomy

The cranial cruciate ligament is one of the primary stabilizers of the canine stifle. It functions to prevent cranial tibial subluxation during weight bearing, limits overextension of the stifle, limits internal tibial rotation, and assists with proprioception [65, 66]. The cranial cruciate ligament is an intra-articular, extrasynovial ligament which is attached to the caudomedial aspect of the lateral femoral condyle and the cranial intercondyloid area on the tibia. The ligament itself is composed of a core region of fascicles containing collagen (predominantly type I collagen) and fibroblasts and is covered by an epiligamentous region composed of synovial intima and loose connective tissue [67]. The ligament receives nourishment via vascular supply from the middle genicular artery and passive permeation of nutrients from the synovial fluid.

1.17 Canine Cranial Cruciate Ligament Disease (CCLD)

Cranial cruciate ligament disease is one of the most common canine orthopedic diseases requiring surgical intervention and has an estimated prevalence of 1.8-2.3% [68, 69]. In 2003, it was estimated that a total of \$1.3 billion dollars was spent on the medical and surgical treatment of this disease [70]. Several underlying etiologies have been evaluated including traumatic rupture of the ligament, avulsion at the attachment site of the ligament, or (most commonly) progressive degeneration of the ligament. Several underlying factors have been investigated which may predispose animals to rupturing a cranial cruciate ligament. These factors include an abnormal gait, obesity, lack of fitness, immune mediated disease, acquired loss or change in blood supply to the midportion of the ligament, septic arthritis, early neutering, genetics, repetitive trauma, neuromuscular disease, and joint laxity [71-76]. Additionally, several conformational abnormalities have been associated with CCLD including straight stifle angles, a narrow intercondylar notch, steep tibial plateau slope, excessive internal tibial rotation, distal femoral torsion, and caudal angulation of the proximal tibia [47, 72, 75, 77-80].

Histopathology of the ruptured cranial cruciate ligament has been performed and was suggestive of a chronic mechanical overload in many cases [67, 81].

Histopathologic findings included metaplasia of ligamentocytes, loss of fibroblasts from the core region, disruption of ligamentous matrix, higher amounts of immature collagen cross-links, and higher amounts of total and sulfated glycosaminoglycans, water content, and matrix metalloproteinases-2.

Diagnosis of cranial cruciate ligament disease can be made based on physical and orthopedic examination. Common findings include lameness and pain localized to the

stifle, palpable stifle effusion, medial buttress, and stifle instability (positive cranial drawer and/or positive tibial thrust) [67]. Further assessment of the stifle is performed with radiographic evaluation of the joint. Common radiographic findings consistent with ligamentous instability of the joint include loss of effacement of the infrapatellar fat pad, osteophyte, and enthesiophyte formation [82]. Additionally, presence of concurrent pathology, including neoplasia, osteomyelitis, and septic arthritis, can be ruled out.

1.18 Treatment of CCLD

Pain and discomfort due to cranial cruciate ligament disease can be due to multiple different factors including increased nociceptive mediators associated with synovitis, inflammation, joint capsule distention, abnormal stifle kinematics, and stifle joint instability [83]. Treatment of cranial cruciate ligament disease focuses on decreasing pain and inflammation (non-steroidal anti-inflammatory drug administration, synthetic opioid administration, and joint lavage), surgical stabilization of the stifle, and improvement in muscle mass and weight bearing function of the limb [84, 85].

Multiple surgical interventions for the treatment of CCLD exist and can be split into 3 main groups: intracapsular repair, extracapsular repair, and osteotomy procedures. Intracapsular repair involves placement of implants within the joint capsule to replace the cranial cruciate ligament. Examples of implants used clinically and experimentally include the patellar ligament (autograft, allograft, and xenograft), bone-patellar ligament-bone grafts, fascia lata, porcine small intestinal submucosa, Gore-Tex, Dacron, and carbon fiber [67, 86-88]. Complications for this procedure include difficulty harvesting the graft and recurrent stifle laxity due to failure of the graft. Although early post-

operative results have been promising, long term success after intracapsular repair is not well documented and therefore is not routinely performed for the canine cranial cruciate ligament deficient stifle [67].

Unlike intracapsular techniques which place the implant within the stifle joint capsule, extracapsular repairs involve stabilization of the stifle with implants placed outside of the joint. Examples of extracapsular procedures include the lateral fabellotibial suture, the Tightrope® technique, and the fibular head transposition [89-91]. Implants are placed to mimic the orientation of the cranial cruciate ligament and stability is maintained with a combination of implant strength and periarticular fibrosis [67]. Complications after extracapsular techniques include recurrent stifle instability (due to breakage or elongation of the suture or ligament), meniscal tear, persistent lameness, infection, and peroneal nerve deficits [91-94].

Osteotomy procedures commonly performed to treat stifle instability due to cranial cruciate ligament rupture include the cranial closing wedge osteotomy, the tibial plateau leveling osteotomy, and the tibial tuberosity advancement. Clinical outcomes after these procedures are relatively similar and the treatment chosen is often based on surgeon preference.

1.19 Tibial Tuberosity Advancement

Tibial tuberosity advancement is a surgery originally developed by Ralph Maquet for treatment of femoropatellar pain due to chondromalacia and osteoarthritis in humans [95, 96]. The procedure utilizes an osteotomy in the proximal tibia to advance the insertion of the patellar ligament cranially. This allows the quadriceps muscle group to

work more efficiently and decreases the force that is transmitted from the femur to the tibia, and from the patella to the femur [95]. Development of this procedure was based on a theory of joint compressive forces originally developed by Ralph Nisell [97-99].

Nisell proposed that the joint compressive force across the stifle is parallel and equal in magnitude to that of the patellar ligament. In humans, when the angle between the patellar ligament and tibial plateau is greater than 100 degrees, an anteriorly directed shear force is generated. Similarly, when the angle between the patellar ligament and the tibial plateau is less than 100 degrees, a posteriorly directed shear force is generated. Femorotibial shear force is approximately zero when the patellar tendon angle is 100 degrees to the tibial plateau angle in humans. When translated to veterinary medicine, a patellar ligament angle of 90 degrees will yield a femorotibial shear force of approximately zero [67].

In 2002 Montavon and Tepic proposed using this procedure for dynamic stabilization of the canine stifle after cranial cruciate ligament tear [100]. During the procedure, the tibial tuberosity is advanced to a predetermined distance, then stabilized with a cage, plate, and bone screws. Pre-surgical planning to determine the advancement required to neutralize the femorotibial shear force is performed on a mediolateral radiographic projection of the stifle. One technique for pre-surgical measurement is the common tangent method [101] (Figure 1). Instructions on how to perform this method are available on the Kyon^a website [102].

Outcome after the TTA procedure has been reported as good to excellent in greater than 90% of dogs [103]. Recovery time is generally between 8 and 12 weeks and is based on the time it takes for radiographic healing of the osteotomy site [104]. Major

complications requiring a second surgical procedure after a TTA have been reported in 1.3-14% of cases [104]. These complications include postliminary meniscal tear (5.3-7.6%), infection (4%), medial patellar luxation (0.4-1.8%), tibial fracture (0.08%), and catastrophic implant failure (0.08%) [103-105].

An in vitro study to evaluate the biomechanical effect of the TTA in dogs was performed by Kim et al in 2009 [106]. This study showed that TTA limited cranial tibial subluxation, decreased internal tibial axial rotation, restored peak contact pressure cranially, and decreased the peak pressure magnitude in the medial compartment of the stifle compared to cranial cruciate ligament deficient stifles prior to stabilization.

Chapter 2

Densitometric Comparison of Autogenous Cancellous Bone Graft and Extracorporeal Shock Wave Therapy for Osteotomy Healing in the Tibial Tuberosity Advancement Procedure in Dogs

2.1 Introduction

Tibial tuberosity advancement (TTA) is a surgical technique originally developed for the treatment of femoropatellar pain in people [95]. In 2002, Montavon and Tepic proposed use of this procedure for dynamic stabilization of the canine stifle after cranial cruciate ligament rupture [107]. Outcome after this procedure in dogs is reported to be good to excellent in the majority of cases and return to normal activity level is permitted after radiographic signs of healing are observed [103-105, 108]. One technique used to promote osseous union after surgery is placement of a bone graft within the tibial osteotomy site. Although this is commonly performed, recent studies have yielded conflicting evidence as to whether the duration and degree of osteotomy healing after TTA is influenced by the addition of bone graft [59, 60].

Extracorporeal shock wave therapy was originally developed for fragmentation of urinary calculi in humans [109, 110]. Orthopedic applications of this treatment modality soon emerged and now include therapy for plantar fasciitis, delayed or non-union of bone fractures, osteoarthritis, supraspinatus tendinopathy, patellar ligament desmitis, and navicular disease in human and veterinary medicine [10, 11, 13, 20, 22-25, 30, 111]. Although the exact mechanism by which extracorporeal shock wave therapy acts on healing bone is not completely understood, previous reports have demonstrated increased osteogenic activity and greater biomechanical strength of treated bone, decreased

inflammation and swelling, short-term analgesia, and improved vascularization to surrounding tissue [1, 4, 12, 13, 15, 19, 20].

Based on the favorable effects seen thus far of extracorporeal shock wave therapy on healing bone, it is possible that this treatment may also benefit osteotomy healing after TTA. The objective of this study was to compare the radiographic density of the osteotomy gap created after a TTA procedure treated with autogenous cancellous bone graft, extracorporeal shock wave therapy, a combination of autogenous cancellous bone graft and extracorporeal shock wave therapy, or neither extracorporeal shock wave therapy nor autogenous cancellous bone graft. Our hypothesis was that dogs treated with either autogenous cancellous bone graft, extracorporeal shock wave therapy, or a combination of the two treatments, would have a greater osteotomy gap density at 4 and 8 weeks after surgery as compared to dogs without any extracorporeal shock wave therapy or autogenous cancellous bone graft treatment.

2.2 Materials and Methods

Study Design

This was a prospective randomized clinical trial utilizing client owned dogs that were presented to the Virginia-Maryland Regional College of Veterinary Medicine's teaching hospital for suspected cranial cruciate ligament rupture. Dogs were excluded from the study if they had a previous surgery on the same stifle, additional orthopedic pathology on the same limb that required treatment (such as patellar luxation), or were skeletally immature. Gender, age, breed, and orthopedic pathology in the contralateral stifle were not factors for exclusion from the study.

Pre-operative Care

The research protocol used and described in this study was approved by the Institutional Animal Care and Use Committee. Additionally, a consent form was signed by all owners allowing data to be collected and used for scientific research and publication. A pre-operative diagnosis of cranial cruciate ligament disease was made based on presence of palpable stifle pathology including effusion, cranial drawer, and tibial thrust. Once admitted, dogs were assigned to one of the following treatment groups based on a random number generator; TTA with autogenous cancellous bone graft (TTA-G, n=10), TTA with autogenous cancellous bone graft and 2 sessions of extracorporeal shock wave therapy (TTA-GS, n=10), TTA with 2 sessions of extracorporeal shock wave therapy (TTA-S, n=10), or TTA with no additional treatment (neither autogenous cancellous bone graft nor extracorporeal shock wave therapy) (TTA-O, n=10). Pre-anesthetic laboratory evaluation of all dogs included hematology, serum biochemistry profile analysis, urinalysis, and a urine culture.

Pre-operative Radiographic Evaluation

Standard calibrated caudocranial and mediolateral digital radiographic views centered on the stifle were obtained under heavy sedation (butorphanolⁱ at 0.2mg/kg and dexmedetomidine^b at 5mcg/kg administered intravenously) with the stifle joint at a standing angle of approximately 135° [107]. Pre-operative measurements for the TTA surgery were performed using the common tangent method [101, 112].

Surgical Procedure

Dogs were premedicated and anesthetized following standard protocols used by the anesthesia service at this hospital. A preoperative intravenous injection of cefazolin^e (22mg/kg) was administered to all animals followed by the same dose every 90 minutes during the procedure and every 8 hours for 24 hours following surgery. A previously described surgical protocol for the TTA procedure^d was performed or supervised by a board-certified surgeon. All menisci were evaluated for tears via medial stifle arthrotomy at the time of the TTA procedure. A partial meniscectomy was performed to remove damaged tissue if present. Dogs in the TTA-G and the TTA-GS groups had autogenous cancellous bone graft placed to fill the osteotomy gap between the distal aspect of the tibial tuberosity to the distal aspect of the TTA cage. To collect the autogenous cancellous bone graft, a window through the medial cortex of the ipsilateral femoral condyle was created and a bone curette was used to collect the graft.

Post-operative Care

Pain and inflammation following surgery were controlled using an injectable opioid for 24 hours and a single dose of a non-steroidal anti-inflammatory drug subcutaneously during the early recovery period. Administration of tramadol (3-6mg/kg by mouth every 8-12 hours) and an oral non-steroidal anti-inflammatory drug (administered for 5-7 days following surgery) were started the morning after surgery.

Post-operative Radiographic Evaluation

Following the surgical procedure (with the patient still under general anesthesia) caudocranial and mediolateral digital radiographic views centered on the stifle at a standing angle of 135 degrees were performed in all animals. An aluminum step wedge consisting of 10 increasing steps of 1mm height each, was placed adjacent to the stifle in all mediolateral views (Figure 1). Radiographs were repeated at 4 and 8 weeks after surgery using the same sedation protocol as previously described for pre-operative radiographs.

Densitometry

The digital radiographic images were analyzed using Osirix imaging software^{®e}. A region of interest (ROI) was placed over each step of the aluminum step wedge (Figure 2) and these optical values were then fit by regression analysis using a non-linear model. The least squares analysis was used to fit the regression line to the formula $y = a e^{-bx}$, where x represents optical density and a and b are constants used to predict y (millimeters of aluminum equivalent [mmAleq]). R-squared (R^2) was recorded for each radiograph to determine goodness-of-fit. Next, an irregular free-hand ROI was placed over the osteotomy gap between the distal aspect of the cage and the mid-proximal aspect of the plate (Figure 2). In addition, 3 rectangular ROI were placed; the first at the cranial cortex of the tibia just distal to the end of the TTA plate, the second at the tibial medullary cavity just distal to the TTA plate, and the third over the distal femur just proximal to the patella. These ROI served as comparative optical values (figure 3). Osteotomy gap density in mmAleq (OGmmAleq) was then determined using the optical density from the

ROI and the above equation. The percent change in OGmmAeq (%OGmmAeq) was calculated for the following time periods; 0-4 weeks, 4-8 weeks, and 0-8 weeks after surgery.

Shock Wave Therapy

Two sessions of extracorporeal shock wave therapy were performed for dogs in the TTA-GS and TTA-S groups; the first was immediately after post-operative radiographs with the dog still under general anesthesia, the second was after obtaining radiographs at the 4-week examination while the dog was still sedated. For each session, coupling gel was applied to the shaved stifle and 1,000 pulses at energy level 6 ($0.15\text{mJ}/\text{mm}^2$) were administered with a 5mm trode [30]. The source of the shock waves was the VersaTron^{®f}.

Statistical Analysis

Primary outcomes were OGmmAeq, the ratio of the optical density of the osteotomy gap to the optical density of the tibial cortex, the ratio of the optical density of the osteotomy gap to the optical density of the tibial medullary cavity, the ratio of the optical density of the osteotomy gap to the optical density of the distal femur, and the %OGmmAeq. A secondary outcome was presence or absence of complications. Primary exposures of interest were treatment (TTA-G, TTA-GS, TTA-S, and TTA-O) and time after treatment. Prognostic factors assessed were age, gender, breed, body weight, body condition score, cage size, and meniscal tear. Outcomes and prognostic factors that were normally distributed were summarized as means $\pm$ standard deviation. Skewed data was

summarized as median (range). Contingency tables were generated for the categorical variables. Associations between treatment group and prognostic factors were tested using one-way analysis of variance (ANOVA) (age, weight, and body condition score), Kruskal-Wallis test (cage size), and Fisher's exact test (breed and meniscal tear). Associations between primary outcomes and prognostic factors were tested using mixed-model analysis of covariance (ANCOVA) (regression within an ANOVA framework). Fixed effects in the ANCOVA model were specified as treatment, time, treatment*time and prognostic factor (s) (the covariate). Dog identification was specified as the random effect to account for repeated measures over time. Effects of treatment and time on primary outcomes without a covariate were tested using mixed-model ANOVA followed by Holm-Tukey's procedure for multiple comparisons. Frequency of complications was compared between treatment groups using Fisher's exact test. Statistical analyses were considered significant at $p < 0.05$. All analyses were performed using a statistical software package^g.

2.3 Results

Signalment

Forty stifles from 39 dogs were included in the study; there were 27 spayed females, and 12 castrated males. Twenty-two out of 39 dogs had normal contralateral stifles, 11/39 dogs had bilateral cranial cruciate ligament disease, and 7/39 dogs did not have a written record for the status of the contralateral cranial cruciate ligament. Of the 11 dogs with bilateral cranial cruciate ligament disease, 6 had previously undergone surgical correction of the contralateral stifle with either a TTA ($n=3$) or an extracapsular

stabilization (n=3). One dog with bilateral cranial cruciate ligament rupture had surgery on one stifle (included in the TTA-O) followed by surgery on the second stifle 9 weeks later (included in the TTA-S group). The mean age was 5.5 years $\pm$ 2.3 years (range, 2-11 years), mean body weight was 33kg $\pm$ 9.8kg (range, 11.8-61.7 kg), and mean body condition score was 3.6 $\pm$ 0.7 (range 2.2-5). The breeds of the dogs were Labrador Retrievers (n=9), mixed breed dogs (n=14), German Shepherd Dogs (n=4), Golden Retrievers (n=2), Alaskan Malamute (n=1), American Bulldog (n=1), Boxer (n=1), Coonhound (n=1), Doberman Pincher (n=1), English Springer Spaniel (n=1), Keeshound (n=1), Mastiff (n=1), Rottweiler (n=2), and Staffordshire Terrier (n=2). There were no significant differences between the 4 groups in the age, body weight, body condition score, gender, or breed of the dogs.

Surgical Findings

A tear of the cranial cruciate ligament was confirmed during surgery in all dogs. Concurrent medial meniscal tear was present in 19/40 stifles and the incidence was not significantly different between groups. The median advancement was 9.75mm (range, 6mm - 15mm) and also was not significantly different between groups.

Complications

There were no major complications during the study that required additional surgery. Minor intra-operative complications occurred in 3/39 dogs and included a medial patellar luxation that was corrected during closure of the arthrotomy (n=1), fracture of the trans cortex during insertion of the distal plate screw that did not require additional

treatment (n=1), and caudal placement of the fork holes that was corrected by re-drilling the fork holes in a more cranial location (n=1).

Minor complication between 0 and 4 weeks occurred in 9/39 dogs and included excessive bruising and swelling (n=1), patellar ligament desmitis (n=1), allergic reaction to oral antibiotic medication (n=1), discharge from incision (n=5), and grade I/IV medial patellar luxation (n=1). Minor complications between 4 and 8 weeks after surgery occurred in 1 dog. This dog had fracture of the distal fork noted on the 88week postoperative radiographs but it was clinically doing well and no additional treatment was required. This dog was in the control group (TTA-O) and was the same dog that required re-positioning of the fork holes intra-operatively. The rate of occurrence of minor complications was not significantly different between groups at any time point.

Prognostic Factors

Prognostic factors were tested (one at a time) for their effects on each outcome. A significant effect was seen for body condition score on osteotomy gap to tibial cortex ratio. Body weight, age, and cage size had a significant effect on OGmmAeq. Multivariable analysis with body weight, age, and cage size in 1 model showed that only body weight had a significant effect on OGmmAeq.

Evaluation of Healing Using Densitometry

Optical data from the aluminum step wedge were fit by regression analysis yielding an $R^2 > 0.94$ for all cases. There were no significant differences between values for R^2 between or within groups at any time point.

A summary of OGmmAeq for all groups is presented in Figure 3 and Table 1. The OGmmAeq was significantly different at 0 weeks compared to 4 weeks, and at 0 weeks compared to 8 weeks for the following groups; TTA-G ($p = 0.028$ and $p = 0.0091$ respectively), TTA-S ($p = 0.0001$ and $p < 0.0001$ respectively) and TTA-O ($p = 0.0012$ and $p < 0.0001$ respectively). The OGmmAeq for TTA-GS did not differ significantly between time points. There were no significant differences between OGmmAeq at 4 weeks compared to 8 weeks in any group.

When comparing OGmmAeq 8 weeks after surgery, there were no significant differences between any groups. At 0 weeks, there was a significant difference between the following groups: TTA-G and TTA-S ($p = 0.0065$), TTA-GS and TTA-S ($p < 0.0001$), and TTA-GS and TTA-O ($p = 0.0010$). When OGmmAeq was adjusted for body weight, there was an additional significant difference between TTA-G and TTA-O at 0 weeks. These numbers indicate a significant difference in OGmmAeq immediately after surgery between dogs that received autogenous cancellous bone graft (TTA-G and TTA-GS) and those that did not (TTA-S and TTA-O). At 4 weeks, a significant difference was only found between TTA-GS and TTA-S ($p = 0.0178$) and between TTA-GS and TTA-O ($p = 0.045$) (Figure 2).

Evaluation of Healing Using Ratios

The change in density of the osteotomy gap was also evaluated by using ratios of the osteotomy gap to the ROIs on the tibial cortex, tibial medullary cavity, and the distal femur. Significant findings are summarized in Figures 6-8 and Tables 2-4. For all ratios evaluated (osteotomy gap to tibial cortex, osteotomy gap to tibial medullary cavity, and

osteotomy gap to distal femur), dogs in the TTA-S group had values that were significantly different at 0 weeks compared to 4 weeks post-operatively ($p = 0.0002$, $p = 0.0301$, and $p = 0.0008$ respectively) and at 0 weeks compared to 8 weeks post-operatively ($p < 0.0001$, $p = 0.0002$, and $p < 0.0001$ respectively). For all ratios used (osteotomy gap to tibial cortex, osteotomy gap to tibial medullary cavity, and osteotomy gap to distal femur), dogs in the TTA-O group also had values that were significantly different at 0 weeks compared to 4 weeks post-operatively ($p = 0.0002$, $p = 0.0005$, and $p < 0.0001$ respectively) and at 0 weeks compared to 8 weeks post-operatively ($p < 0.0001$, $p < 0.0001$, and $p < 0.0001$ respectively).

Dogs in the TTA-G group had values that were significantly different at 0 weeks compared to 4 weeks post-operatively ($p = 0.0016$), and at 0 weeks compared to 8 weeks post-operatively ($p = 0.0048$) using only the osteotomy gap to tibial medullary cavity ratio. When using the osteotomy gap to tibial cortex ratio, dogs in the TTA-G group had values that were significantly different at 0 weeks compared to 8 weeks post-operatively ($p = 0.0272$). When using the osteotomy gap to distal femur ratio, dogs in the TTA-G group had values that were significantly different at 0 weeks compared to 4 weeks post-operatively only ($p = 0.018$). There were no significant differences between any of the ratios for dogs in the TTA-GS group at any time point. Additionally, there were no significant differences noted using any of the ratios between 4 weeks and 8 weeks after surgery in any group.

At 0 weeks, there was a significant difference between the following groups using the osteotomy gap to tibial cortex, osteotomy gap to tibial medullary cavity, and osteotomy gap to distal femur: TTA-G and TTA-S ($p = 0.0004$, $p = 0.0005$, and $p =$

0.0024 respectively), TTA-G and TTA-O ($p = 0.0005$, $p = 0.0011$, and $p = 0.0094$ respectively), TTA-GS and TTA-S ($p < 0.0001$, $p < 0.0001$, and $p = 0.0005$ respectively), and TTA-GS and TTA-O ($p < 0.0001$, $p < 0.0001$, and $p = 0.0022$ respectively). There were no significant differences at any time point with any ratio between groups that received bone graft (TTA-G and TTA-GS). Additionally, there were no significant differences between groups that did not receive bone graft regardless of whether they were treated with ESWT or not (TTA-S and TTA-O).

By 4 weeks post-operatively, there was no significant difference between any groups when using the ratio between osteotomy gap and distal femur. When using the osteotomy gap to tibial cortex and the osteotomy gap to tibial medullary cavity, there was a significant difference between dogs in the following groups: TTA-G and TTA-S ($p = 0.0386$, $p < 0.0001$ respectively), TTA-GS and TTA-S ($p = 0.0026$, $p < 0.0001$ respectively), and TTA-GS and TTA-O ($p = 0.0049$, $p = 0.0024$ respectively). Additionally, when using the osteotomy gap to tibial medullary cavity, there was a significant difference between TTA-G and TTA-O ($p = 0.0026$). At 8 weeks post-operatively, there were no significant differences between any groups using the osteotomy gap to tibial cortex ratio or the osteotomy gap to distal femur ratio. When using the osteotomy gap to tibial medullary cavity, there was a significant difference between dogs the following groups: TTA-G and TTA-S ($p = 0.0165$), TTA-G and TTA-O ($p = 0.0299$), and TTA-GS and TTA-S.

Percentage Change in Osteotomy Gap Density

The %OGmmAeq for all groups is summarized in Figure 4 and Table 2. A significant difference was noted for %OGmmAeq for 0-4 weeks compared to 4-8 weeks for TTA-S ($p = 0.0031$) and TTA-O ($p = 0.0086$). Additionally, there was a significant difference noted between %OGmmAeq for 0-8 weeks compared to 4-8 weeks in the same groups; TTA-S ($p = 0.0013$) and TTA-O ($p = 0.0005$). The %OGmmAeq was not significantly different for 0-4 weeks and 0-8 weeks for any group. There were no significant differences in %OGmmAeq at any time point (0-4, 0-8, and 4-8 weeks) for TTA-G or TTA-GS.

The %OGmmAeq from 0-4 weeks was not significantly different between any of the groups. The %OGmmAeq from 0-8 weeks was significantly different for TTA-O compared to TTA-GS ($p = 0.0112$), and significantly different for TTA-S compared to TTA-GS ($p = 0.0360$).

2.4 Discussion

When comparing OGmmAeq, our results do not indicate a significant difference between groups 8 weeks after surgery regardless of the treatment modality used. Thus, we were not able to accept our hypothesis that dogs treated with autogenous cancellous bone graft, autogenous cancellous bone graft and extracorporeal shock wave therapy, or extracorporeal shock wave therapy would have greater healing of the osteotomy gap than control animals at the conclusion of this study.

Several methods for evaluation of bone healing, specifically after a TTA procedure, are previously described and include a grading scheme based on the number

of sites of bridging bone, subjective assessment of callus density at specific locations on the osteotomy, and opacity at the graft site [60, 103, 113]. It is possible that different results could have been attained if an alternate method for assessment of bone healing was used. Densitometry was chosen for this study because it is more quantitative and assigns a value to the density of the osteotomy gap rather than relying on subjective measures of healing.

Densitometry allows comparison of optical density across multiple radiographic images while controlling for other factors that may influence results such as exposure parameters. This method of analysis involves use of a reference penetrometer (aluminum step wedge) to obtain baseline optical values with which to compare healing bone. Currently, it is reportedly used for evaluation of human dental radiographs, bone mineral density measurement, for comparison of radiographic films to digital images, and to evaluate bone healing [53, 57, 61, 64]. In order to use this method, a linear relationship between steps of the aluminum step wedge must be confirmed. The least squares analysis was used to fit our data points from the step wedge to a straight line and revealed an $R^2 > 0.94$ for all radiographs. This indicated a strong linear relationship between steps of the aluminum step wedge and allowed comparison between different images. The gold standard for the evaluation of bone density is dual-energy x-ray absorptiometry [53, 114]. However, use of this technique is limited due to the associated cost and need for specialized equipment. Densitometry remains an excellent option for objective measurement of bone density when more specialized equipment is not available.

In addition to densitometry, ratios between the osteotomy gap and each ROI were obtained to compare the density of the healing osteotomy gap to that of cortical and

medullary bone. Significant differences were identified on the immediate post-operative radiographs (0 weeks) between groups that received ACBG and those that did not. Dogs that received ACBG had ratios that were closer to 1 indicating that the density of the osteotomy gap in these dogs had a value closer to normal bone (both medullary and cortical). Dogs that did not receive ACBG had ratios farther from 1 likely due to the absence of radiodense bone particles within the osteotomy gap. At the 4-week recheck evaluation, this difference was still significant only for 2 of the ratios (osteotomy gap to tibial cortex and osteotomy gap to tibial medullary cavity). No difference was noted between groups at the 4-week or 8-week recheck using the osteotomy gap to distal femur. One possible explanation for this is that increased density of soft tissue structures overlying the distal femur as compared to the proximal tibia may have prevented identification of subtle differences. If utilizing a ratio to compare bone density across images, the osteotomy gap to distal femur is likely the least sensitive of the ratios used in this study. Previous reports have indicated that this method is less sensitive in areas of greater soft tissue coverage (such as the proximal limb)[53]. Based on our findings, we would not recommend comparison of the site of interest to the distal femur for assessment of bone healing. The ratio of the osteotomy gap to the tibial cortex yielded results equivalent to those obtained with densitometry. This site has less soft tissue coverage compared to the distal femur and may be the best option for comparison of healing bone out of the 3 ROIs used in this study.

The TTA procedure was originally described with use of cancellous bone graft within the osteotomy site to promote faster bone healing and reduce the risk of implant associated complications [59, 107]. Similar to the findings of a previous report, use of

autogenous cancellous bone graft alone during the TTA procedure in this study did not significantly affect the osteotomy gap density or occurrence of complications after surgery [60]. Based on our findings, use of bone graft during TTA procedures may not be necessary.

For this study, autogenous cancellous bone graft was chosen over allograft due to its increased osteogenic potential [37, 42]. The distal femur was chosen as the graft collection site instead of the proximal tibia so that graft could be harvested through the same surgical incision and would not interfere with the site of interest for post-operative evaluation of healing. Although the exact quantity of autogenous cancellous bone graft was not standardized across cases, enough graft was harvested to completely fill the osteotomy gap for each dog. A previous study concluded that healing across a bone defect was not influenced by the volume of bone graft as long as the osteotomy gap was completely filled [43]. A standard quantity of bone graft may be helpful in subsequent studies evaluating the benefits of bone grafting during TTA procedures

Extracorporeal shock wave therapy utilizes acoustic waves of high pressure and high velocity to exert effect on bone [8, 29]. Although previous studies have shown enhanced healing of acute fractures following extracorporeal shock wave therapy [13, 14, 115], no significant differences were noted between dogs treated with extracorporeal shock wave therapy alone (TTA-S) compared to control dogs (TTA-O) at any time point in this study. Therefore, we cannot recommend using extracorporeal shock wave therapy alone after TTA to enhance bone healing using this treatment protocol.

It is possible that a different protocol for administration of extracorporeal shock wave therapy, such as a greater number of treatments or a smaller interval between

treatments, could result in greater beneficial effects. The optimal interval and number of extracorporeal shock wave therapy treatments is not yet determined. Previously reported treatment intervals range from 1 to 9 weeks [10, 11, 21-23] and beneficial effects of ESWT are documented to persist beyond the immediate treatment period. Enhanced radiographic evidence of bone healing after extracorporeal shock wave therapy has been shown to persist 9 weeks after treatment compared to untreated bone [10].

Neovascularization and enhanced release of beneficial cytokines have also been noted within 1 week of treatment and are documented to continue for 8-12 weeks after only 1 session [17]. A 4-week treatment interval was chosen for this study to evaluate for differences in early healing, minimize the number of visits with required sedation for each patient, and to ensure owner compliance. However, it is possible that a shorter treatment interval, or higher number of treatments, could potentiate the beneficial effects of extracorporeal shock wave therapy and therefore alter the results. In another study evaluating the effect of extracorporeal shock wave therapy on bone healing, differences in callus size between treated and untreated bones were not significantly different until the end of 12 weeks [13]. Therefore, it is also possible that a longer follow-up period would have yielded additional significant differences between groups.

The effect of extracorporeal shock wave therapy on bone graft is not yet determined. However, at the time of the 4-week examination, there was a significant difference between TTA-GS compared to TTA-S and TTA-O using OGmmAeq, the ratio between the osteotomy gap to the tibial cortex, and the ratio between the osteotomy gap to tibial medullary cavity. This may indicate that the combination of extracorporeal shock wave therapy and autogenous cancellous bone graft was most beneficial for

osteotomy gap healing during this period. When cancellous bone is treated with extracorporeal shock wave therapy, there are multiple interfaces between soft tissue and bone, so energy is deposited in a number of acoustic interfaces [2, 116]. If this is also true for treatment of cancellous bone graft with extracorporeal shock wave therapy, this may explain why the effects of extracorporeal shock wave therapy are enhanced when the 2 treatment modalities are combined. This beneficial effect did not seem to persist beyond the 4-week examination.

Minor complications occurred in a total of 13/39 dogs during the course of the study. The majority of complications arose during the first 4 weeks after surgery with the most common being swelling and drainage from the incision. Although occurrence of minor complications was not significantly different between groups, 7 out of 9 dogs with minor complications between 0 and 4 weeks were in groups that received extracorporeal shock wave therapy (5 in the TTA-GS group, 2 in the TTA-S group). Possible explanations for this include accumulation of fluid in the soft tissues or increased capillary density after extracorporeal shock wave therapy as previously described [6]

One limitation of this study is the small sample size which may have lead to a type II error. Greater numbers would be needed to increase the power of the study. An additional limitation is the inability to control for confounding factors that may influence bone healing such as home activity level or concurrent orthopedic disease leading to a change in load bearing of the treated limb.

In conclusion, we found that treatment with autogenous cancellous bone graft and extracorporeal shock wave therapy yielded a greater OGmmAleq at 4 weeks after surgery compared to treatment with extracorporeal shock wave therapy alone or control dogs.

Regardless of the treatment modality chosen, no significant differences were noted in OGmmAleq at 8 weeks after surgery using densitometry. Use of extracorporeal shock wave therapy and autogenous cancellous bone graft did not significantly alter the complication rate. Additionally, densitometry using an aluminum step wedge is a feasible method for the assessment of bone density in the osteotomy gap after the TTA procedure.



Figure 2. Immediate postoperative mediolateral stifle radiograph showing the position of the aluminum step wedge.



Figure 3. Same mediolateral stifle radiograph as shown in Figure 2. A rectangular ROI is drawn on the first step of the aluminum step wedge and placement of the irregular ROI is shown in the osteotomy gap.

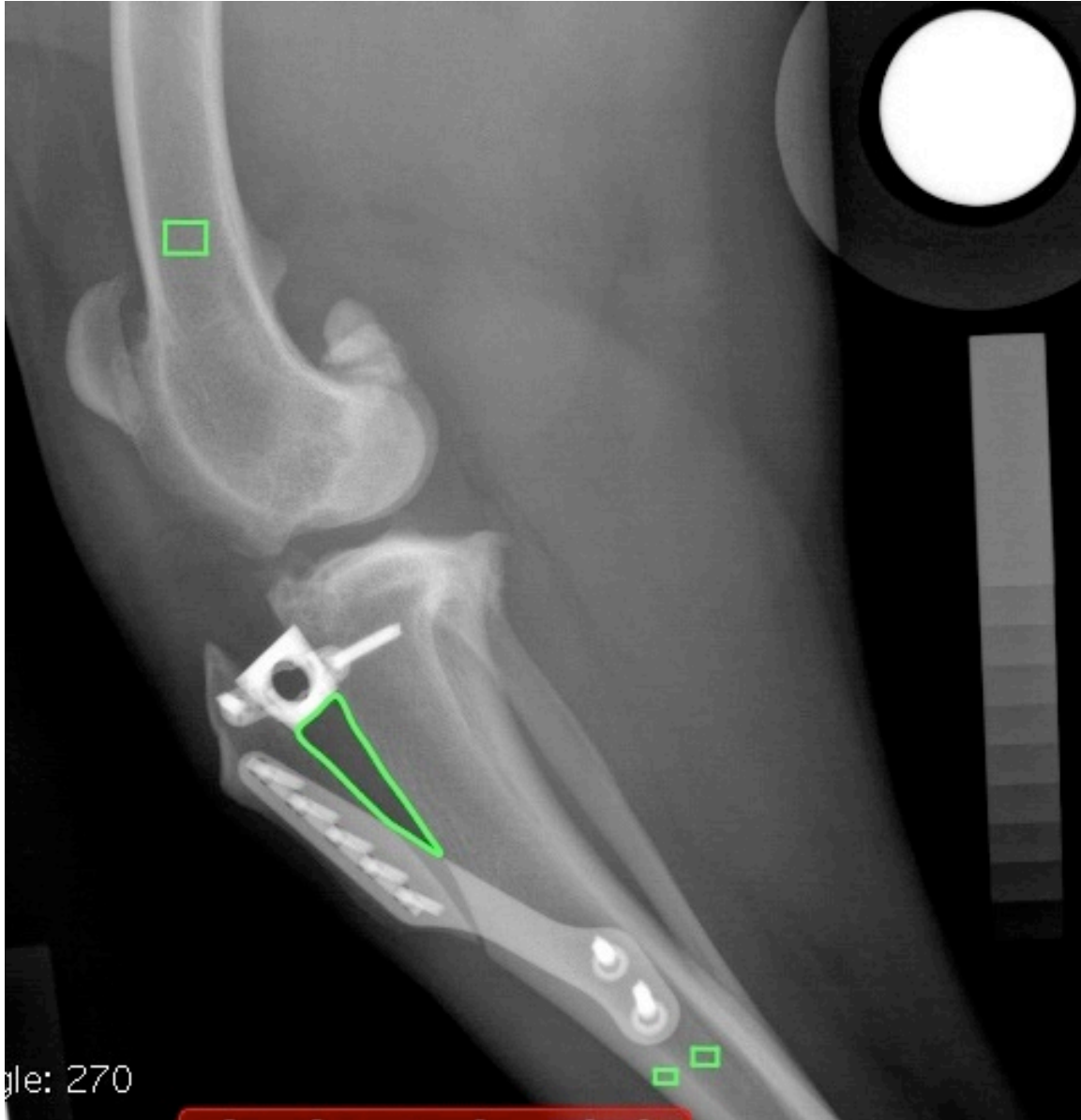


Figure 4. Same mediolateral stifle radiograph as shown in Figure 2. Rectangular ROIs are drawn on the tibial cortex, tibial medullary bone, and distal femur.

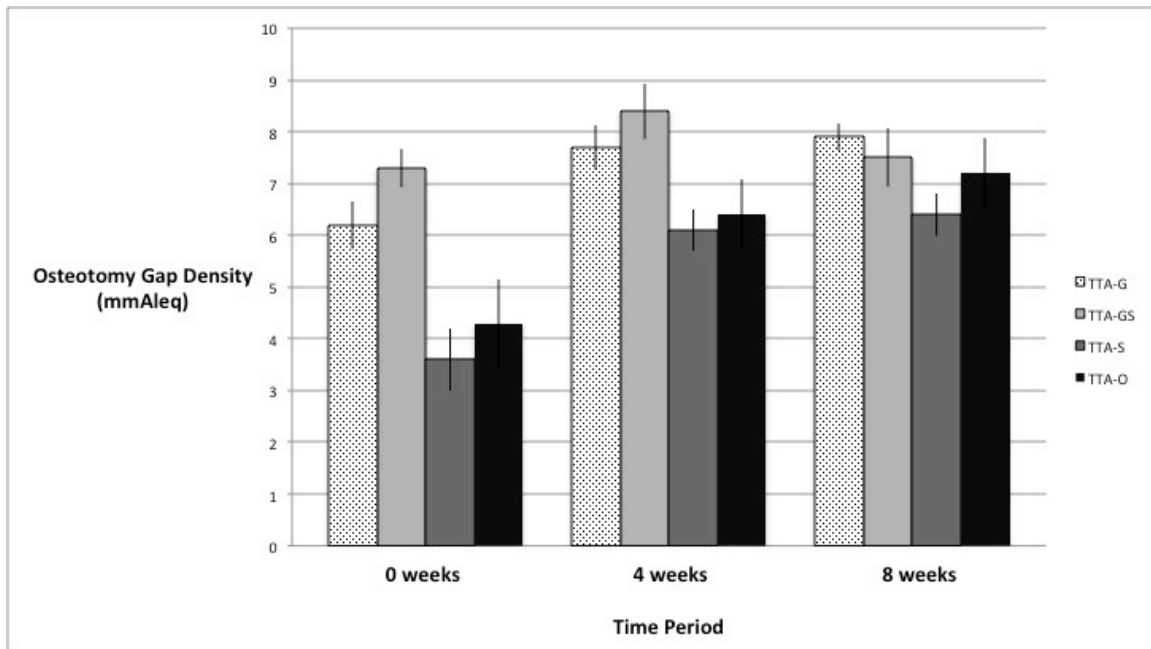


Figure 5. Bar chart showing the osteotomy gap density in millimeters of aluminum equivalent (mmAeq) after tibial tuberosity advancement. Results are expressed as a mean $\pm$ the standard error. At 0 weeks, there is a significant difference in osteotomy gap density between groups that received bone graft (TTA-G and TTA-GS) and those that did not (TTA-S and TTA-O). This difference is no longer significant by 8 weeks after surgery. TTA-G: tibial tuberosity advancement with autogenous cancellous bone graft, black dot pattern; TTA-GS: tibial tuberosity advancement with autogenous cancellous bone graft and extracorporeal shock wave therapy, light grey pattern; TTA-S: tibial tuberosity advancement with extracorporeal shock wave therapy, dark grey pattern; TTA-O: tibial tuberosity advancement with no additional therapy, black pattern.

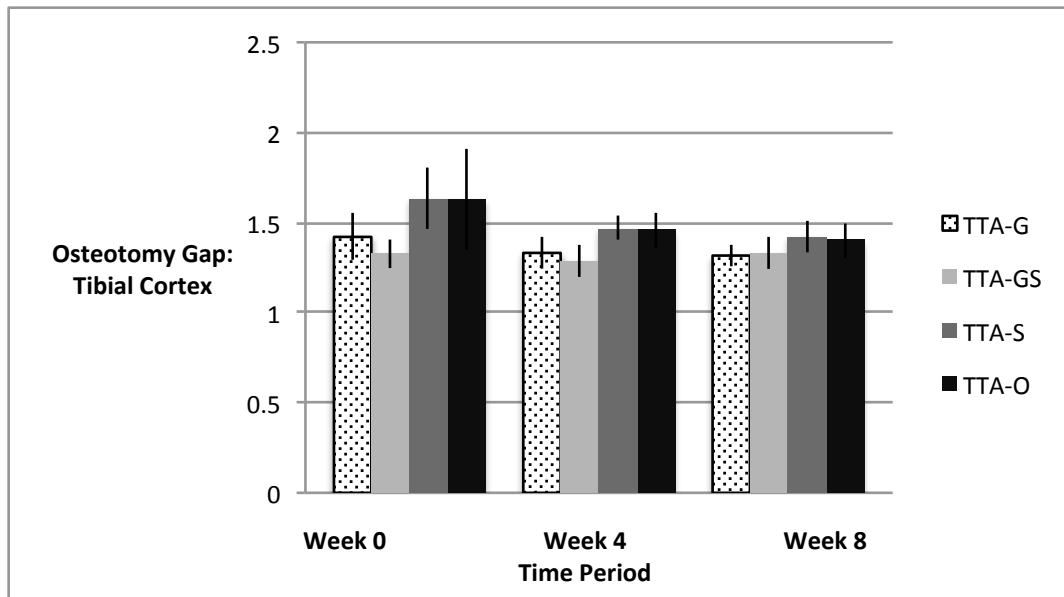


Figure 6. Bar chart showing the ratio of the optical density of the osteotomy gap compared to the optical density of the tibial cortex after tibial tuberosity advancement. Results are expressed as a mean $\pm$ the standard error. At 0 weeks, there is a significant difference in osteotomy gap density between groups that received bone graft (TTA-G and TTA-GS) and those that did not (TTA-S and TTA-O). This difference is no longer significant by 8 weeks after surgery. TTA-G: tibial tuberosity advancement with autogenous cancellous bone graft, black dot pattern; TTA-GS: tibial tuberosity advancement with autogenous cancellous bone graft and extracorporeal shock wave therapy, light grey pattern; TTA-S: tibial tuberosity advancement with extracorporeal shock wave therapy, dark grey pattern; TTA-O: tibial tuberosity advancement with no additional therapy, black pattern.

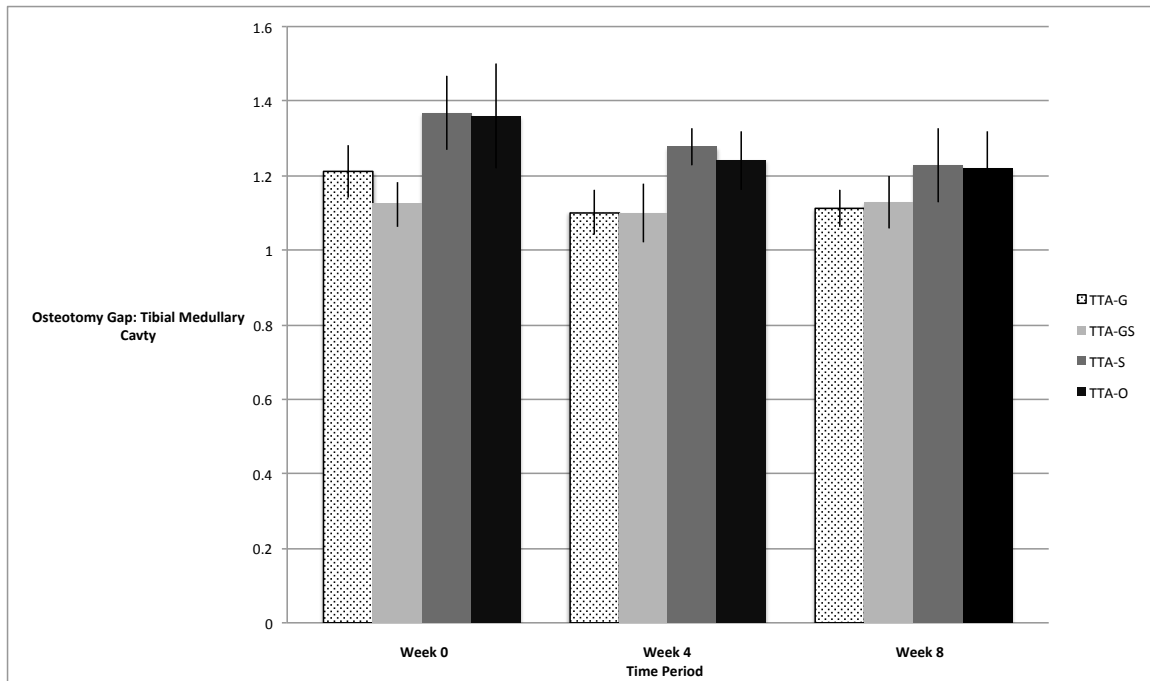


Figure 7. Bar chart showing the ratio of the optical density of the osteotomy gap to the optical density of the tibial medullary bone after tibial tuberosity advancement. Results are expressed as a mean $\pm$ the standard error. At 0 weeks, there is a significant difference between groups that received bone graft (TTA-G and TTA-GS) and those that did not (TTA-S and TTA-O). This difference is still significant by 4 and 8 weeks after surgery. TTA-G: tibial tuberosity advancement with autogenous cancellous bone graft, black dot pattern; TTA-GS: tibial tuberosity advancement with autogenous cancellous bone graft and extracorporeal shock wave therapy, light grey pattern; TTA-S: tibial tuberosity advancement with extracorporeal shock wave therapy, dark grey pattern; TTA-O: tibial tuberosity advancement with no additional therapy, black pattern.

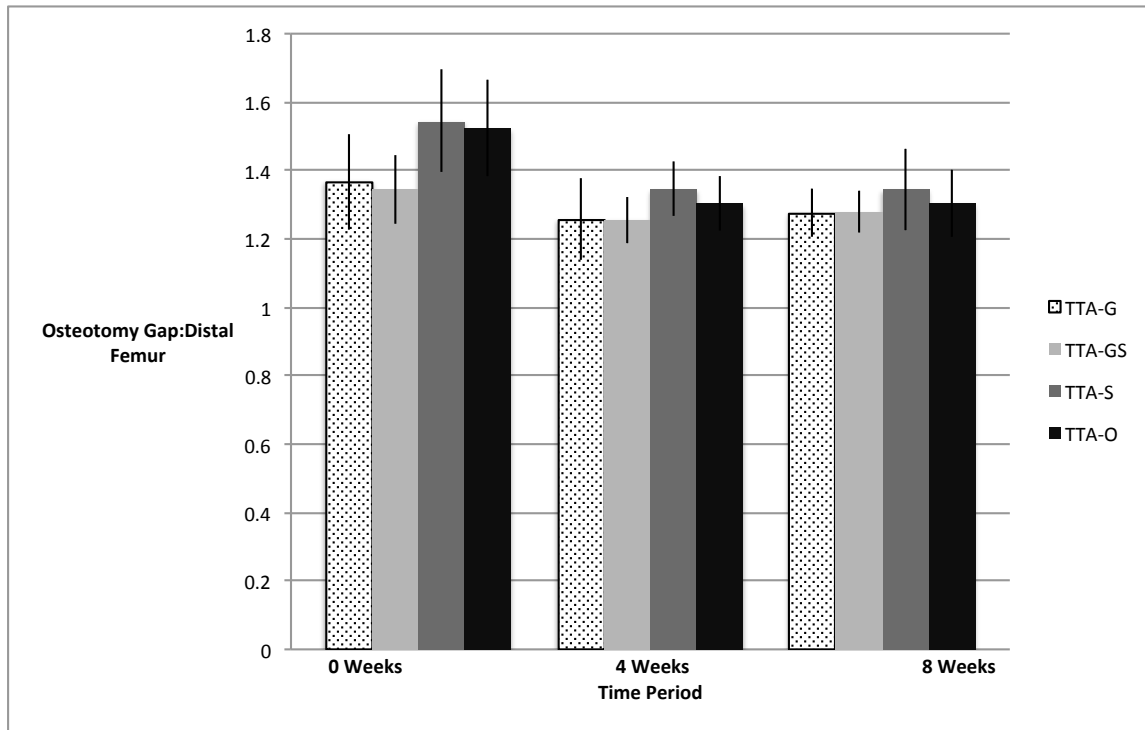


Figure 8. Bar chart showing the ratio of the optical density of the osteotomy gap compared to the optical density of the distal femur after tibial tuberosity advancement. Results are expressed as a mean $\pm$ the standard error. At 0 weeks, there is a significant difference between groups that received bone graft (TTA-G and TTA-GS) and those that did not (TTA-S and TTA-O). TTA-G: tibial tuberosity advancement with autogenous cancellous bone graft, black dot pattern; TTA-GS: tibial tuberosity advancement with autogenous cancellous bone graft and extracorporeal shock wave therapy, light grey pattern; TTA-S: tibial tuberosity advancement with extracorporeal shock wave therapy, dark grey pattern; TTA-O: tibial tuberosity advancement with no additional therapy, black pattern.

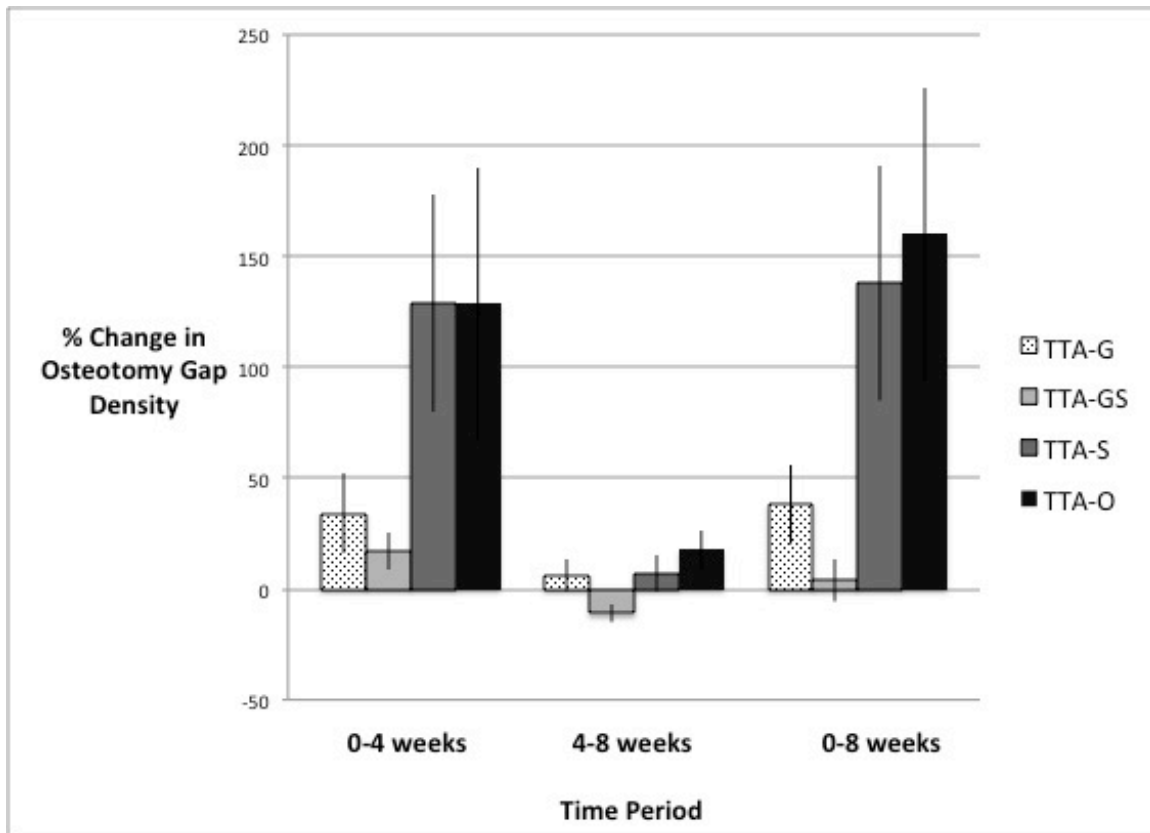


Figure 9. Bar chart showing the percentage change in osteotomy gap density over time. Results are expressed as a mean $\pm$ standard error. Note that a significantly greater change in osteotomy gap density occurred within the first 4 weeks after surgery for groups that did not receive bone graft (TTA-S and TTA-O). No other significant differences were noted. TTA-G: tibial tuberosity advancement with autogenous cancellous bone graft, black dot pattern; TTA-GS: tibial tuberosity advancement with autogenous cancellous bone graft and extracorporeal shock wave therapy, light grey pattern; TTA-S: tibial tuberosity advancement with extracorporeal shock wave therapy, dark grey pattern; TTA-O: tibial tuberosity advancement with no additional therapy, black pattern.

Treatment group	Time after tibial tuberosity advancement		
	0 weeks	4 weeks	8 weeks
TTA-G	6.2±0.5 ^{aA}	7.7±0.4 ^b	7.9±0.3 ^{bA}
TTA-GS	7.3±0.4 ^{aA}	8.4±0.5 ^{aA}	7.5±0.6 ^{aA}
TTA-S	3.6±0.6 ^{aB}	6.1±0.4 ^{bB}	6.4±0.4 ^{bA}
TTA-O	4.3±0.8 ^{aB}	6.4±0.7 ^{bB}	7.2±0.7 ^{bA}

Table 1. Osteotomy gap density (mean ± SE) in millimeters if aluminum equivalent.

Within each row, mean values that do not share a common lower case superscript letter are significantly different ($p<0.05$). Within each column, mean values that do not share a common upper case superscript letter are significantly different ($p<0.05$)

Treatment group	Time after tibial tuberosity advancement		
	0 weeks	4 weeks	8 weeks
TTA-G	1.42 ± 0.04^{aA}	1.33 ± 0.03^a	1.31 ± 0.02^{bA}
TTA-GS	1.33 ± 0.03^{aA}	1.29 ± 0.03^{aA}	1.33 ± 0.03^{aA}
TTA-S	1.63 ± 0.05^{aB}	1.47 ± 0.02^{bB}	1.42 ± 0.03^{bA}
TTA-O	1.63 ± 0.06^{aB}	1.46 ± 0.03^{bB}	1.40 ± 0.03^{bA}

Table 2. Ratio of the optical density of the osteotomy gap compared to the optical density of the tibial cortex after tibial tuberosity advancement. Within each row, mean values that do not share a common lower case superscript letter are significantly different ($p < 0.05$). Within each column, mean values that do not share a common upper case superscript letter are significantly different ($p < 0.05$)

Treatment group	Time after tibial tuberosity advancement		
	0 weeks	4 weeks	8 weeks
TTA-G	1.21 ± 0.02^{aA}	1.1 ± 0.02^{bA}	1.11 ± 0.02^{bA}
TTA-GS	1.12 ± 0.02^{aA}	1.1 ± 0.03^{aA}	1.13 ± 0.02^{aA}
TTA-S	1.37 ± 0.03^{aB}	1.28 ± 0.02^{bB}	1.23 ± 0.03^{bB}
TTA-O	1.36 ± 0.04^{aB}	1.24 ± 0.03^{bB}	1.22 ± 0.03^{bB}

Table 3. Ratio of the optical density of the osteotomy gap compared to the optical density of the tibial medullary cavity after tibial tuberosity advancement. Within each row, mean values that do not share a common lower case superscript letter are significantly different ($p < 0.05$). Within each column, mean values that do not share a common upper case superscript letter are significantly different ($p < 0.05$)

Treatment group	Time after tibial tuberosity advancement		
	0 weeks	4 weeks	8 weeks
TTA-G	1.37 ± 0.05^{aA}	1.26 ± 0.04^{bA}	1.28 ± 0.02^A
TTA-GS	1.34 ± 0.03^{aA}	1.26 ± 0.02^{aA}	1.28 ± 0.02^{aA}
TTA-S	1.54 ± 0.05^{aB}	1.38 ± 0.02^{bA}	1.35 ± 0.04^{bA}
TTA-O	1.52 ± 0.04^{aB}	1.3 ± 0.03^{bA}	1.30 ± 0.03^{bA}

Table 4. Ratio of the optical density of the osteotomy gap compared to the optical density of the distal femur after tibial tuberosity advancement. Within each row, mean values that do not share a common lower case superscript letter are significantly different ($p < 0.05$). Within each column, mean values that do not share a common upper case superscript letter are significantly different ($p < 0.05$)

Treatment group	Time after tibial tuberosity advancement		
	0-4 weeks	4-8 weeks	0-8 weeks
TTA-G	33.9±18.4 ^{aA}	6.3±7.4 ^{aA}	38.3±18 ^a
TTA-GS	16.8±8.3 ^{aA}	-11±3.9 ^{aA}	4±9.6 ^{aA}
TTA-S	129±48.7 ^{aA}	7.3±8.4 ^{bA}	138±52.8 ^{aB}
TTA-O	128±60.7 ^{aA}	17.9±9 ^{bA}	160±66.3 ^{aB}

Table 5. Percentage change in osteotomy gap density after tibial tuberosity advancement.

Within each row, mean values that do not share a common lower case superscript letter are significantly different ($p<0.05$). Within each column, mean values that do not share a common upper case superscript letter are significantly different ($p<0.05$)

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