

The Effect of Exogenous Canine Growth Hormone on Bone Healing in Dogs: Radiographic, Scintigraphic, Functional, and Biomechanical Effects

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Abstract

The healing of long bone fractures in dogs is an unsure and lengthy process if the bones cannot be anatomically reconstructed or if the physical status of the patient is less than ideal. If not assisted, the most likely result is a non-union. A non-union is when the fracture site heals without bone formation. The resulting fibrous tissue forms a pseudo joint which moves when subjected to muscle forces, causing considerable pain in the patient. Long bone fractures are usually corrected through the use of internal or external fixation devices. These devices lengthen healing time and usually require a surgical procedure to implant or fix them in place. Delayed healing can also lead to muscle atrophy, joint stiffness, and even refracture of the bone.

The purpose of this project is to examine the effect of the addition of canine growth hormone on the healing of long bone fractures in dogs. Growth hormone has effects on cartilage and bone and has been proved to enhance fracture healing in laboratory rodents. There are also reports of its use in the treatment of delayed union fractures in geriatric humans.

This experiment will investigate the effects of recombinant growth hormone administered via a subcutaneous implant on bone healing in a non-rigid fracture healing model. Non-rigid refers to the fact that during the healing process, the ends of the fractured bones will not be fixed or splinted in any way. Mature dogs, which have a low growth hormone concentration and production compared to immature dogs, will be used. The degree of healing will be assessed radiographically, photodensitometrically, and scintigraphically. Force plate gait analysis will be used to determine the

functional use of the affected limb. Finally, biomechanical tests in three point bending will evaluate the mechanical strength of the bone healing by determining the stiffness and ultimate strength of the healed bone in relation to that of the intact radius.

This study will provide important information about the quality and time of bone healing in dogs treated with exogenous growth hormone. Although this study will concentrate only on bone healing in dogs, valuable comparative data will be obtained. Future studies may be able to extrapolate this and other data to the study of human fracture healing.

Motive and History

Serious fractures of the long bones are common in dogs. Due to small bone fragment size or missing fragments, such fractures can not always be reduced or stabilized through the use of fixation devices. Secondary bone healing, or healing through the formation of a bony callus around the fracture site, occurs when there is a gap wider than 1 mm between fracture ends, or when the fracture site is non-rigid. Missing bone fragments can cause extraordinarily large forces to be placed on fixation devices. These forces coupled with the delayed healing time can cause premature failure of the device and can lead to refracture of the bone.

The effects of the growth hormone somatotropin (ST) on cells of bone and cartilage have been investigated both in the body and in a laboratory environment. ST stimulates bone growth along the axis of the bone by stimulating the local production of insulin-like growth factor I, which is one of the key factors in bone repair. Most of the early studies

have focused on using rats with human or bovine growth hormone. Despite the fact that these studies used a wide range of dose levels, length of treatment, and source, nearly all of them showed a stimulatory effect on bone healing in patients subjected to growth hormone.

To date no complete studies have focused on the influence of canine growth hormone on the radiographic, scintigraphic, functional, and biomechanical aspects of the healing of long bone fractures in dogs. A radial ostectomy, or the removal of a piece of the front limb bone known as the radius, will allow the evaluation of fracture healing in a non-rigid model while minimizing patient discomfort. The discomfort of the patient will be minimized by leaving the ulna, next to the radius, intact and allowing the weight of the animal to be shared by the intact bone.

Radiographic changes are one of the most widely used indicators used to assess fracture healing. Radiographic analysis is done by injecting a harmless radioactive isotope into the animal at the fracture site. This isotope is taken up by the tissue surrounding the site and when examined through a device which allows the viewing of the isotope, a picture of the healing site can be generated. Photodensitometry provides a more accurate estimate of bone mineral content by comparing the absorbance of light through a point on the radiograph to the absorbance through a standard medium.

Nuclear scintigraphy is a good indicator of the level of bone cell and vascular activity. Technetium 99 is a commonly used radionuclide because its uptake correlates well with active bone metabolism as well as capillary budding. During fracture healing, capillaries bud into the fracture

site and provide extra nutrients to the osteoblastic, or bone producing, cells. Growth hormone has a positive effect on capillary budding. The use of growth hormone may increase the amount of budding taking place in the fracture and increase the level of bone production, hence speeding the healing process.

Weightbearing by the injured limb can provide effective information about the functional use of the fracture during healing. Force plate gait analysis provides information regarding the vertical, horizontal, breaking, and propulsive forces placed on the injured limb. Because one of the goals of this study is to decrease the healing time and return the patient to full use of the fractured limb as soon as possible, this information is vital to the assessment of the quality of the bone healing. If the growth hormone has a positive effect on the healing time, the dogs treated with it should place more weight on the injured limb sooner than the untreated dogs.

Biomechanical testing will provide data regarding the actual strength and stiffness of the bone after fracture healing. A load vs. displacement curve is generated when the bones are tested in three point bending as shown in figures 1 and 2.

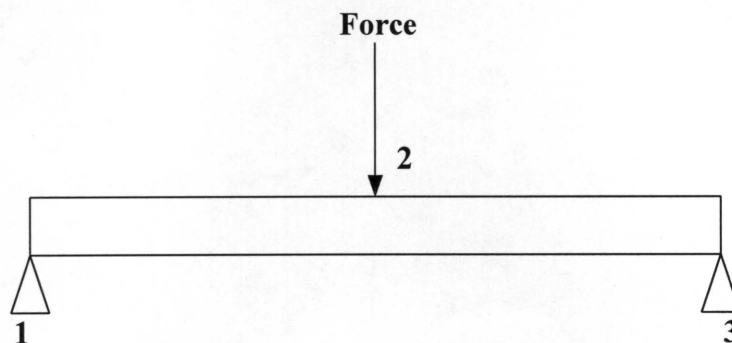


figure 1: Example of three point bending

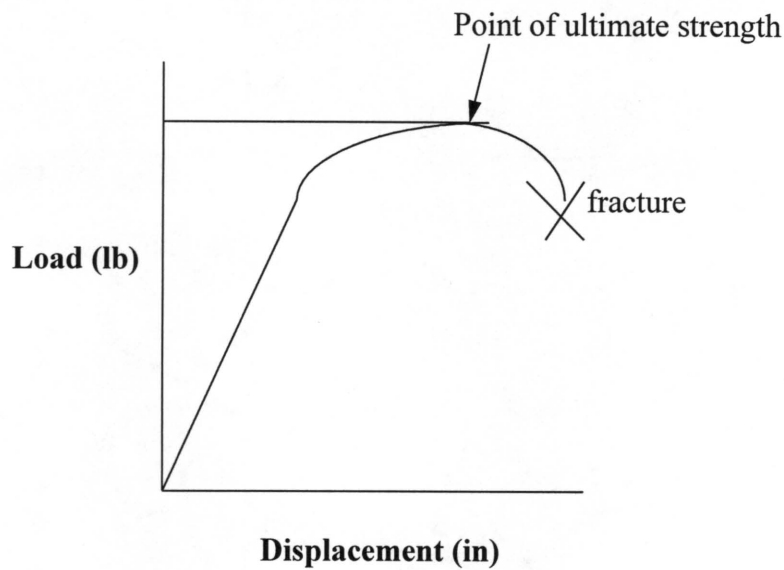


figure 2: Sample load vs. displacement curve

To account for physical variations among the dogs, the biomechanical information from the fractured radius will be compared to the data for the intact radius for each dog.

Materials and Methods

Eight healthy, mature, female beagle dogs weighing between 20 and 25 lb were allocated into two groups of four. The dogs then underwent a radial ostectomy of a random radius. A 3 mm section of the radius was removed from one radius of each dog. One group of dogs received somatotropin growth hormone via a subcutaneous implant during healing while the other control group received a saline placebo. The implants release 4 mg of somatotropin per day for 42 days. These implants were a gift from the Monsanto Company (Dr. Frances Buonomo). The dogs were kept at a relatively constant weight level throughout the study. They were

allowed unrestrained movement during healing to promote the non-rigid fracture model.

Radiographic Evaluation

Dogs were sedated with acepromazine (.05 mg/lb) and butorphanol (.1 mg/lb). Radiographs from the lateral and front views were taken prior to surgery, immediately following surgery, and at 2, 4, 6, and 8 weeks during the course of the project. A standard aluminum stepwedge was placed on the radiograph to provide radiographic density controls for photodensitometric scanning.

Fracture healing was assessed blindly by a board certified veterinary radiologist (Dr. Greg Daniel). Healing was assessed by examining the callus formation, cellular reaction, and gap distance between bone ends. Photodensitometric measurements were taken to determine the bone density.

Nuclear Scintigraphy

While the dogs are sedated for the radiographic tests, nuclear scans of the fractured radius were made. These scans were also performed prior to surgery as well as 2, 4, 6, and 8 weeks during the course of the study. Scans were run by administering 20 mc of ⁹⁹Technitium methylene diphosphonate by intravenous injection. Two hours following injection, a large field gamma camera was used to produce images of the healing bone. Each picture was assessed by Dr. Greg Daniel to evaluate isotope accumulation at the fracture site in relation to the normal adjacent bone.

Uptake of the iosotope in the fractured limb was compared to that of the intact limb.

Force Plate Gait Analysis

An AMTI Model OR6-6 force plate was used to obtain information on the functional use of the injured limb. Tests were run prior to surgery and at 2, 4, 6, and 8 weeks after surgery. The dogs were were trotted across the plate at velocities from 2.1 to 2.4 m/s for three trials for each dog. Through this test, the peak vertical force, breaking force, and propulsive forces were measured.

Biomechanical Testing

At the end of the eight week testing period, the dogs were euthanized and both the intact and fractured radii from each dog were surgically removed. The bones were then radiographed and then subjected to three point bending tests performed on an Instron testing machine. A downward deflection was applied at 2 mm/min until failure of the specimen was achieved. A load vs. displacement curve was generated for each tested bone. From these curves, the stiffness (elastic modulus), ultimate strength, and area under the curve were evaluated for the intact and fractured radii.

Results

Radiographic results:

Radiographs of the dogs treated with somatotropin showed increased fracture healing. There was a great deal of callus formation and nearly reached union at the end of the 8 week period. Untreated dogs had little callus formation and slow fracture healing. The bones were progressing to non-union. Charts 1-3 show the total length, thickness and amount of callus measured for the treated vs. untreated dogs.

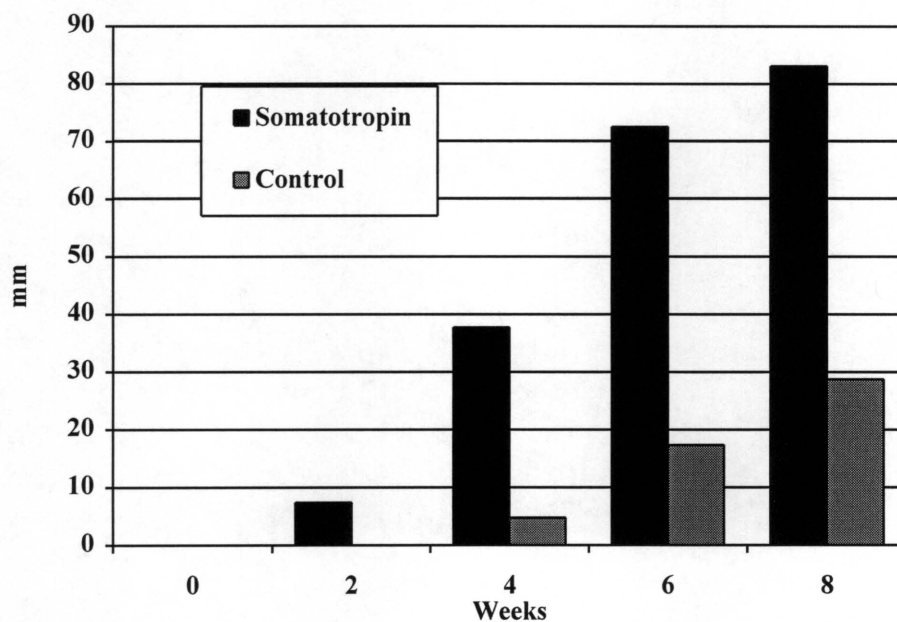


chart 1: Bone callus length

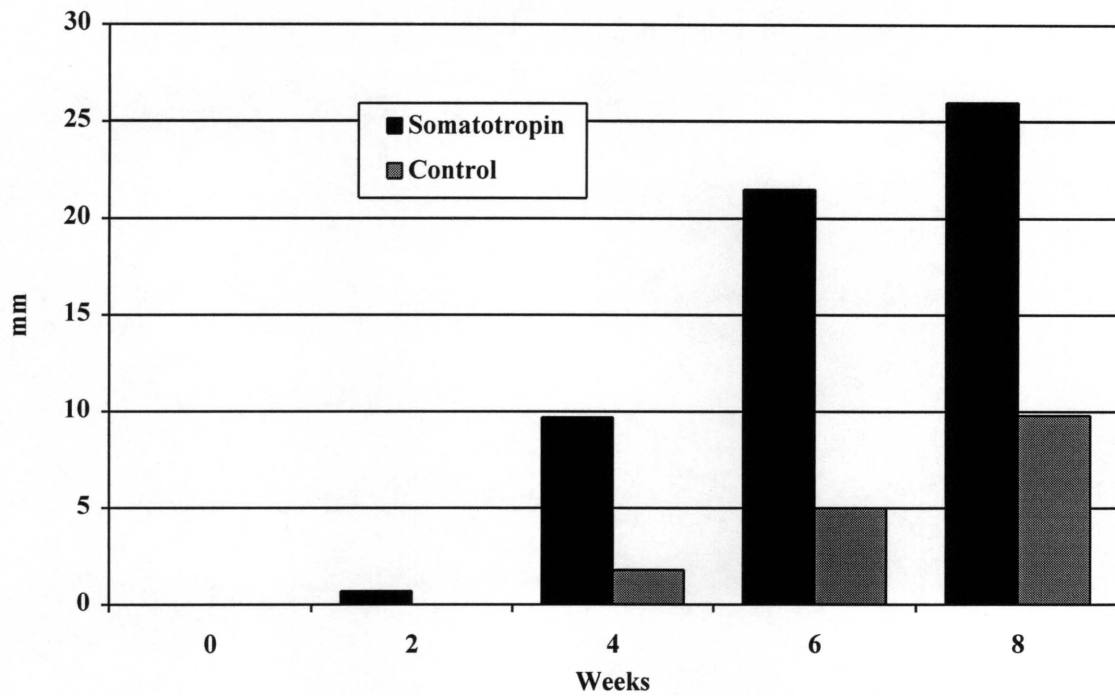


chart 2: Bone callus thickness

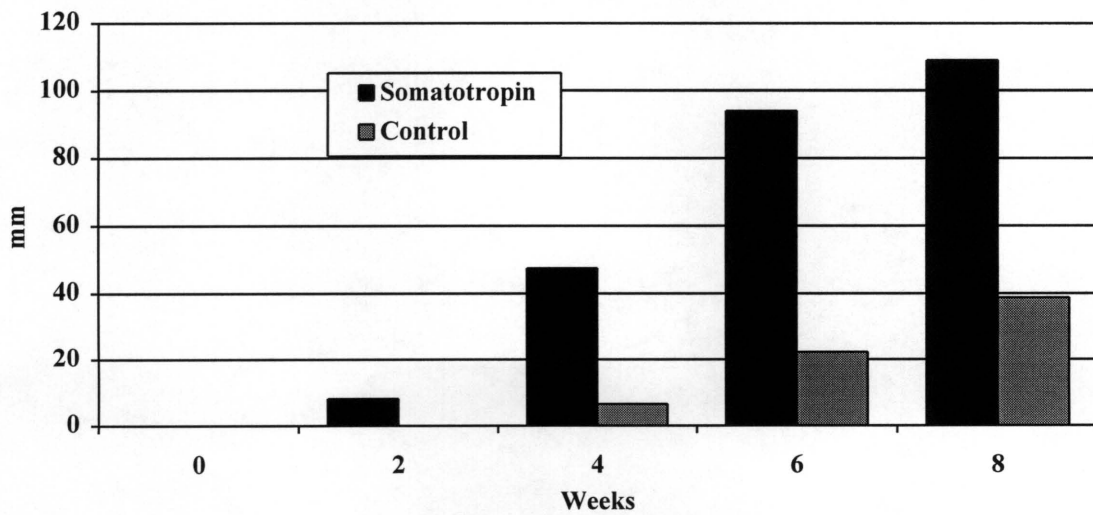


chart 3: Total bone callus measured

The total length, thickness, and amount of the callus was nearly 3 times greater in the dogs receiving somatotropin than in the control dogs.

Nuclear Scintigraphic Results

All dogs showed increased ^{99}Tc absorption at the fracture sites. Some remodeling was noticed in the ulnas due to the increased force placed on them during weight bearing. The somatotropin treated dogs showed a single active region at the fracture site while the untreated dogs maintained 2 separate regions.

Through ostectomy analysis, the bone and muscle density ratios for the dogs were calculated. The bone ratio of the humerus was 30% greater at 2 weeks in the treated dogs. This increase persisted throughout the study. The muscle count density ratio was the same for 4 weeks, but by 6 and 8 weeks, the ratios were 90% and 60% greater in treated dogs, respectively. Charts 4 and 5 show these results graphically. Chart 6 shows the comparisons of the bone area, mineral content, and bone mineral densities for the control and treated dogs.

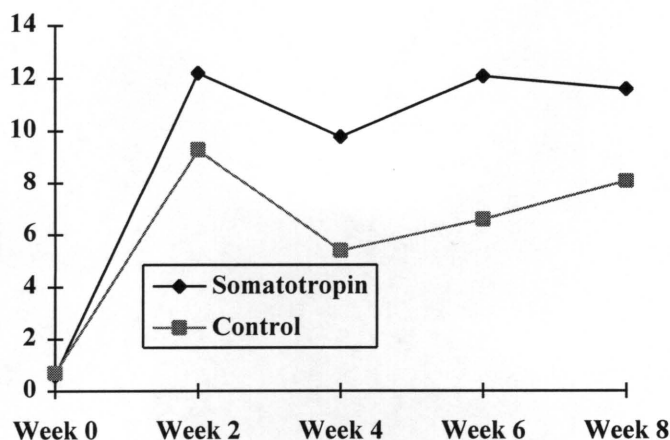


chart 4: Humerus bone density ratios

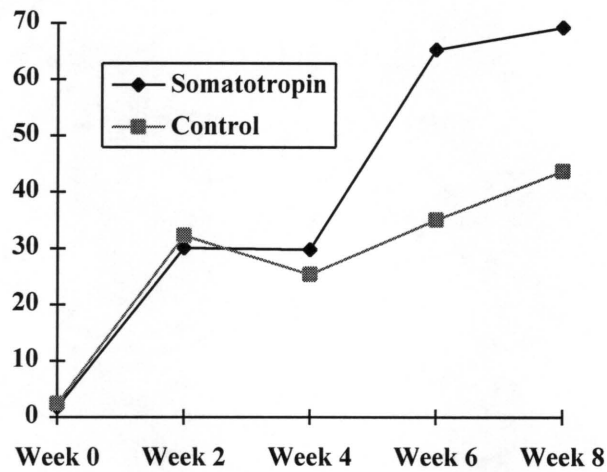


chart 5: Triceps muscle density ratios

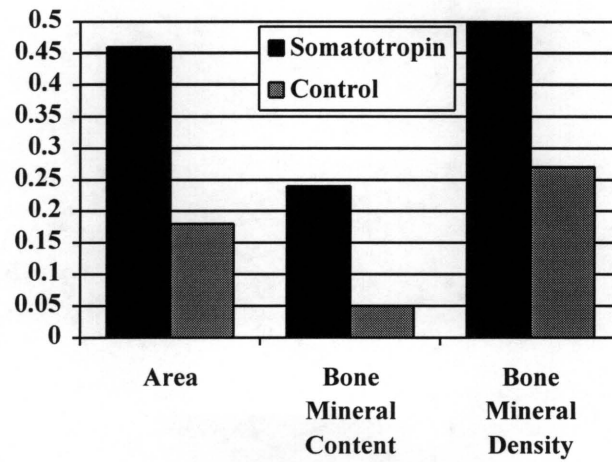


chart 6: Bone comarisons at fracture site

The area, bone mineral content, and bone mineral density of the healing fracture site were 2.5, 5, and 2 times greater, respectively, in dogs treated with somatotropin than in untreated dogs.

Biomechanical Results

The three point bending tests yielded the ultimate strength and stiffness of the intact and fractured radii. The fractured radii of dogs treated with somatotropin were 3 times stronger than fractured radii of untreated dogs (chart 7). The somatotropin did not have an effect on the unfractured contralateral radii (chart 8). Fractured radii of dogs treated with somatotropin were 5-6 times stiffer than fractured radii of untreated dogs (chart 9). Once again, the somatotropin did not have an effect on the unfractured contralateral radii (chart 10).

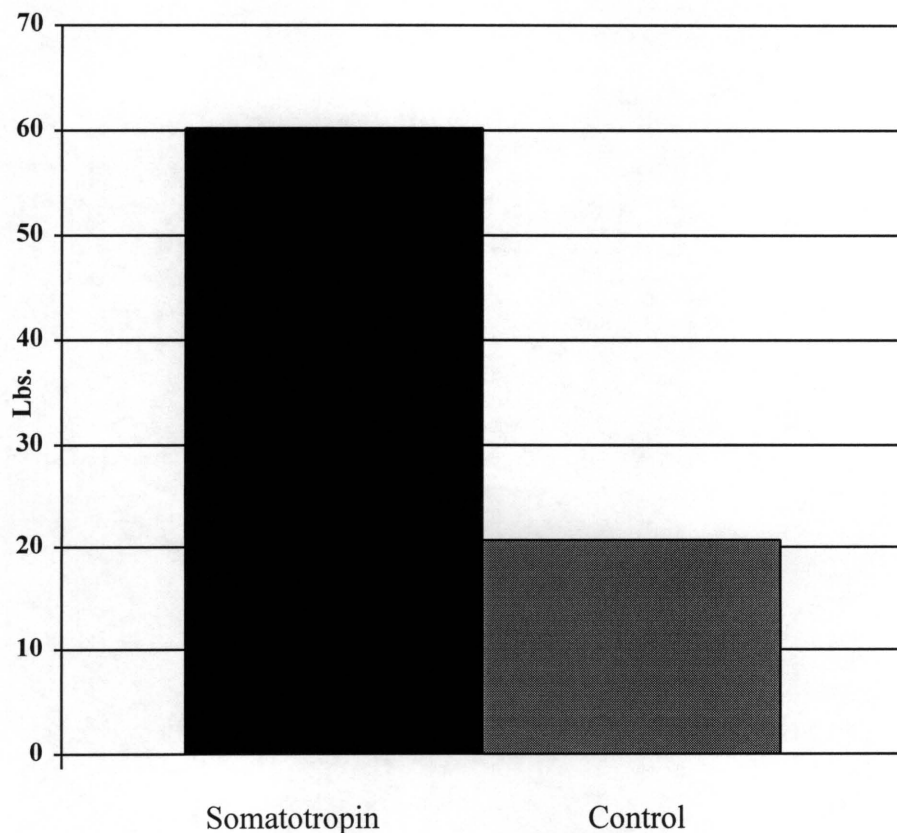


chart 7: Ultimate strength of fractured radii

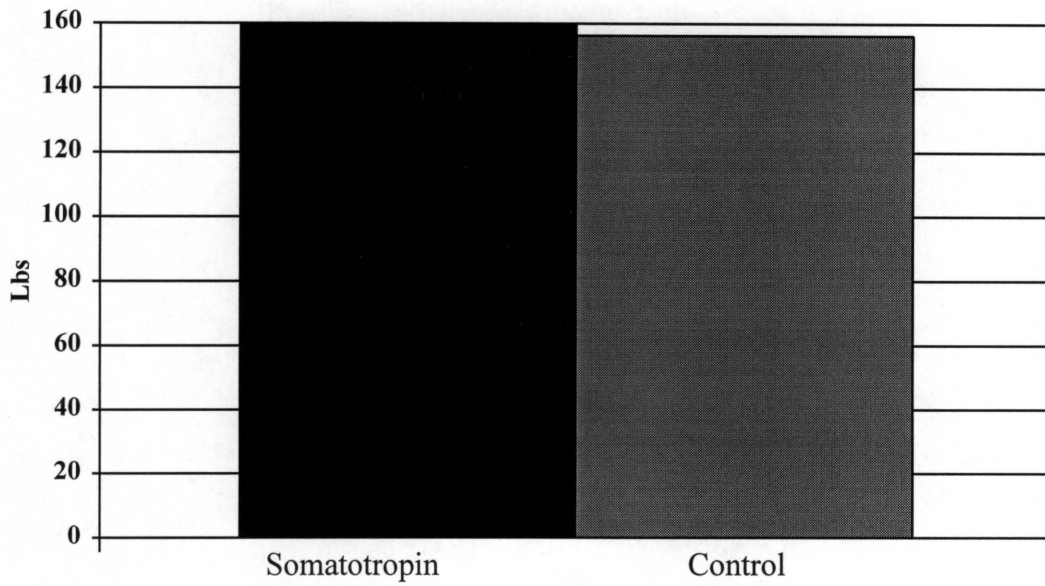


chart 8: Ultimate strength of intact radius

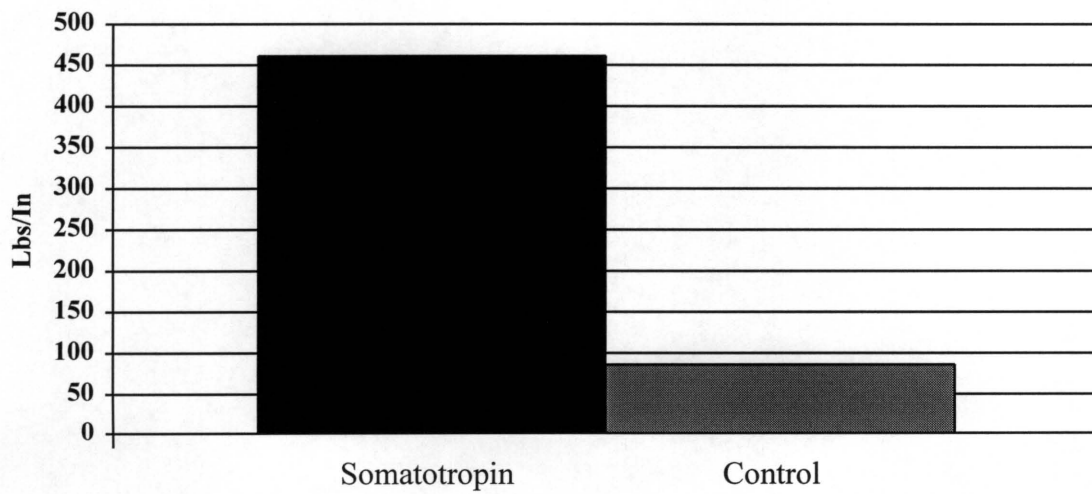


chart 9: Stiffness of fractured radii

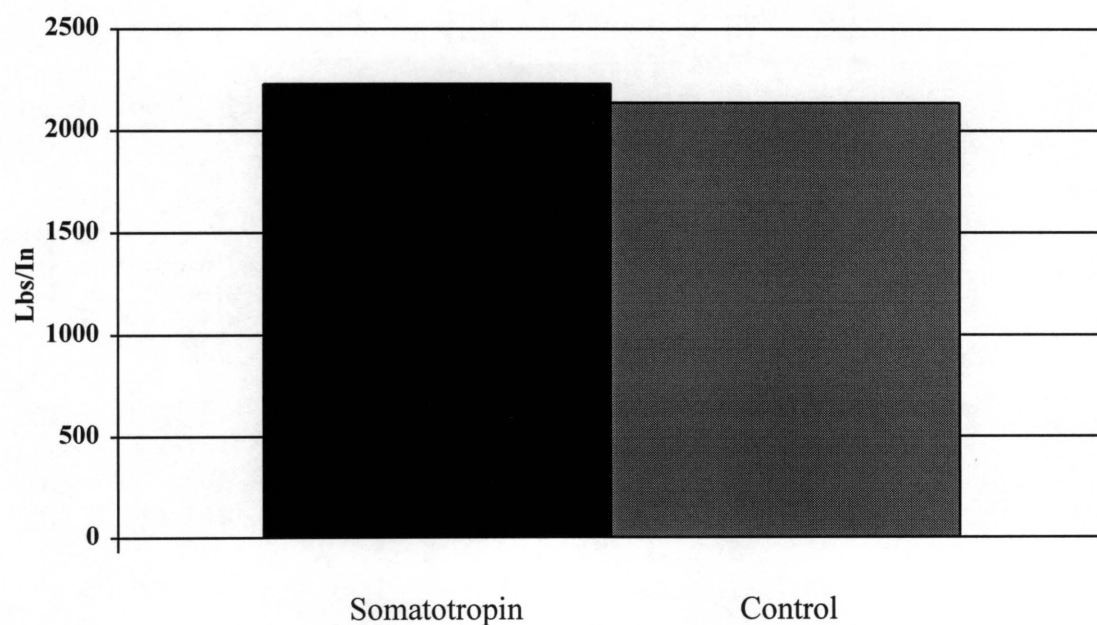


chart 10: Stiffness of intact radii

Conclusion

The addition of somatotropin has a profound positive effect on the healing of fractures in dogs. The radiographs all showed increased bone callus and bone density in the treated dogs vs. the untreated dogs. The fracture site showed a unified active site under scintigraphic analysis for the treated dogs. This means that the fracture was progressing to a bony union. The untreated dogs maintained two distinct active sites at the point of fracture, meaning that the bone was progressing to a nonunion.

The ultimate test of fracture healing is the strength of the bone under load bearing. Strength and stiffness of bone can be related to the total volume of bone, the bone mineral content present, and the density of the bone. In all of the treated dogs, the ultimate strength was about 3 larger and the stiffness was 5 to 6 times larger than those of the untreated dogs.

Future Studies

Future studies should be run to provide other necessary information about the use of somatotropin for fracture healing. Valuable information should include finding an optimal dose level and method of administration of the hormone. Physiological repercussions such as systemic short term and long term effects may be studied as well. Other fracture models besides non-rigid should be examined. Finally, clinical studies should also be pursued.