WEEKLY SPRINT EXERCISE INCREASES BONE STRENGTH AND IMPACTS BONE MARKERS OF JUVENILE ANIMALS

By

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ABSTRACT

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Previous research determined that maintaining young animals in stalls is detrimental to their bone health, while the addition of 50 to 82-m sprints 5 d/wk aids in counteracting the reduction to bone health. This research aimed to determine if 1 or 3 d/wk of sprinting would yield the same benefits to bone as 5 d/wk of sprinting compared to confined animals. Twenty-four Holstein bull calves were obtained from the MSU Dairy Cattle Teaching and Research Center. At 9 weeks of age, calves were randomly assigned to treatments of 0, 1, 3, or 5 d/wk of sprint exercise. Individual sprinting bouts included a single sprint down a 71-m concrete aisle. For the duration of the 6-wk study, calves were housed in individual stalls at the MSU Beef Cattle Teaching and Research Center. Serum was collected weekly via jugular venipuncture to obtain concentrations of osteocalcin (OC) and C-telopeptide crosslaps of type I collagen (CTX-1) - markers of bone formation and degradation. On d 42, calves were humanely euthanized and both front limbs were harvested. Computed tomography scans and mechanical testing were performed on the left fused third and fourth metacarpal bones. Serum OC concentration was highest for calves sprinted 5 d/wk ($P < 0.001$). All exercise treatments experienced greater dorsal cortical widths compared to control animals ($P < 0.01$). Through mechanical testing, fracture forces of all sprinting treatments were determined to be greater than the control treatment ($P < 0.02$). Results from this study support that sprinting 1, 3, or 5 d/wk during growth can improve bone health and cause favorable alterations in bone markers. This study demonstrates the remarkably few strides at speed needed to enhance bone strength and emphasizes the danger to skeletal strength if sprinting opportunities are not afforded.

This thesis is dedicated to Jetta and Spike, the horses who started it all,
and to Boomer, who offered unwavering support.

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CHAPTER 1: Review of Literature

THE HORSE AND HORSE RACING INDUSTRIES

The powerful horse that is recognized today evolved by using speed and athleticism as primary defenses to escape predators on the North American Plains. Selective breeding has further enhanced the innately athletic horse: Arabians trot and canter for up to 160 km in endurance races and Quarter horses launch into speeds as high as 88 km/h over short distances (Hinchcliff and Geor, 2008). Owners have been showing off their successful breeding and training for centuries. Records as early as 1674 indicate matched races of Quarter Horses sprinting down village streets and fields - winning plantations and monetary awards (Ross and Dyson, 2011). Today, travel and agriculture are not dependent upon the power of the horse, but at one point they were. In the nineteenth century, horses could be seen plowing fields, pulling barges along bodies of water, aiding railroad construction, and transporting individuals on a daily basis (Rawson, 2011). At the advent of industrialized travel and livelihood, the population of horses in the United States (U.S.) decreased from 25 million in 1920, to 3 million in 1960. The U.S. horse population now stands at 7.2 million according to the American Horse Council Foundation (2018). The most recent rise in the horse population can be attributed to the interest in horses for competition and entertainment, such as racing.

In 2017 there were 74,243 new horses registered to the American Quarter Horse Association (AQHA). In 1970, Quarter Horse racing had 10,493 total starters and $9,427,886 in purses. In 2017, AQHA races had 13,764 total starters with purses totaling $116,255,967. (AQHA, 2010; AQHA, 2017). The Jockey Club (2019a) estimated that in North America there were 49,390 starters in Thoroughbred races in 2018 and 22,388 in 1950. Gross purses in Thoroughbred races have increased from $714.5 million in 1990 to $1,118 million in 2018 (The

Jockey Club, 2019b). While the use of horses for work and as a primary mode of transportation has severely declined, the use of horses for entertainment and recreation are still valued and, rightly, the health and welfare of these horses need to be supported, evaluated, and researched.

The rise in interest in racing is not without risk due to the nature of the sport and prevalence of injuries. Injuries to racehorses can yield career-ending retirement, death of horses, injury and death of jockeys, adverse perception of horse racing by the public, and detrimental effects to the industry's economics (Stover, 2003). Career lengths of horses involved in racing are much shorter than other equine sports. Racing Thoroughbreds in Australia had a mean career length of 19 months with an average of 15 career starts (Velie et al., 2012) and Thoroughbreds racing in New Zealand had an average career length of 2 to 3 years (Tanner et al., 2012). In other disciplines such as dressage and eventing, horses compete for an average of 4 and 5.8 years respectively (Sloet van Oldruitenborgh-Oosterbaan et al., 2010). Musculoskeletal injuries in young Thoroughbred racehorses are the greatest cause of missed training days and racehorse turnover (Stover, 2003; Ramzan and Palmer, 2011). Musculoskeletal injuries are also the largest cause of wastage in all equine disciplines, not just racing (Rogers, 2012). Appropriate, science based of training of young horses can be a valuable tool to decrease racing-related injuries; such as timely introduction of appropriate exercise and reduction of disuse. Comprehension of bone structure and bone function in horses leads to better understanding of musculoskeletal injuries and how utilization of exercise can reduce these injuries. The following sections detail on a cellular and tissue level why the skeleton of young animals and adolescent humans is more adaptable to exercise than their mature counterparts.

BONE FUNCTIONS AND CHARACTERISTICS

Bone is a connective tissue, which has functions involving locomotion, soft tissue support and protection, storage of calcium and phosphate, and to contain bone marrow (Florencio-Silva et al., 2015). An important characteristic of bone is the ability to adapt to its environment, and the forces (strains) placed on it, according to Wolff's Law (Woo et al., 1981). This response of bone to strains allows for change while animals and humans grow. Optimizing the response of bone to strain while young can optimize the structure and strength through youth as well as maturity (Guadalupe-Grau et al., 2009). While bone may be perceived as static, it is constantly changing through two similar, yet separate processes: bone remodeling and modeling. Remodeling is the process by which old bone is formed into new bone, while modeling is the process of bone acquirement and removal in growing individuals (Florencio-Silva et al., 2015). Bone remodeling and modeling employ two unique cells: osteoclasts and osteoblasts.

Osteoblasts originate from stem cells located in the bone marrow and are involved in bone formation through the production of bone matrix constituents (Florencio-Silva et al., 2015). Bone matrix is crucial to the structure and function of bone and is secreted from osteoblasts in the form of non-mineralized osteoid which is then mineralized over weeks to form bone matrix. Matrix is composed largely of an inorganic mineral hydroxyapatite, which orients around collagen fibers. Other constituents of bone matrix are type 1 collagen, water, and noncollagenous proteins, such as the vitamin-K dependent hormone protein osteocalcin. The collagenous make-up of bone matrix contributes to tensile strength, while mineralization via hydroxyapatite crystals contributes to compressive strength. Material and mechanical properties, such as elastic modulus, are related to the degree of mineralization. In young horses, the mineralization level is low and

increases as the animal matures, thus leading to material changes which affect the mechanical properties of bones (Lee et al., 2000; Goodship and Smith, 2008; Florencio-Silva et al., 2015).

Osteoblasts have multiple fates at the end of the bone-forming phase, and functions that go with each fate; the most common fates are to undergo apoptosis, become osteocytes, or become bone-lining cells (Franz-Odenaal et al., 2006; Florencio-Silva et al., 2015). The rate of apoptosis, or programmed cell death, of osteoblasts increases as animals age. An osteoblast may undergo apoptosis as a response to microdamage of bone or by early incorporation into the bone matrix as a result of an influx of osteoblast transformation to osteocytes. Near the end of a bone formation cycle, about 15% of osteoblasts will become embedded in matrix and differentiate to osteocytes. Research provides a few ideas as to why some osteoblasts transform to osteocytes and some succumb to other fates. Some of these hypotheses are that osteoblasts are at many different developmental stages along bone surfaces or that bone cells communicate to determine the fate of an osteoblast.

Osteocyte function and structure vary based on age. Newer osteocytes are similar to osteoblasts, they will release some matrix, but are smaller in size. Older osteocytes are deeper in bone than their younger counterparts. Osteocytes function as receptors of strain and communicate with nearby osteocytes or cells on bone surface through a network of cellular processes that run through the microscopic canals in bone matrix (Franz-Odenaal et al., 2006; Hadjidakis and Androulakis, 2006; Goodship and Smith, 2008).

Osteoblasts that have not undergone apoptosis or transformed into osteocytes may transform into bone-lining cells. These are flat-shaped osteoblasts that remain on bone surfaces where neither bone resorption nor bone formation are taking place. Depending on the status of a bone, the bone-lining cells can reacquire their secretory activity, changing to a cuboidal

appearance. Their function is not comprehensively understood, but they do prevent direct interaction of the osteoclasts with the bone matrix when bone resorption should not be occurring. It is also thought that they participate in differentiation of osteoclasts (Florencio-Silva et al., 2015).

Similar to osteoblasts, osteoclasts originate from stem cells in bone marrow, but these bone cells are tasked with the resorption of bone. Osteoclasts are multinucleated and contain numerous golgi complexes, mitochondria, and vesicles which allow for the resorption function of these bone cells. Bone resorption is accomplished by the acidification and proteolysis of bone matrix and hydroxyapatite crystals. To resorb bone, osteoclasts will attach to bone surface via podosomes. Through the continuous assembly and disassembly of podosomes, osteoclasts can move across bone surfaces and resorb bone (Hadjidakis and Androulakis, 2006). Osteoclasts and osteoblasts are critical cells in the bone processes aforementioned: bone modeling and bone remodeling.

The function of bone modeling is to either form or resorb bone at a specific surface to maintain and alter bone shape. Formation modeling occurs with the use of osteoblasts, while resorptive modeling employs osteoclasts. Modeling adapts bone to better endure strain it is experiencing and is most active during growth and development of the immature animal. While the adult skeleton undergoes some modeling, it is not as common as in the immature skeleton. For this reason, while animals and children are growing, their skeletal strength is highly influenced by the strains their bones undergo through daily use and exercise (Allen and Burr, 2013). Short-term dynamic exercise as an adolescent can lead to beneficial changes in bone morphology, increased fracture force, and reduced fracture risk at maturity (Warden et al., 2009). Dynamic strain changes, such as those from cyclical impact exercises like sprinting, will lead to

changes in bone formation. Exercises in which loading of bone is static or not loaded, such as swimming, do not signal changes in bone formation. Studies on humans have shown that swimming leads to reduced or unchanged bone mineral content and does not positively influence bone morphology compared to dynamic loading sports, such as soccer (Ferry et al., 2011; Allen and Burr, 2013; Mohr et al., 2015). Factors in the strain environment of a bone that elicit remodeling responses include magnitude of strain, rate of change in strain, as well as spread of dynamic strain (Rubin and Lanyon, 1984; Lanyon and Rubin, 1984; Guadalupe-Grau et al., 2009). A topic frequently discussed in relation to strain and strain rate is the threshold of a bone, which has been shown to be at 1000 με or at 0.1% change in length of the bone (Robling et al., 2014). If bone undergoes a load elevated above its threshold, the bone cells are signaled to increase bone matrix by synthesizing new bone through formation modeling. Consequently, if bone experiences a reduction in loading well below the threshold, resorption modeling commences and bone removal occurs (Allen and Burr, 2013). Systematically, formation and resorption may be occurring at the same time during bone modeling, but they do not need to be happening at the same location on a bone. In bone modeling, osteoblasts and osteoclasts are not tied to the same sequential time frame as they are in bone remodeling (Allen and Burr, 2013).

Bone remodeling normally exists in a coordinated relationship between bone resorption and formation. The function of remodeling is to renew bone over time, through replacement of bone matrix and repair of microdamage. Bone resorption by osteoclasts and bone formation by osteoblasts occur sequentially, at the same location on bone surface. Both osteocyte apoptosis and microdamage to bone will signal osteoclasts to a specific location to begin remodeling (Allen and Burr, 2013). Due to their location in the bone matrix, osteocytes are tasked with the translation of a mechanical stimulus into biochemical signals and therefore, are regulators of

bone remodeling (Florencio-Silva et al., 2015). During the first stage of bone remodeling, known as resorption, osteoclasts will absorb old bone. The next stage of remodeling is reversal; during reversal, mononuclear cells appear on the bone surface in preparation for osteoblasts to form new bone. Formation is the final stage of bone remodeling. During formation, osteoblasts lay down new matrix until the absorbed bone is completely replaced (Hadjidakis and Androulakis, 2006). The resorption and formation stages of bone remodeling are not always in an equal balance, especially when there is injury or disease present (Allen and Burr, 2013). Osteoclasts can remove bone tissue in a time frame of a few days to 2 weeks. Unfortunately, the formation of bone by osteoblasts can take months. In areas of bone under repair, injury is of higher possibility as bone has been removed, yet not completely replaced (Stover, 2003).

Bone is stiffer than other tissues in the body, but still has dynamic abilities to adapt to changes in demand from loading or disuse. Ideally, a skeleton should be stronger than the maximum load it undergoes in daily use. For most land-dwelling mammals, limb bones can undergo loads that deform them 3 to 4 times the amount they are normally deformed in peak activity. This concept is known as a safety factor. In horses, the most distal portions of the limbs are at higher risk for fracture. Mass in the distal portion of the limbs is minimized to reduce limitations on locomotor speed. However, the decrease in mass leads to a decrease in the safety factor, and thus the distal portions of limbs are at higher risk for fracture than the proximal portions of limbs (Skerry, 2008). Counteracting musculoskeletal injuries would not be affectively accomplished by an increase in the mass of equine bones. In the distal limbs of horses, extraneous mass would negatively affect the speed and locomotion of the animal due to increased energy needs to move larger bones (Smith and Goodship, 2008).

Reduction of catastrophic injury to bone can come from quantifying strain which improves bone and strain, or lack thereof, which induces injury. Taking into account strain environments which illicit bone formation or resorption is crucial when considering exercise which affects skeletal strength. An understanding of the functions of osteoblasts and osteoclasts and their roles in bone modeling and remodeling clarifies the relationships between exercise activities of the horse, and modifications at the cellular level.

CHANGES TO BONE IN YOUNG ANIMALS

Horses are born ready to bear weight on their bones; quite unlike humans, they stand up within minutes of birth and are sprinting within hours. Most of the weight of a horse is borne by the third metacarpal (MC III). At birth, the MC III is ready to support a foal immediately. Changes to the MC III occur before parturition to allow for early strength and weight-bearing (Firth, 2006). Skeletal development of young horses does not lessen after birth, in fact it quickly increases. Within 9 days of birth, foals can travel up to 10 km/d (Rogers et al., 2012). At the age of 6 weeks, birth weight is doubled in Thoroughbred foals. At 12 months of age, a yearling is 90% of its mature height and 66% of its mature weight and by 4 years of age growth has completed (Firth, 2006).

Bone growth solely occurs in young animals and adolescent humans, making this period critical to bone acquisition (Guadalupe-Grau et al., 2009). Bones of young animals have a greater response to stimuli compared to their older, mature counterparts. The bones of young horses are most responsive to stimulus from the yearling stage up to 2 years of age (Rogers et al., 2012). If loading occurs between these ages, there is opportunity for the animal to be able to resist damage to and failure of bone in adulthood. Horses that are intended for athletic events may benefit from exercise induced early on, while their skeleton can still be influenced. Epidemiological studies

have revealed that Thoroughbred and Standardbred horses entered in race training at 2 years of age had more race starts and greater earnings than those who entered training later in life. Regardless of whether the horses that entered training at 2 years of age actually raced at that age, the positive stimulus of exercise from training provided advantages to those who started training at 2 years of age (Rogers et al., 2012). A recent review of literature has determined that age is a risk factor for catastrophic musculoskeletal injury of racing Thoroughbreds, finding that older horses were of greater risk (Hitchens et al., 2019).

Permitting and prescribing exercise to young animals has the potential to reduce injuries to bone later in life, especially if the animals are being raised for an athletic career. Exposing the influenceable skeletal structure of young horses to loading patterns during development could optimize the skeletal structure through maturity, thus leading to a lower chance of musculoskeletal injury (Smith and Goodship, 2008; Rogers et al., 2012). Warden et al. (2007) determined that cyclical compression induced onto a rat's forelimb for 7 wk during adolescence can lead to maintained bone quality and strength lasting into maturity compared to non-exercised rats. Greater strength during maturity compared to non-exercised animals suggests that exercise while growing can provide lifelong benefits. Young animals undergoing exercise experience more loading than those who are confined with limited access to exercise. In response to greater frequency of loading, exercised animals respond with greater strength to bone (Warden et al., 2007; Rogers et al., 2012).

EVALUATION OF BONE ACTIVITY

Given the location and function of bone, it can prove difficult to monitor the activity of bone and bone cells in live animals. However, bone markers and imaging have proven useful to equine researchers and veterinarians as their availability and ease of use continue to increase. Osteocalcin, produced by osteoblasts and an indicator of osteoblastic activity, is a noncollagenous vitamin K-dependent hormone protein. Two forms of osteocalcin exist, carboxylated and undercarboxylated. Vitamin K is a mandatory cofactor in the carboxylation of osteocalcin. Due to a conformational change, the carboxylated form of osteocalcin shows preference for calcium and hydroxyapatite crystals in bone matrix, while the undercarboxylated form does not have a strong affinity for minerals and will be transported to the bloodstream to reach other organs such as adipose tissue, testis, and the pancreas (Florencio-Silva et al., 2015; Lee et al., 2000). Undercarboxylated osteocalcin may be an indicator of poor bone health as it appears to increase in concentration with age and is an indicator of hip fracture in elderly women (Lee et al., 2000). However, intact osteocalcin concentration measured in most commercial assays is an indicator of osteoblastic activity, and therefore bone formation. Commercial assays for osteocalcin function off the affinity of carboxylated osteocalcin for hydroxyapatite, and therefore do not measure undercarboxylated osteocalcin unless specifically designed to do so. Serum osteocalcin concentration will reflect 10 to 40% of osteocalcin that is produced but not incorporated in bone matrix (Lee et al., 2000; Hiney et al., 2004a,b).

Use of bone markers, such as osteocalcin, is minimally invasive as concentrations can be determined from blood or urine. Due to the ease of collection methods for blood and urine, bone marker evaluation to determine bone activity is a commonality in equine research (Matsuo et al., 2014). When a bone formation marker is evaluated, a bone degradation marker such as

deoxypyridinoline or C-terminal telopeptides of Type 1 collagen is typically evaluated as well to visualize the bigger picture of bone health in the animal. A study performed by Hoekstra et al. (1999) evaluated osteocalcin and deoxypyridinoline, markers of bone formation and degradation respectively. Lower osteocalcin and higher deoxypyridinoline concentrations in stall-reared horses helped to determine that stall-reared horses experienced bone loss compared to those reared on pasture.

Imaging technologies are also used to observe bone activity in response to exercise and confinement. Radiographic images can be utilized to determine changes in bone mineral content. Radiographic bone aluminum equivalence (RBAE) utilizes an aluminum stepwedge as a comparison against bone for mineral content. Often, analysis of radiographs using RBAE is done at the nutrient foramen of the MC III, as it is a clear and discernable anatomical marker. Use of RBAEs by Nielsen et al. (1997) detected changes in cortical bone mineral content of the left MC III in response to stall-confinement and race training. Radiographs are taken with the animal standing, and current machines are usually portable, making it easier to take images for research studies and clinical use. Computed tomography (CT) scans can prove more difficult to use as animals are typically exposed to general anesthesia in order to lie down for extremity access to the gantry and machines are not portable. Gantry size can also be a limitation, as most gantries are only large enough for the extremities of horses. However, CT scans still provide useful information to analyze bone activity as they can generate 360 degrees of imaging, cross-sectional images, and 3-dimensional reconstructions unlike radiographs (Ross and Dyson, 2011).

Hiney et al. (2004a) performed CT scans on the fused third and fourth metacarpal (MC III & MC IV) of calves postmortem to determine cross-sectional morphology and bone mineral density (BMD) of animals that were sprinted 5 d/wk, confined with no exercise, or group-housed

with no exercise. Analysis of CT scans found that calves in the sprinting group had decreased medullary cavities, rounder bones, increased dorsal cortical thickness, and increased total BMD in the MC III & IV when compared to the confined and group-housed animals. Another imaging technology that has entered the equine research scene is micro CT. Micro CT employs a higher resolution capable of detecting local apparent density of bone and characterizing trabecular bone structure (Leahy et al., 2010). It has been determined by Leahy et al. (2010) through the use of micro CT that there are correlations between density and mechanical properties, especially when utilizing low apparent density portions of trabecular bone in the distal equine MC III. Without subjecting the bones of animals to mechanical testing, bone strength can be determined with the use of micro CT, allowing researchers to determine mechanical properties of bones in live animals.

Utilization of these imaging technologies and bone markers can aid in the difficult task of monitoring and determining bone activity in horses. It is up to researchers to determine which bone markers and imaging technologies fit best with their objectives. While portability and size seem to limit the use of CT and micro CT to terminal studies, the rest of the discussed options are minimally invasive to the animal and realistic to use throughout a study to determine baseline and progression of bone activity. Detection, as well as monitoring the progression of bone injuries is important in reduction of injury as well as prescribing proper treatment for animals. Using information gathered from bone markers and imaging techniques can help to make research, and clinical, diagnoses with the end goal of a reduction in injuries to athletic horses.

FREQUENCY AND EFFECTS OF INJURIES TO THE HORSE

Skeletal injuries put horses, as well as their riders at risk, and lead to negative impacts on the horse industry and economy (Stover, 2003). Skeletal injuries are a leading cause of catastrophic race-related injury as well as training-related injuries. Unfortunately, training-related injuries are difficult to record as there is no standard for reporting and observation of injuries during race training. Along with career-ending catastrophic injuries during races, there are also career-ending skeletal injuries that occur during training and prevent some young horses from ever starting in a race (Ross and Dyson, 2011). Skeletal injuries which are preventable need to be addressed so that animals entering the athletic population do so prepared for the skeletal demands (Stover, 2003).

Mohammed et al. (1991) evaluated common risk factors for musculoskeletal injuries in racing Thoroughbreds. From 1986 to 1988, 310 breakdowns were reported by the chief veterinarian of the New York Racing Association. The most common musculoskeletal injuries and their frequencies in the 310 breakdowns were lameness (16%), metacarpal bone fracture (13%), tendonitis (10%), and carpus fracture (8%). Of the horses that broke down, 56 had to be put down due to the severity of their injuries. Most breakdowns were seen during a horse's first or second season of racing. Horses with less starts were more likely to be injured than those with more starts. The researchers believed that the risks for injuries presented themselves early in the horse's life, before they had many starts in races. Most musculoskeletal injuries are repetitive overuse injuries which are caused by stress-related bone injuries.

As young horses undergo training their bones change, especially the MC III, as a result of the novel forces from increased exercise. Nielsen et al. (1997) performed a study using 53 Quarter Horses that entered race-training at 18 months of age. Before training, these horses were

kept on pasture; once training started, they were kept in stalls which they were maintained in for the duration of the study. Once race training started, declines were visible in bone mineral content. Of the 53 horses in the study, 15 had bone-related injuries. Being stalled during conditioning and training could have influenced the onset of injury as the animals were no longer experiencing normal strains on bone while on pasture. The extended stalling periods during training may have caused the decrease in bone mass and subsequent bone injuries, not the gallops or other exercises during race training. Horses maintained on pasture have higher bone mineral content and cover at least twice as much distance voluntarily compared to those kept in stalls with or without exercise (Graham-Thiers and Bowen, 2012). Thoroughbred racing horses inflicted with stress-induced bone injuries and were treated with rest and free choice exercise on a paddock were able to return to racing with no decrease in performance or class (Tull and Lawrence, 2011).

Horses involved in racing are not the only ones at risk of musculoskeletal injuries. An evaluation of non-racing Quarter Horses unveiled the injuries prevalent in common western performance disciplines. Competitive futurities place a high incentive for trainers to begin working horses as young as 18 months. As is typical for many training protocols, young horses in reining training are mainly maintained in stalls with minimal to no free exercise on pasture. Young horses in training are not allowed much rest, and undergo strenuous, repetitive training sessions while still being maintained in a stall with little to no turn-out time which allows free exercise. Injuries of non-racing Quarter Horses are typically chronic, and not associated with a specific injurious event, similar to racing Quarter Horses and Thoroughbreds. Allowing time for rest and recovery from injuries could reduce the severity and incidence of further injury (Scott, 2008).

Injuries that involve musculoskeletal structures are an endangerment not only to horses, but to their riders as well. From 2007 to 2012, data were collected in California from Thoroughbred and Quarter Horse race tracks regarding the causes of jockey falls and injuries. During this study, 707 horses experienced an injury or sudden death that was race-related. Catastrophic injury to the horse, or sudden death, caused 32% of jockey falls in this study. Quarter Horse races were found to have higher frequencies of horse fatalities, which lead to more jockey falls and injuries. Forelimb musculoskeletal injuries were most likely to cause a fall of both horse and rider (Hitchens et al., 2016). In both racing Thoroughbreds and Quarter Horses, the forelimbs are the most common locations for catastrophic musculoskeletal injuries (Beisser et al., 2011). As it is, horse racing is a dangerous event to both horses and their jockeys. When a horse is injured in a race and falls, the jockey also falls, often in a dangerous fashion. The reduction of equine breakdowns on the track would benefit the health of both horses and riders. Implementing sprinting to increase bone strength of young horses as a protocol before training starts, could reduce the risk for a dangerous forelimb musculoskeletal injury.

FATIGUE FRACTURES

The skeleton is designed such that it should not fracture as a result of damage from repeated strains of normal activities. Microdamage to bones is normal and is typically repaired through the bone remodeling process. Bone resorption will occur as a result of osteoclasts responding to microdamage in bone. However, if loading continues, bone tissue is being removed from a region of bone that is still enduring high strains. If damage occurs faster than repairs can be made to a bone, fatigue fractures become a possibility. These fatigue fractures are frequent with prolonged exercise (Carter, 1984). Periosteal callus and stress remodeling noticed along fracture interfaces of equine bones such as the femur and MC III suggest that overuse

injuries lead to fractures. Development of a periosteal callus, as well as the process of remodeling, takes time, proving that repetitive overuse over time causes microdamage before leading to a fracture (Stover, 2003). Fatigue fractures do not occur due to an instantaneous misstep of the animal; instead, a fracture arises after a build-up of small injuries to the bone that go unrepaired. Remodeling at the locations of strains may be occurring, but the rebuilding of new bone happens much slower than the resorption of bone. Dorsal metacarpal disease ("bucked shins") is caused by this lag time of bone formation during remodeling. This injury is commonly seen in 2-year-old racing Quarter Horses as well as racing Thoroughbreds. Afflicted animals will exhibit varying degrees of discomfort ranging from an uneven gait, sensitivity to the touch of the MC III, and poor race performance due to pain (Ross and Dyson, 2011). The most effective treatment is appropriate rest before returning to training so that microfractures do not continually occur in an area of bone that is already weakened.

In general, there are two ways to prevent bone fractures related to load bearing; the load can be lessened, or the strength of the bone itself can be increased through exercise. The skeleton's response to exercise through bone modeling is greatest in young animals, suggesting exercise prescribed to increase bone mass is most effective at a young age (Judex et al., 1999). To gain beneficial bone adaptation, only a few cycles of loading are required. As few as 4 and 36 cycles of loading were needed to maintain or increase bone mineral content respectively in a study imposing cyclical loads on rooster ulnae. A large amount of loading cycles are not needed to impose deformation which produces a stimulus leading to achievement and maintenance of bone mass (Rubin and Lanyon, 1984). Short dynamic loading periods in young horses will lead to a response to stimulus via bone modeling which increases bone mineral content and therefore

skeletal strength (Skerry, 2008). Adding short sprints before training young horses could lead to a lower risk of bucked shins and fatigue fractures as training commences.

DISUSE AND STALL CONFINEMENT

Removing or significantly decreasing exercise can cause bone to undergo disuse and become compromised. Disuse, in terms of bone, represents a reduction in use to below what the skeleton had been previously adapted. Given its relative description, disuse does not occur at a defined level of activity, or lack thereof, nor at a specific age. Instead, occurrence of disuse depends on the normal activity of the animal and the strains to which the skeleton is accustomed. For example, a racing Thoroughbred is habituated to strains much higher than a horse that solely walks on trails and will experience disuse at very different levels of strain than the trail-riding horse (Skerry, 2008). Disuse by way of reduction in exercise, immobilization, or confinement has deleterious effects on bone (Snow et al., 2001; van Harreveld et al., 2002). In the absence of loading, the skeleton will revert to its genetic minimum, a reduced bone mass that can support basic function without failure (Skerry, 2008). Animals may be exposed to disuse while recovering from an injury, resting from a competition season, or being maintained long-term in stalls. Counteracting disuse, even during rest, could maintain equine bone strength and reduce risk of injury from bone loss.

Valued horses are often kept in stall confinement, for fear of injury if turned out to pasture. Exposure to pasture may be limited in time and space for these animals, if pasture is utilized at all in management. A study performed by Hoekstra et al. (1999) evaluated differences in bone mineral content between box-stall and pasture-reared yearling Arabian horses. After an 84-d pre-training period on their respective stall or pasture treatments, horses started training and were ridden 5 d/wk. Box stalled horses were placed on a mechanical walker for 1 hour on non-

riding days, and pasture-kept horses were allowed free exercise as they desired. The box-stalled horses had an overall decrease in bone mineral content of the medial and lateral cortices as determined by RBAE. Exercise from training and the walker did not counteract bone loss in the stalled horses. Young horses housed in stalls may experience detriment to their skeletal health compared to young horses raised on pasture. Maintaining horses in stalls without providing exercise beyond training may also be detrimental to their musculoskeletal health. Bones of young horses need to develop and strengthen through cycles of loading and unloading. If young horses are not allowed these opportunities before training begins, they will be starting their athletic careers already in a deficit (Scott, 2008).

Immobilization will also lead to reduction in bone mineral content of the MC III. A study by van Harreveld et al. (2002) used five sound horses 22 to 26 months of age that underwent immobilization of one forelimb via a fiberglass cast for 7 weeks. Following removal of the cast, all horses were at least a grade 2 lameness localized to the metacarpophalangeal joint and were put on an 8-week period of gradually increasing exercise on a treadmill. Lameness did improve in some horses following the exercise protocol, but 2 horses experienced lameness of the same degree or worse than when the cast came off. Cortical bone mineral content of the MC III was decreased for all immobilized limbs at the end of 7 wks of immobilization, as determined by radiography. An improvement in mineral content was reported by the researchers after the 8-wk exercise protocol. The authors of this study concluded that persisting lameness after 8 wks of remobilization could be due to either immobilization or the exercise protocol. It should also be noted that this immobilization due to casting is not the same as disuse due to stall confinement or reduction of normal training activity. However, the reduction of bone mineral content in the immobilized limbs certainly lends to support adaptation of bone in relation to strains, which

follows Wolff's Law that bone responds to strains or lack of strains placed on it (Woo et al., 1981).

A third example of bone undergoing disuse is through reduction in activity of intercollegiate competing female gymnasts at Oregon State University. Athletes were evaluated during two 8-month training seasons and two 4-month off-seasons over two consecutive years. During the training seasons, BMD of the spine increased 3.5% the first year and 3.7% the second year. Spine BMD declined 1.5% and 1.3% during the off-seasons. Total hip BMD increased 2.3% and 1.9% during the two training seasons, followed by declines of 1.5% and 1.2% respectively. Overall, during both off-seasons, athletes underwent loss of bone mineral content in both the spine and hip (Snow et al., 2001).

Stall confinement, limb immobilization, and reduction of activity are all examples of relative disuse in which bone health declined. The skeleton responds to increasing load magnitude and increases BMD to meet heightened demands. Likewise, when the stimulus is removed or decreased, reductions in BMD occur to meet the lower demands (Hoekstra et al., 1999; van Harreveld et al., 2002; Snow et al., 2001). Careful balance of skeletal preparation through exercise, rest, and avoidance of disuse during training can help young horses entering training to do so in good skeletal health with a potentially lowered risk of catastrophic injury.

CALVES AS JUVENILE EQUINE MODELS

Two studies performed by Hiney et al. at Michigan State University provide evidence that prescribed exercise in young animals can influence bone characteristics (2004a,b). The first study utilized Holstein bull calves as a model for horses, and the second study implemented similar treatments as the bull calf study on weanling horses and verified that calves serve as a viable bone model for young horses.

In the calf study, 18 bull calves were randomly assigned to three treatment groups: group-housed (GR), confined without exercise (CF), and confined with exercise (EX). The EX group was sprinted once daily 5 d/wk for 50 m on concrete. Calves entered the study at 8 wk of age, stayed on the study for 6 wk, and were euthanized at 14 wk of age. Blood samples were taken weekly on all calves to analyze markers of bone formation and resorption. Computed tomography scans were performed on the MC III & IV to determine cross-sectional morphology and BMD. The 5 d/wk sprinting of the EX group resulted in rounder bone and greater dorsal cortex thickness than the CF or GR groups ($P < 0.01$). Dorsal, palmar, and total BMD in EX were greater than GR ($P < 0.05$). Serum osteocalcin concentrations normalized from d 0 were greater in EX compared to CF ($P < 0.05$). Fracture force of EX calves tended to be higher than CF calves ($P < 0.10$). Results from this study support that the 5 d/wk exercise protocol for calves in treatment EX increased bone formation and altered bone shape when compared to treatments CF and GR.

The second study utilized 18 Quarter Horse weanlings. At 4 months of age, the animals were weaned and placed in box stalls for a 5-wk adjustment period. At the end of the adjustment period, weanlings were randomly assigned to treatment groups: GR, CF, and EX. Horses assigned to GR were maintained in a 992 m^2 dry lot, affording free access to exercise. The

horses on the CF group remained in 3.7 m x 3.7 m stalls for the duration of the study. Animals assigned to EX stayed in the same sized stalls as CF except during their 82 m/d, 5 d/wk sprints. These animals stayed on their treatments for 56 days. Unlike the calves, the weanlings were not euthanized at the end of the study. On d 0, 28, and 56, radiographs of the MC III were taken. The dorsopalmar medullary cavity was smaller in EX compared to GR ($P=0.027$). Dorsal and medial cortical width increased in the EX animals ($P<0.01$). The smaller dorsopalmar medullary cavity was due to the increases in cortical widths. Parallel to the results in the bovine study, the EX treatment increased cortical widths suggesting that short-duration exercise improves skeletal strength in horses. The results from these two studies support that short-duration sprint exercise positively impacts bone strength and morphology, as well as justifies that calves are an acceptable skeletal model for young horses.

OBJECTIVE AND HYPOTHESIS

The purpose of this study was to extend the studies performed by Hiney et al. (2004a,b) and further quantify weekly exercise needed for skeletal strength. Study objective and hypothesis are as follows:

1. Objective: determine if sprinting 1 d/wk or 3 d/wk provides the same benefits to bone mineral content, density, and strength as does sprinting 5 d/wk compared to animals not exercised.
2. Hypothesis: sprinting 1 and 3 d/wk will have the same beneficial impacts to bone health of juvenile animals as sprinting 5 d/wk, when compared to confined animals. Sprinting will have an effect on Osteocalcin and the fracture force of exercised animals will be greater than controls.

LITERATURE CITED

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Allen, M. R. and D. B. Burr. 2014. Bone modeling and remodeling. In: D. B. Burr and M. R. Allen, editors, Basic and Applied Bone Biology. Academic Press, San Diego, CA. p.75–90.

American Horse Council Foundation. 2018. Economic Impact of the U.S. Horse Industry Michigan Economic Impact Study.

American Quarter Horse Association. 2010. Annual Report. https://www.aqha.com/media/8793/2010_annualreport_web1.pdf (Accessed November 11 2018).

American Quarter Horse Association. 2017. Annual Report. https://www.aqha.com/media/24096/2340-18-64-2017-annual-report.pdf (Accessed November 11 2018).

Beisser, A. L., S. McClure, C. Wang, K. Soring, R. Garrison, and B. Peckham. 2011. Evaluation of catastrophic musculoskeletal injuries in Thoroughbreds and Quarter Horses at three Midwestern racetracks. J. Am. Vet. Med. Assoc. 239 (9): 1236-1241. doi:10.2460/javma.239.9.1236.

Carter, D. R. 1984. Mechanical loading histories and cortical bone remodeling. Calcified Tissue Int. 36:19–S24. doi:10.1007/BF02406129.

Ferry, B., M. Duclos, L. Burt, P. Therre, F. Le Gall, C. Jaffré, and D. Courteix. 2011. Bone geometry and strength adaptations to physical constraints inherent in different sports: comparison between elite female soccer players and swimmers. J. Bone Miner. Metab. 29 (3): 342-351. doi:10.1007/s00774-010-0226-8.

Firth, E. C. 2006. The response of bone, articular cartilage and tendon to exercise in the horse. J. Anat. 208: 513–526. doi:10.1111/j.1469-7580.2006.00547.x.

Florencio-Silva, R., G. R. D. S. Sasso, E. Sasso-Cerri, M. J. Simões, and P. S. Cerri. 2015. Biology of bone tissue: structure, function, and factors that influence bone cells. Biomed. Res. Int. 2015: 1-17. doi:10.1155/2015/421746.

Franz-Odendaal, T. A., B. K. Hall, and P. E. Witten. 2006. Buried alive: How osteoblasts become osteocytes. Dev. Dyn. 235:176–190. doi:10.1002/dvdy.20603.

Graham-Thiers, P. M. and K. Bowen. Improved ability to maintain fitness in horses during large pasture turnout. 2012. J. Equine Vet. Sci. 33 (2013): 581-585. doi:10.1016/J.JEVS.2012.09001.

Goodship, A. E. and R. K. W. Smith. 2008. Skeletal physiology: responses to exercise and training. In: K. Hinchcliff, R. Goer, A. Kaneps, editor, Equine exercise physiology. Saunders/Elsevier, Edinburg, NY. p. 81-105.

Guadalupe-Grau, A., T. Fuentes, B. Guerra, and J. A. L. Calbet. 2009. Exercise and bone mass in adults. Sports Med. 39: 439–468. doi:10.2165/00007256-200939060-00002.

Hadjidakis, D. J. and I. I. Androulakis. 2006. Bone Remodeling. Ann. N. Y. Acad. Sci. 1092: 385–396. doi:10.1196/annals.1365.035.

Hinchcliff, K. W. and R. J. Geor. The horse as an athlete: a physiological overview. In: K. Hinchcliff, R. and Goer, A. Kaneps, editor, Equine Exercise Physiology. Saunders/Elsevier, Edinburg, NY. p. 81-105.

Hiney, K. M., B. D. Nielsen, D. Rosenstein, M. W. Orth, and B. P. Marks. 2004a. High-intensity exercise of short duration alters bovine bone density and shape. J. Anim. Sci. 82: 1612–1620. doi:/2004.8261612x.

Hiney, K. M., B. D. Nielsen, and D. Rosenstein. 2004b. Short-duration exercise and confinement alters bone mineral content and shape in weanling horses. J. Anim. Sci. 82: 2313–2320. doi:10.2527/2004.8282313x.

Hitchens, P.L., A.E. Hill, and S. M. Stover. 2016. The role of catastrophic injury or sudden death of the horse in race-day jockey falls and injuries in California, 2007-2012. Equine Vet. J. 48: 50–56. doi:10.1111/evj.12392.

Hitchens, P.L., A.V. Morrice-West, M. A. Stevenson, and R. C. Whitton. 2019. Meta-analysis of risk factors for racehorse catastrophic musculoskeletal injury in flat racing. Vet. J. 245: 29-40. doi: 10.1016/J.TVJL.2018.11.014.

Hoekstra, K. E., B. D. Nielsen, M. W. Orth, D. S. Rosenstein, H. C. Schott, and J. E. Shelle. 1999. Comparison of bone mineral content and biochemical markers of bone metabolism in stall- vs. pasture-reared horses. Equine Vet. J. 30: 601–604. doi:10.1111/j.2042-3306.1999.tb05292.x.

Judex, S., W. C. Whiting, and R. F. Zernicke. 1999. Exercise-induced bone adaptation: Considerations for designing an osteogenically effective exercise program. Int. J. Ind. Ergon. 24: 235–238. doi:10.1016/S0169-8141(98)00056-0.

Lau, R. Y. and X. Guo. 2011. A review on current osteoporosis research: with special focus on disuse bone loss. J. Osteoporos. 2011: 1–6. doi:10.4061/2011/293808.

Lanyon, L. E. and C. T. Rubin. 1984. Static vs dynamic loads as an influence on bone remodeling. J. Biomech. 17: 897-905. doi:10.1016/0021-9290(84)90003-4.

Leahy, P. D., B. S. Smith, K. L. Easton, C. E. Kawcak, J. C. Eickoff, S. S. Shetye, and C. M. Puttlitz. 2010. Correlation of mechanical properties within the equine third metacarpal with trabecular bending and multi-density micro-computed tomography data. Bone. 46 (2010): 1108-1113. doi:10.1016/j.bone.2010.01.366.

Lee., A. J., S. Hodges, and R. Eastell. Measurement of osteocalcin. 2000. Ann. Clin Biochem. 37: 432-446. doi:10.1177/000456320003700402.

Matsuo, A., A. Togashi, K. Sasaki, B. Devkota, T. Hirata, and N. Yamagishi. 2014. Diurnal variation of plasma bone markers in Japanese black calves. J. Vet. Med. Sci. 76 (7): 1029-1032. doi:10.1292/jvms.14-0021.

Mohammed, H. O., T. Hill, and J. Lowe. 1991. Risk factors associated with injuries in Thoroughbred horses. Equine Vet. J. 23: 445–448. doi:10.1111/j.20423306.1991.tb03758.x.

Mohr, M., E. W. Helge, L. F. Peterson, A. Lindekskov, P. Weihe, J. Mortensen, N. R. Jørgensen, and P. Krustrup. 2015. Effects of soccer vs swim training on bone formation in sedentary middle-aged women. Eur. J. Appl. Physiol. 115 (12): 2671-2679. doi:10.1007/soo421-015-3231-8.

Nielsen, B.D., G.D. Potter, E.L. Morris, T.W. Odom, D.M. Senor, J.A. Reynolds, W.B. Smith, and M.T. Martin. 1997. Changes in the third metacarpal bone and frequency of bone injuries in young quarter horses during race training - Observations and theoretical considerations. J. Equine Vet. Sci. 17: 541–549. doi:10.1016/S0737-0806(97)80227-4.

Ramzan, P.H.L. and L. Palmer. 2011. Musculoskeletal injuries in Thoroughbred racehorses: A study of three large training yards in Newmarket, UK (2005-2007). Vet. J. 187: 325–329. doi:10.1016/j.tvjl.2009.12.019.

Rawson, M. 2011. A horse is a horse, of course, but also much more: recovering the animal contribution to the urbanization and industrialization of America. J. Urban Hist. 37(4): 614-618. doi:10.1177/0096144211403094.

Robling, A. G., R. K. Fuchs and D. B. Burr. 2014. Bone modeling and remodeling. In: D. B. Burr and M. R. Allen, editors, Basic and Applied Bone Biology. Academic Press, San Diego, CA. p.175-204.

Rubin, C. T. and L. E. Lanyon. 1984. Regulation of bone formation by applied dynamic loads. J Bone Joint Surg. Am. 66: 397-402.

Rogers, C.W., C. F. Bolwell, J. C. Tanner, and P. R. van Weeren. 2012. Early exercise in the horse. J. Vet. Behav. 7: 375–379. doi:10.1016/j.jveb.2012.01.003.

Ross, M. W., and S. J. Dyson. 2011. Diagnosis and management of lameness in the horse. 2nd ed. Elsevier/Saunders, St. Louis, MO.

Scott, M. 2008. Musculoskeletal Injuries in Nonracing Quarter Horses. Vet. Clin. North Am. Equine Pract. 24: 133–152. doi:10.1016/j.cveq.2007.11.006.

Skerry, T. M. 2008. The response of bone to mechanical loading and disuse: Fundamental principles and influences on osteoblast/osteocyte homeostasis. Arch. Biochem. Biophys. 473: 117–123. doi:10.1016/j.abb.2008.02.028.

Sloet van Oldruitenborgh-Oosterbaan, M. M., W. Genzel, and P. R. Van Weeren. 2010. A pilot study on factors influencing the career of Dutch sport horses. Equine Vet. J. 42 (s38): 28-32. doi: 10.1111/j.2042-3306.2010.00251.x

Smith, R. K. W. and A. E. Goodship. 2008. The effect of early training and the adaptation and conditioning of skeletal tissues. Vet. Clin. North Am. Equine Pract. 24: 37–51. doi:10.1016/j.cveq.2007.11.005.

Snow, C. M., D. P. Williams, J. LaRiviere, R. K. Fuchs, and T. L. Robinson. 2001. Bone gains and losses follow seasonal training and detraining in gymnasts. Calcified Tissue Int. 69: 7–12. doi:10.1007/s00223-001-0014-5.

Stover, S. M. 2003. The epidemiology of thoroughbred racehorse injuries. Clin. Tech. Equine Pract. 2: 312–322. doi:10.1053/j.ctep.2004.04.003.

Sun, T. C., C. M. Riggs, N. Cogger, J. Wright, and J. I. Al-Alawneh. 2018. Noncatastrophic and catastrophic fractures in racing Thoroughbreds at the Hong Kong Jockey Club. Equine Vet. J. 51(1): 77-82. doi:10.1111/evj.12950.

Tanner, J. C., C. W. Rogers, and E. C. Firth. 2012. The association of 2-year-old training milestones with career length and racing success in a sample of Thoroughbred horses in New Zealand. Equine Vet. J. 45 (1): 20-24. doi:10.1111/j.2042-3306.2011.00534.x.

The Jockey Club. 2019. Size of field and starts per horse (Fact Book Index). http://jockeyclub.com/default.asp?section=FB&area=10 (Accessed March 7 2019)

The Jockey Club. 2019. Gross purses (Fact Book Index). http://www.jockeyclub.com/default.asp?section=FB&area=7 (Accessed March 7 2019).

Tull T. M., and L. R. Bramlage. 2011. Racing prognosis after cumulative stress-induced injury of the distal portion of the third metacarpal and third metatarsal bone in Thoroughbred racehorses: 55 cases (2000-2009). J. Am. Vet. Med. Assoc. 238 (10): 1316-1322. doi:10.2460/javma.238.10.1316.

van Harreveld, P. D., J. D. Lillich, C. E. Kawcak, A. S. Turner, and R. W. Norrdin. 2002. Effects of immobilization followed by remobilization on mineral density, histomorphometric features, and formation of the bones of the metacarpophalangeal joint in horses. Am. J. Vet. Res. 63: 276–281. doi:10.2460/ajvr.2002.63.282.

Velie, B. B., C. M. Wade, and N. A. Hamilton. 2012. Profilin careers of Thoroughbred horses racing in Australia between 2000 and 2010. Equine Vet. J. 45(2): 182-186. doi: 10.1111/j.2042-3306.2012.00614.x.

Warden, S. J., R. K. Fuchs, A. B. Castillo, I. R. Nelson, and C. H. Turner. 2007. Exercise when young provides lifelong benefits to bone structure and strength. J. Bone Miner. Res. 22: 251–259. doi:10.1359/jbmr.061107.

Woo, S. L. Y., S. C. Kuei, D. Amiel, M. A. Gomez, W. C. Hayes, F. C. White, and W. H. Akeson. 1981. The effect of prolonged physical training on the properties of long bone: A study of Wolff's law. J. Bone Joint Surg. 63-A:780-787.

CHAPTER TWO: Short sprints lead to greater dorsal cortical width and fracture force of the fused third and fourth metacarpal of juvenile animals

INTRODUCTION

Horse racing has experienced increases to starters and purses in Quarter Horse and Thoroughbred racing over the past few decades, despite a reduction in the United States horse population (American Quarter Horse Association, 2010; American Quarter Horse Association 2017; American Horse Council Foundation, 2018; The Jockey Club, 2019a,b). The rise of interest and participation in racing comes with inherent risks due to the fast-paced, high-intensity nature of the sport and resulting prevalence of injuries. Injuries to racehorses can lead to career-ending retirement, death of horses, injury to or death of jockeys, adverse perceptions of horse racing by the public, and detrimental effects to the horse industry's economics (Stover, 2003).

Catastrophic musculoskeletal injuries occur at a rate of 1.33 injuries per 1,000 race starts during Quarter Horse races (Beisser et al., 2014) and fatal injuries occur at a rate of 1.61 per 1,000 starts in Thoroughbred races (The Jockey Club, 2018). Beyond affecting horses while racing, musculoskeletal injuries are the main cause of missed training days and wastage of racing horses and horses of all other disciplines (Rogers, 2012). One such musculoskeletal injury is dorsal metacarpal disease, which is commonly seen in 2-year-old racing Quarter Horses as well as racing Thoroughbreds. Dorsal metacarpal disease is characterized by stress fractures in the dorsal region of the metacarpal, caused by the lag time between bone formation during remodeling, as rebuilding of bone happens much slower than the resorption of bone. Young animals are often afflicted with dorsal metacarpal disease likely because they have not been accustomed to the strains of racing as they have been removed from pasture, kept in stalls, and not afforded voluntary exercise with strides at speed (Ross and Dyson, 2011). Past research has

depicted that bone mineral loss in the third metacarpal occurs as a result of horses being removed from pasture and kept in stalls at the onset of race training and that the loss of bone mineral content leads to increased incidence of injuries as training progresses (Nielsen et al., 1997). Bone responds to strains placed on it as, sprints between 50 and 82-m performed 5 d/wk for 6 wk increased bone mineral content in Quarter Horse weanlings and bone mineral density (BMD) in Holstein bull calves. Bone morphology was also positively impacted in sprinted animals compared to confined counterparts (Hiney et al., 2004a,b). Simultaneously, Hiney et al. also determined that calves were an applicable skeletal model for juvenile horses (2004a,b). Furthermore, low impact exercise, such as that from endurance training over long distances, does not alter bone density compared to young horses kept on pasture with no additional exercise (Spooner et al., 2008). The intensity of exercise is important to consider when evaluating the effects of exercise on bone health. The knowledge that 5 d/wk of short-duration high-intensity exercise increases bone health in young animals leads to the inquiry of how many days of sprint exercise are truly needed to experience benefits to bone health which counteract bone loss from stalled confinement.

In this current study, Holstein bull calves were sprinted 0, 1, 3, or 5 d/wk to evaluate the effects to bone morphology, BMD, and bone strength as well as biomarkers of bone formation and resorption. Calves were used as a model for juvenile horses allowing for post-mortem mechanical testing of bone strength. It was hypothesized that sprinting 1 or 3 d/wk would have the same beneficial impacts to bone health of juvenile animals as sprinting 5 d/wk, when compared to confined animals.

MATERIALS AND METHODS

Animals and management

This project was approved by the Michigan State University Institutional Animal Care and Use Committee (Protocol 10/16-183-00). Twenty-four Holstein bull calves were obtained from the Michigan State University Dairy Cattle Teaching and Research Center over a span of 6 months. Calves were age-matched before entering the study, to have a span of no more than 10 d between the oldest and youngest calf in each group. Groups contained between 3 and 6 calves (Table 1). At 7 and 8 wk of age, all calves underwent two halter training sessions in which they were walked 4 laps on a 10 m long concrete barn aisle. Calves were weaned from milk replacer at 8 wks of age, after which their diet consisted of 3.6 kg of a commercially available pelletized calf starter daily (Ampli-Calf STR 20P R50 DBZ9.1 Medicated, Purina Animal Nutrition LLC) and ad libitum access to water. This pelletized calf starter meets the National Research Council's requirement of Ca and P for young calves (NRC, 2001). At d 0, each calf was presented with 3.6 kg of calf starter. Fresh calf starter was given every 24 hr. If a calf left less than 0.5 kg of calf starter uneaten, then they were presented with 4.1 kg of calf starter. Each time a calf left less than 0.5 kg of calf starter uneaten, their presented amount of calf starter was increased by 0.5 kg.

Study design

Each group of calves entered the study when their average age was 9 wks, at which time the group was transported to the Michigan State University Beef Cattle Teaching and Research Center where they were housed for the duration of the 42-d study. Individuals from each group were randomly assigned to one of 4 treatments, leading to 6 calves per treatment (Table 1). Treatments were based on how many days of sprinting an animal would perform each week: 0, 1,

3, or 5 d/wk. Calves sprinted 0 d/wk served as a control group and spent the duration of the 6-wk study confined.

Table 1: Treatment assignment of calves based on age-matched groups

Group	Treatment 0 d/wk	Treatment 1 d/wk	Treatment 3 d/wk	Treatment 5 d/wk	Total calves
1	2	1	1	1	5
2	1	1	1	1	4
3	0	1	1	1	3
4	1	2	2	1	6
5	1	1	0	1	3
6	1	0	1	1	3
Total calves	6	6	6	6	24

For a sprinting session, animals were individually walked out of their stalls, down a 71-m concrete aisle away from their stalls, released, then verbally encouraged to sprint the 71-m back down the aisle. At the end of the aisle, the calf was collected and walked back to their stall. Regardless of treatment, all animals were housed in stalls that were 93 cm in width and 175 cm in length. The size of these stalls ensured that calves could stand up, lie down, and turn around with ease, but could not get any extraneous exercise beyond controlled sprints determined by treatment assignment. Height, weight, and length of each calf were taken on d -1 and d 41. Additionally, hoof angles of the calves were measured by an equine farrier on d -1 and d 41. On d 42, calves were transported from the MSU Beef Cattle Teaching and Research Center to the MSU Meat Lab, where the animals were humanely euthanized by a captive bolt pistol, with a United States Department of Agriculture inspector present.

Sprint videos

On d 7 and continuing weekly until d 42, calves were videotaped while sprinting. Calves were not videotaped while sprinting until d 7 to permit calves an acclimation period to the exercise treatments. The video camera was positioned so that calves were running towards it during their entire sprint. The entire 71-m sprint was videotaped, but only the middle 21 m of the sprint was analyzed to calculate stride frequency and sprint velocity. Calves were recorded in the middle 21 m of their 71-m sprint to avoid confounding sprint analysis with acceleration and deceleration of calves. The middle 21-m of the 71-m barn aisle was marked with bright tape on a wall along the aisle so that the middle 21-m portion was identical and visible for all sprints. Stride frequency was calculated by counting from the recorded video the amount of impacts the left front limb made during the middle 21-m portion of the sprint. Sprint velocity was determined from the recorded video by calculating the time it took a calf to complete the 21-m middle portion of the sprint and dividing 21 m by that span of time. Videos needed to meet the criteria that all impacts of the left front limb during a calf's sprint could be counted during the middle 21 m. Videos that did not meet this criterion due to video quality, field of view, or calf behavior were excluded from the dataset. Four calf sprints were removed from the dataset due to not meeting the video quality criterion. Two of the calves that were removed sprinted 1 d/wk, 1 calf sprinted 3 d/wk, and 1 calf sprinted 5 d/wk.

Sample collection

Starting on d 0 and continuing weekly until d 42, blood was collected via jugular venipuncture into non-heparinized vacutainers between 0730-0830 h. Blood was allowed to coagulate for one hour, then centrifuged at 1,000 x g for 15 min to attain serum separation. Serum samples were pipetted into microcentrifuge tubes in quadruplicate, then frozen at -20° C

for later analysis. Serum was analyzed for concentrations of osteocalcin (OC) and C-telopeptide crosslaps of type I collagen (CTX-1) - respective markers of bone formation and degradation.

After euthanasia on d 42, both front limbs from each calf were removed above the carpal bones. The left limb was immediately placed into a -20° C freezer for later computed tomography (CT) analysis and mechanical testing. From the right limb, synovial fluid samples were collected from the middle carpal joint for analysis of collagen type I and II cleavage (C1, 2C) - a biomarker of collagen degradation. Synovial fluid samples were snap frozen in liquid nitrogen and dry ice, then kept in a -80° C freezer until further analysis. Articular surfaces of carpal joints of the right limb were observed for lesions.

Sample analysis

Calf serum samples were analyzed for OC utilizing the commercially available MicroVue Osteocalcin EIA (Quidel, San Diego, CA). Serum OC reflects osteoblastic activity and is a marker of bone formation (Lee et al., 2000; Hiney et al., 2004a,b). Serum samples utilized for osteocalcin analysis were diluted with deionized water at a 1:15 ratio. Serum samples were analyzed for CTX-1 concentration using the commercially available Serum CrossLaps (CTX-1) ELISA (Immunodiagnostics Systems Gaithersburg, MD). Concentration of CTX-1 in serum is a marker of bone resorption used in both equine and bovine research (Donabedian et al., 2008; Matsuo et al., 2014). Serum samples utilized for CTX-1 analysis were run neat. Synovial fluid samples were analyzed for C1, 2C using a commercial ELISA kit obtained from IBEX (Quebec, Canada). To reduce the viscosity of the sample, synovial fluid was digested with 50 units/mL of hyaluronidase from bovine testes at 37° C for 40 min. This method of hyaluronidase digestion was similar to that performed by O'Connor-Robison et al. (2014). Synovial fluid samples were

diluted at a ratio of 1:1.1 as a result of hyaluronidase digestion. All assays were performed according to the instructions provided by the manufacturer.

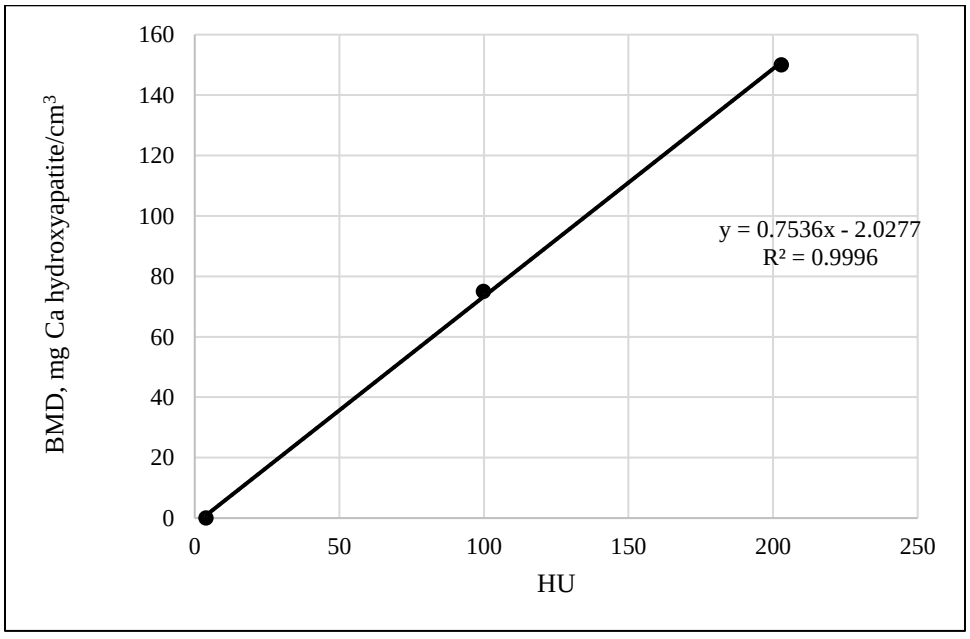
Computed tomography

The left limb was kept in a -20° C freezer until CT scans were performed. Before CT scans were performed, limbs were removed from the freezer and placed in a chiller to thaw for 2 to 3 days at 4.8° C. Scans were performed by a technician at the Michigan State University College of Veterinary Medicine. Computed tomography scans were performed with the following settings: 120 kV, 320 mAmp, 0.625 mm slice thickness, 2,000 slices per scan, and lumbar spine position using a GE Revolution Evo scanner (General Electric Healthcare, Princeton, NJ). All limbs were positioned perpendicular to the gantry as straight as possible. Solid calcium hydroxyapatite phantoms (Image Analysis, Inc; Columbia, KY) with rows representing 0, 75, and 150 mg/cm^3 Ca were scanned along with limbs for BMD comparison. Analysis of CT scans was completed using Mimics 21.0 (Materialise, Leuven, Belgium). For each calf, a midpoint of the left fused third and fourth metacarpal (MC III & IV) was calculated for cross sectional measurements. The distal and proximal ends of the fused MC III & IV were found, and the average slice between the two ends was calculated and denoted as the midpoint of the whole-bone (MID). Internal and external diameters of cortical bone, as well as the dorsal, palmar, lateral, and medial cortical widths (CW), were measured at MID for the left fused MC III & IV for each calf. Internal diameters represented the diameter of the medullary cavity. Diameters and CW were calculated with a mask at threshold of 400 Hounsfield Units (HU), this mask permitted that only bone, and not soft tissue was included in the reported value. Using an angle measurement tool in Mimics, perpendicular dorsopalmar and mediolateral lines were

drawn to measure the internal and external diameters, as well as to identify the locations to measure CW.

Bone mineral density was measured in a cross-sectional view at MID. Values for average BMD were collected from the dorsal, lateral, medial, and palmar cortices and whole slice at MID. A mask with a threshold value of 400 HU was used to collect BMD. Squares measuring 10 mm^2 were used to determine the average BMD in the 4 cortices. For whole slice BMD, the entire slice was highlighted with the mask, and BMD of the entire cortical bone at MID was recorded. Values collected from CT scans for average BMD were reported in HU. Average HU were recorded at each of the three concentrations of calcium along the length of the phantom at 10 locations for each individual scan. The HU values were then averaged for each scan and compared in a scatter plot to the known concentrations of the phantom (Fig. 1). The equation produced from the regression line converted density in HU to mg Ca hydroxyapatite/cm^3. This method of determining BMD is supported by Robison and Karcher (in press).

Figure 1: Graph used to calculate bone mineral density (BMD) of values obtained from computed tomography (CT) scans analyzed with Mimics

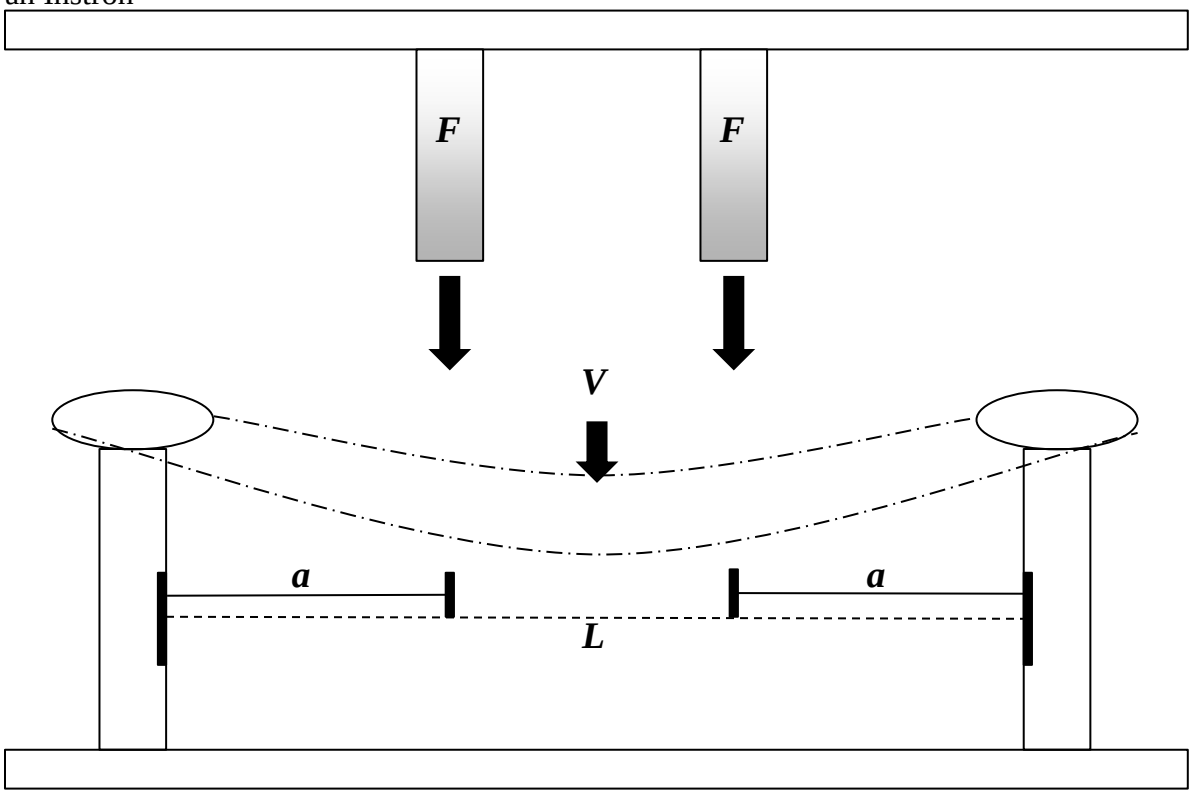


Cross-sectional and cortical areas were determined with a mask at threshold of 350 HU. This mask threshold was selected so that medullary contents were included in the cross-sectional area. Cross-sectional area was obtained by using a tool which reports the area of an entire ellipse, including the medullary cavity. Cortical area was calculated by determining the area of the inner medullary cavity and subtracting that from the cross-sectional area, to yield only the area of cortical bone selected at MID. After CT scanning, limbs were placed back into the -20° C freezer until mechanical testing was performed.

Mechanical testing and calculations

Before mechanical testing, the left limbs were removed from the -20° C freezer and thawed for 4 days at 4.8° C. Once thawed, the limbs were skinned and remaining tissues were removed from the fused MC III & IV. The bones were wrapped in paper towel, covered in phosphate buffer saline, and kept in an upright refrigerator in individual plastic bags overnight until mechanical testing. Mechanical testing was performed via four-point bending on an Instron machine (Model number: 4202, Serial number: 537) at room temperature. Fused MC III & IV were placed with the palmar aspect of the bone facing upwards toward the force applicators, and the dorsal portion facing the bottom supports. For all bones tested, loading speed was 10 mm/min, span (L) between the bottom supports was 100 mm, and the load cell used was 10kN (Fig. 2, adapted from Pruyn et al., 2000).

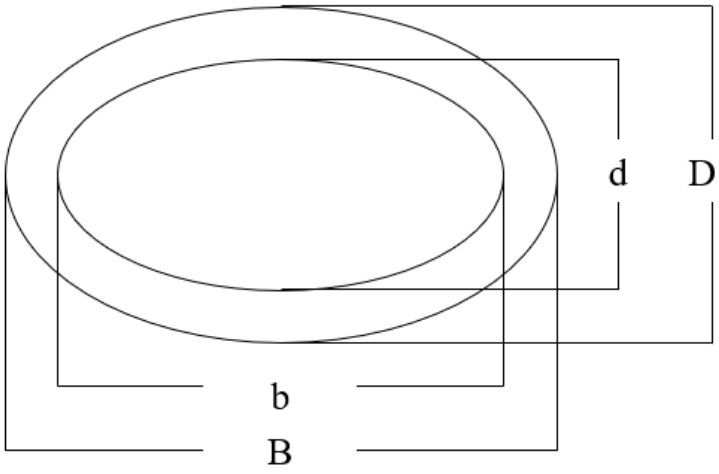
Figure 2: Diagram of a bovine fused third & fourth metacarpal subjected to 4-point bending on an Instron



Moment of inertia (I, mm^4) was determined with the calculation for a hollow ellipse described in ASABE standards (2017, Fig. 3, adapted from Hiney et al., 2004a):

$$I = 0.049[(B * D^3) - (b * d^3)]$$

Figure 3: Cross-section of bovine fused third and fourth metacarpal illustration *Cortical diameters included: B = exterior lateromedial diameter, b = interior lateromedial diameter, D = exterior dorsopalmar diameter, d = interior dorsopalmar diameter*



Flexural rigidity (EI, N mm^2) and Young's modulus of elasticity (E, N/mm^2) were determined based on calculations appropriate for four-point bending with an Instron:

$$EI=(F/V)(a^2/12)(3L-4a)$$

$$E=EI/I$$

While calculating EI, the slope of the force deformation curve (*F/V*) was calculated from 4 to 5.5 mm of deformation. For all calves, 4 to 5.5 mm of deformation was part of the linear portion of the curve, with an R^2 of 0.99. The value of *a* was calculated individually for all calves. At the time of bone breaking, the distance between the two downward force applicators was measured for each calf. This value was subtracted from L and then divided by 2 to determine *a*. On average, *a* had a value of 38.6 ± 0.4 mm.

Statistical Analysis

Results are reported as means ± SEM. All reported data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Bone marker and video data were evaluated with a model containing fixed effects of treatment and wk, interaction of treatment and wk, as well as repeated measures of wk with calf as subject. Calf size parameters, concentration of C1,2C, CT data, fracture force, and data obtained from mechanical property calculations were analyzed with a model containing only the fixed effect of treatment. All data, except for stride frequency and C1,2C concentration, were deemed to be normally distributed. Abnormally distributed data were transformed as 1/y and subsequently followed a normal distribution after transformation. This transformation was selected as a box-cox 95% confidence interval performed with the TRANSREG procedure included a lambda of -1, which yields an inverse transformation of 1/y. Significance was set at $P \leq 0.05$ and trends were observed at $P \leq 0.10$.

RESULTS

The average age of calves when entering the project was not different between treatments (Table 2). Calves started and finished the project with initial and final heights, weights, and lengths that were not different between exercise treatments. Average daily gain was not different between treatments either (Table 2). There were no differences in initial and final hoof angles by treatment (Table 3).

Table 2: Calf size parameters measured at the beginning and end of the study

Treatment	Calf age at d 0	Initial height, cm	Final height, cm	Initial weight, kg	Final weight, kg	Initial length, cm	Final length, cm	ADG, kg/d
0	62 ± 1	90 ± 1	100 ± 1	89 ± 3	115 ± 7	67 ± 1	75 ± 2	0.6 ± 0.2
1	62 ± 1	92 ± 1	101 ± 1	85 ± 3	117 ± 7	67 ± 1	75 ± 2	0.8 ± 0.2
3	63 ± 1	93 ± 1	101 ± 1	85 ± 3	119 ± 7	68 ± 1	74 ± 2	0.8 ± 0.2
5	63 ± 1	91 ± 1	101 ± 1	79 ± 3	116 ± 7	65 ± 1	75 ± 2	0.9 ± 0.2
P Values	0.99	0.49	0.90	0.16	0.97	0.49	0.99	0.60

Table 3: Initial and final mean calf hoof angles by treatment

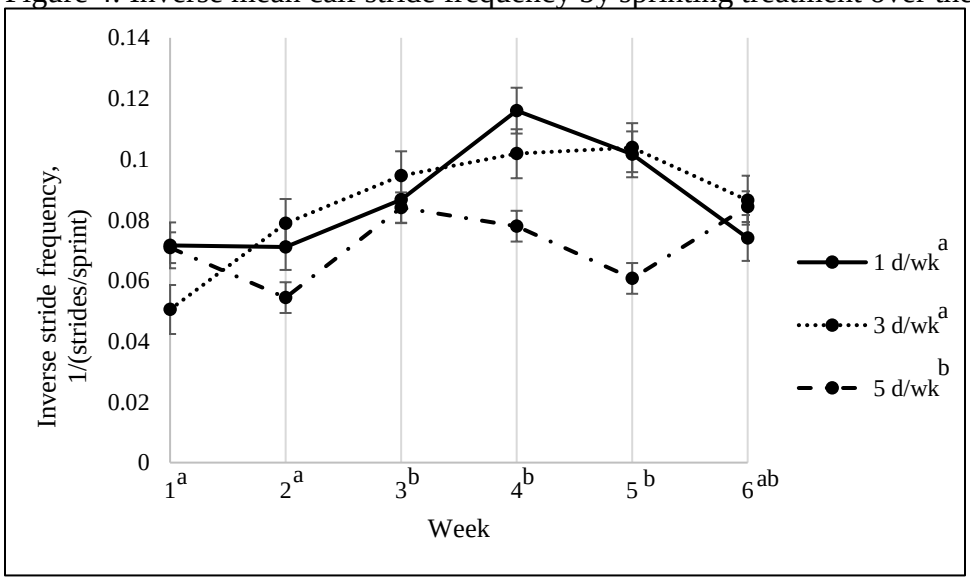
Treatment	Initial hoof angle	Final hoof angle
0 d/wk	54 ± 1	56 ± 1
1 d/wk	55 ± 1	55 ± 1
3 d/wk	53 ± 1	56 ± 1
5 d/wk	54 ± 1	54 ± 1
P Values	0.56	0.63

Video data

There was no treatment*week interaction in terms of stride frequency (Figure 4). There was a difference between treatments ($P < 0.05$), with calves sprinting 5 d/wk lower inverse stride frequency, indicative of higher stride frequency. There was a difference between weeks ($P < 0.05$), with weeks 1 and 2 having lower inverse stride frequency, indicative of higher stride frequency. There was no treatment*week interaction in terms of mean sprinting velocities

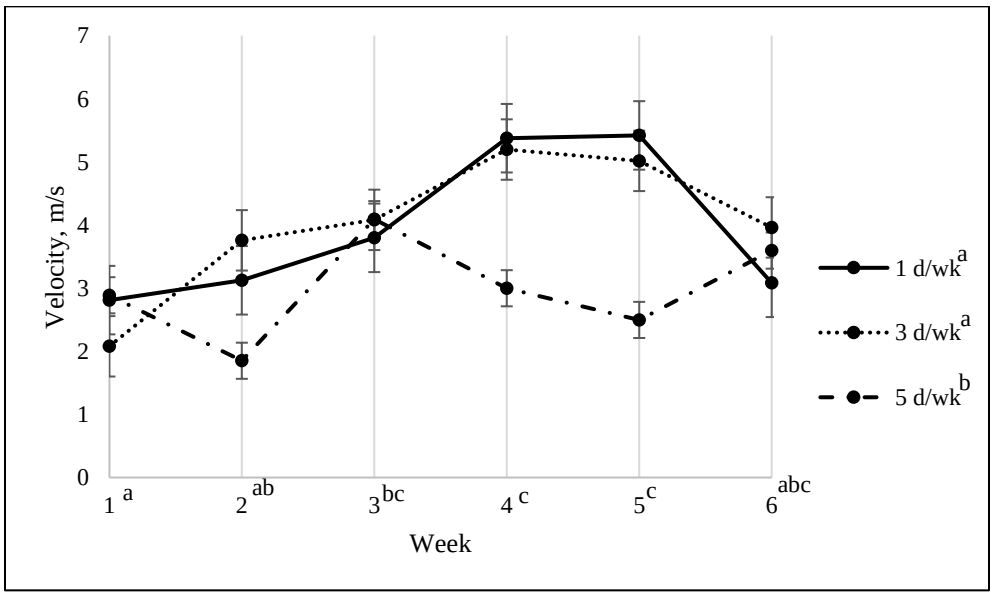
(Figure 5). However, there was a difference between treatments ($P < 0.05$), with calves sprinting 5 d/wk having the lowest mean velocity. There was also a difference between weeks ($P < 0.05$), with week 1 having lower velocity than weeks 3, 4, and 5, and week 2 having lower velocity than weeks 4 and 5.

Figure 4: Inverse mean calf stride frequency by sprinting treatment over the 6-wk study period



[a,b]Treatments lacking common superscripts differ ($P < 0.05$), weeks lacking common superscripts differ ($P < 0.002$)

Figure 5: Mean calf sprinting velocity by treatment over the 6-wk study period

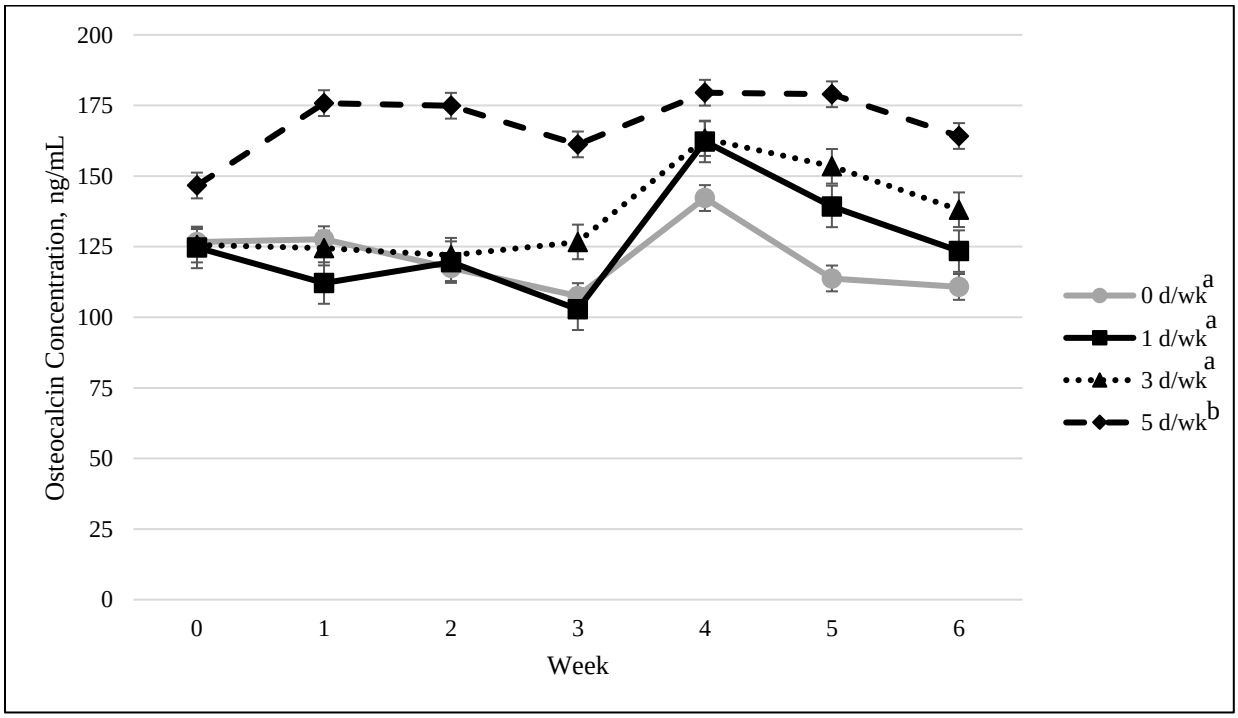


[a,b]Treatments lacking common superscripts differ (P < 0.01)
[a-c]Weeks lacking common superscripts differ (P < 0.01)

Bone and biological markers

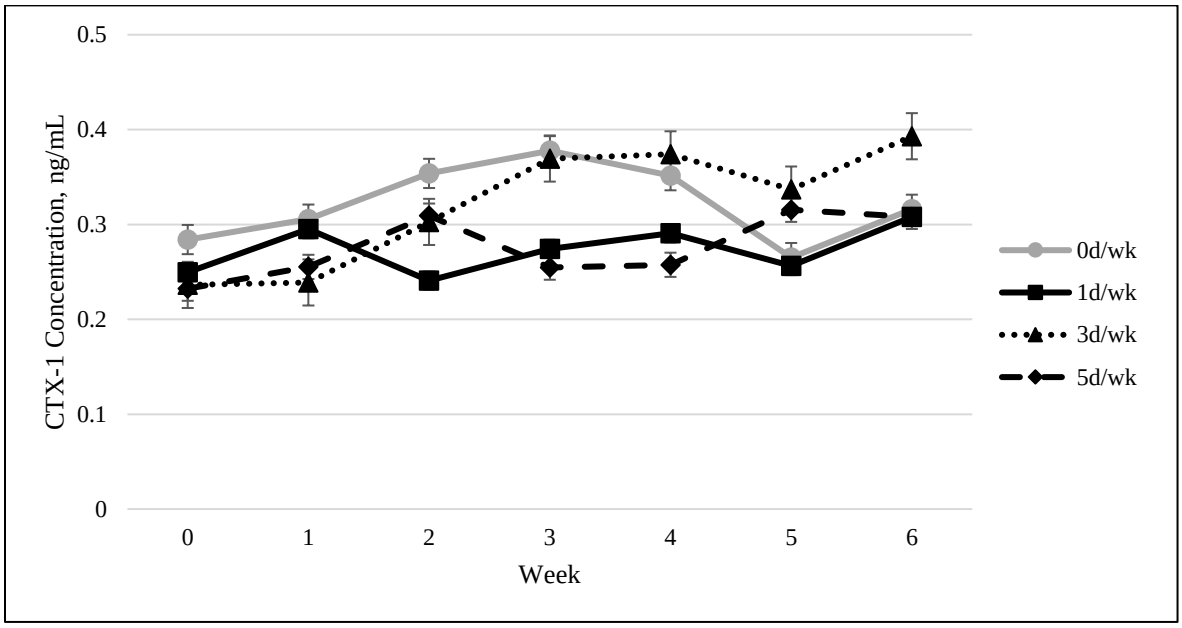
There was a difference between treatments in mean OC concentrations (P < 0.001, Fig. 6). The 5 d/wk exercise group had the greatest mean OC concentration compared to all other treatments (P < 0.05). There were no differences between weeks or interactions between treatment*week. For CTX-1, there were no differences between treatments or weeks, nor was there an interaction between treatment*week (Fig. 7).

Figure 6: Mean calf serum osteocalcin concentration by treatment throughout the 6-wk study period



[a,b]Treatments lacking common superscripts in legend differ (P < 0.001)

Figure 7: Mean C-terminal telopeptides of type 1 collagen (CTX-1) concentration in calf serum by treatment throughout the 6-wk study period



There were no treatment differences in inverse C1,2C concentrations (P = 0.73; Table 4). There were no observed articular surface lesions on the carpal bones of any calves involved in the study.

Table 4: Inverse mean concentration of collagen type I and II cleavage (C1,2C) in calf synovial fluid in the middle carpal joint on d 42, separated by treatment

Treatment	Inverse C1,2C concentration, 1/(μg/mL)
0 d/wk	4.39
1 d/wk	4.67
3 d/wk	4.05
5 d/wk	5.00
SEM	0.61
P Value	0.73

Computed tomography and mechanical testing

Calves sprinted 1 d/wk tended to have increased internal ML diameter at MID (Table 5; P < 0.10). Dorsal CW at MID was greater for all exercise treatments compared to non-exercised calves (Table 6; P < 0.01). There were no differences in cross sectional areas or cortical areas at MID between treatments (Table 7). Likewise, there were no differences between treatments in cortical bone densities or whole-slice bone densities at MID (Table 8).

Table 5: Internal (Int) and external (Ext) dorsopalmar (DP) and mediolateral (ML) diameters at MID

Treatment	MID ML Int, mm	MID ML Ext, mm	MID DP Int, mm	MID DP Ext, mm
0	17.4[x]	26.8	11.9	20.3
1	18.9[y]	28.2	11.6	20.5
3	17.0[x]	27.3	11.0	20.7
5	17.3[x]	27.3	11.2	20.3
SEM	0.5	0.7	0.5	0.4
P Values	0.06	0.48	0.53	0.89

[x,y]Values lacking common superscripts tend to differ (P < 0.10)

Table 6: Cross sectional cortical widths (CW) at MID

Treatment	Dorsal CW, mm	Palmar CW, mm	Lateral CW, mm	Medial CW, mm
0	4.3[a]	4.1	4.5	4.9
1	4.9[b]	3.9	4.9	4.7
3	5.5[b]	4.2	4.9	5.3
5	4.9[b]	4.1	5.0	5.1
SEM	0.2	0.2	0.2	0.2
P Value	0.006	0.67	0.16	0.24

[a,b]Values lacking common superscripts differ ($P < 0.01$)

Table 7: Cross sectional and cortical areas at MID

Treatment	MID cross-sectional area, mm^2	MID cortical area, mm^2
0	437	288
1	465	309
3	451	320
5	453	318
SEM	20	15
P Values	0.80	0.43

Table 8: Cortical and whole-slice bone densities at MID

Treatment	Whole-slice, mg Ca hydroxyapatite/cm^3	Dorsal, mg Ca hydroxyapatite/cm^3	Lateral, mg Ca hydroxyapatite/cm^3	Medial, mg Ca hydroxyapatite/cm^3	Palmar, mg Ca hydroxyapatite/cm^3
0	996	1,210	1,220	1,230	1,060
1	997	1,130	1,250	1,240	1,040
3	987	1,210	1,220	1,210	1,070
5	982	1,220	1,220	1,210	1,050
SEM	13	28	20	17	18
P Value	0.82	0.13	0.57	0.55	0.78

In terms of mechanical properties, no differences by treatment were found in moment of inertia, flexural rigidity, or Young's modulus. However, fracture force did exhibit a difference between treatments (P = 0.01). Calves sprinting 1, 3, or 5 d/wk had increased fracture force compared to control calves as determined by four-point bending (Table 9). All exercise treatments had similar fracture force, even just 1 d/wk of sprinting had a greater fracture force of 7,940 N compared to the 6,300 N fracture force of calves sprinted 0 d/wk (P = 0.004).

Table 9: Fracture force and calculated mechanical properties of the fused third & fourth metacarpal of calves separated by treatment

Treatment	Moment of inertia, mm^4	Flexural rigidity, x 10^7 Nmm^2	Young's modulus, $N \cdot mm^{-2}$	Fracture force, N
0	9,660	2.3	2,530	6,300[a]
1	10,400	2.6	2,490	7,940[b]
3	10,800	2.4	2,210	7,850[b]
5	10,200	3.0	3,220	7,550[b]
SEM	813	0.5	543	358
P Value	0.81	0.78	0.61	0.01

[a,b]Values lacking common superscripts differ (P = 0.01)

DISCUSSION

Disuse by way of exercise reduction, immobilization, or confinement has deleterious effects on bone (Snow et al., 2001; van Harreveld et al., 2002). In the absence of loading, the skeleton reverts to its genetic minimum, a reduced bone mass that can support basic function without failure (Skerry, 2008). Immobilization for 6 wk has been shown to lead to decreased BMD, ultimate load, and stiffness in rats (Inman et al., 1999). Calves subjected to stall confinement for 6 wk in this study did not experience alterations to BMD, but ultimate load (fracture force) determined through four-point bending was negatively impacted as a result of confinement. All three exercise treatments experienced an 23% increase in fracture force compared to the non-sprinted calves, with just 1 d/wk of sprinting leading to an 26% increase in fracture force compared to calves sprinted 0 d/wk. When calves were sprinted 50 m 5 d/wk, fracture force tended to be greater in sprinted animals compared to exercised animals (Hiney et al., 2004b). Differences between studies, such as mechanical testing method used and length of

sprints, may be the cause of differing results in terms of fracture force. In the current study, mechanical testing was performed through four-point bending, while three-point bending was utilized by Hiney et al (2004a). It has been noted in polymer testing that differences are present in mechanical properties between three-point and four-point bending of the same specimen (Mujika, 2006). This phenomenon, coupled with the fact that sprint lengths were 50-m in the initial study and 71-m in the current study may explain why only a trend was noted in the initial study, but a treatment difference was present in fracture force in the present study.

Similar to Hiney et al (2004b) the exercised calves in this study had greater terminal dorsal CW compared to the confined group. As mentioned in the results, confined calves had similar BMD as exercised calves. However, the calves exercised 1, 3, and 5 d/wk all had increased dorsal CW, suggesting that they had more physical bone of similar BMD than the confined calves. Greater dorsal CW in exercised calves may have contributed to the greater fracture force of exercised calves, as force during four-point bending was applied to the palmar cortex with the dorsal cortex facing the bottom supports.

Lack of differences in external and internal lateromedial and dorsopalmar diameters lead to a lack of differences in moment of inertia. As Young's modulus of elasticity is calculated based on moment of inertia and flexural rigidity, no differences between treatments could be expected. Absence of differences in flexural rigidity may be caused by the lack of differences in area of cortical bone between treatments. While dorsal CW at MID was greater for sprinted treatments, cross sectional and cortical area at MID were not different. Values for cross sectional and cortical area were far greater than values for dorsal CW, subsequently, increased dorsal CW in exercised calves was not great enough to lead to changes in area. In future studies, a change of bone morphology from baseline could better detect responses to exercise. Dynamic strains to

bone, such as those from sprinting, are known to lead to bone formation (Allen and Burr, 2014). Unfortunately, formation of bone by osteoblasts can take months, while resorption of bone by osteoclasts can occur in the time span of a few days to two weeks (Stover, 2003). For this reason, differences in area and diameter of the cortical bone may not have yet been detectible at the end of this 6-wk study but may have been detectible if the study period spanned a few months.

Bone morphology was found to be affected when calves were sprinted 50 m 5 d/wk, leading to sprinted calves having a smaller medullary cavity and larger cortical bone area than confined counterparts (Hiney et al., 2004a). In the original calf exercise study, confined calves were kept in tie-stalls in which they could stand-up and lie down only. In the current study, calves were maintained in stalls which afforded room to stand-up and lie-down as well as turn-around 360°. Additional loading opportunities afforded to the calves in the current study may explain why minimal differences in bone morphology were detected. In retrospect, video analysis of calf behavior or use podometers could depict how often calves moved in their stalls. The additional bone strength and dorsal cortical width attained in sprinted calves in this study should not be discounted, as all calves had equal opportunities for movement and loading in their stalls.

Removing animals from pasture and confining them to stalls does have an influence on markers of bone formation and bone degradation (Hoekstra et al., 1999). A difference between treatments was evident in OC concentration - calves exercised 5 d/wk had greater OC concentration compared to all other treatments. In calves sprinted 5 d/wk, higher OC concentration suggests greater osteoblastic activity and therefore greater bone formation (Lee et al., 2000). Higher OC in calves sprinted 5 d/wk may be a result of the greater distance covered during the study by calves exercised 5 d/wk. Calves exercised 5 d/wk sprinted 2,130 m over the

6-wk study while calves exercised 1 d/wk sprinted 426 m during the same period. The number of sprints per week did not differ in influence on dorsal CW or fracture force, but OC does appear to be impacted by amount of sprints/wk suggesting that OC is affected by loading cycles while bone size and strength are affected by presence of sprints. Calves sprinted 50 m 5 d/wk also experienced greater OC concentration compared to confined animals (Hiney et al., 2004b). Concentration of OC has been shown to increase in horses in response to simulated race training on a treadmill composed of 2 minutes of trotting, 2 minutes of galloping, then 2 more minutes of trotting 5 d/wk (Frisbie et al., 2008).

Lack of differences in overall CTX-1 concentrations between treatments are not surprising as calves were housed individually in small stalls and not afforded exercise before the study began, beyond the two short walking sessions for halter training. Calves sprinted 0 d/wk did not experience changes to their normal activity once the study started as the stimuli their skeleton was accustomed to had not changed as a result of study initiation. The lack of a difference in CTX-1 concentration between weeks suggests that initiation of exercise did not lead to increased bone resorption, nor did maintenance in stalls. Serum deoxypyrodinoline, another marker of bone resorption, also did not differ between treatments in Hiney et al (2004a). If the calves in this study were maintained in pastures or in large group-housed pens before being housed in confinement during the study, then there may have been evident changes to CTX-1 concentration in the calves sprinted 0 d/wk. The minimal differences in CTX-1 concentration between treatments are reasonable given the lack of changes between pre-study housing and confinement during the study. The calves in this study were all at a juvenile age of 9 wk, at which point skeletal growth was occurring and would continue to occur if calves were maintained in calf-hutches of similar size to the stalls used in this study, as is normal industry

practice. Concentration of CTX-1 was not expected to differ between treatments as a result of sprint exercise, or lack thereof.

Similar to CTX-1, the lack of changes in C1,2C concentration by treatment are not surprising. Increased concentrations of C1,2C appear to be consistently found in horses inflicted with injury to articular surfaces (Frisbe et al., 2008; Nicholson et al., 2010). In Thoroughbred racehorses with osteochondral injuries, C1,2C concentration obtained from synovial fluid in carpal joints was greater compared to C1,2C concentration in uninjured young and adult horses (Nicholson et al., 2010). Observation of articular joint surfaces post mortem did not reveal any visual signs of osteochondral injuries to carpal joints in calves on the current study, suggesting that C1,2C concentration should not differ among treatments in this study. An increase to C1,2C concentration in the carpal bones of normal horses in response to exercise has been recorded (Frisbie et al., 2008). Synovial fluid was only collected on d 42 of the current study, preventing a progression of response to exercise to be evaluated. If synovial fluid was collected on d 0 and weekly thereafter, concentrations of C1,2C from d 0 to d 42 could potentially differ in response to exercise. The results of the current study propose that sprint exercise 1, 3, and 5 d/wk does not lead to a concentration of C1,2C on d 42 that is different than sprint exercise 0 d/wk.

While calves were sprinted on a hard-concrete surface for this study, young horses in training are typically exercised on a dirt or turf track. Calves sprinted at slower speeds than horses do, with Quarter Horses peaking at 24 m/s, Thoroughbreds at 18 m/s, and calves sprinting an average of 3.6 m/s in this study. Strides at speed are relative to the species of interest. Swiss heifers have been found to walk normally on a treadmill at an average speed of 1.33-1.40 m/s (Meyer et al., 2007). While traveling at 3.6 m/s may not be a sprint for horses, a velocity of 3.6 m/s is fast in terms of the normal traveling speed of a bovine and results in dynamic loading

which yields a skeletal response. The slower sprinting speed of calves compared to horses, and short distance of the sprints, justifies the use of a concrete surface to obtain dynamic strains similar to those experienced by a racehorse. It has been demonstrated by Hiney et al. (2004a,b) that calves sprinted on concrete and weanling horses sprinted on grass yield similar changes to cortical bone morphology and bone markers of resorption and formation. Video analysis confirmed that calves sprinted 1 and 3 d/wk sprinted at similar speeds. Response of bone in terms of CW and fracture force was the same for all sprinted treatments, even though calves sprinted 5 d/wk did not sprint as fast as calves sprinted 1 and 3 d/wk. Calves sprinted 5 d/wk also took more strides during each sprint, as a result of the lower sprint velocity. As mentioned above, distance covered may have had an impact on concentration of OC, but presence of strides at speed, not quantity of strides at speed, seems to have a notable influence on bone health via increased cortical bone width and ultimate strength.

It is important to note that calves may have underwent a gradual increase to speed at the beginning of the study, as sprint exercise was not incorporated into their management until d 0. At the beginning of the study calves were not oriented to the performance of sprints, nor the direction of travel. Faster velocities towards the end of the 6 wk study period suggest that calves had acclimated to sprint exercise by week 3. Stride frequency was greater during the first two weeks of the study, suggesting that calves took more strides at the beginning of the study while they were traveling at slower velocities. After week 3 calves took fewer strides to complete a sprint and completed their sprints at higher velocities. Calves increased in height and length during the 6 wk study, potentially attributing to the increase in sprint velocity and decrease in stride frequency. Familiarity to the sprint exercise as the study progressed also attributed to the changes in sprint velocity and stride frequency. Few cycles of dynamic loading are needed to

produce a stimulus which leads to the achievement and maintenance of bone mass. In roosters, as few as 4 and 36 cycles of loading were needed to maintain or increase long bone mineral content respectively (Rubin and Lanyon, 1984).

Egg-laying hens are also known to have increased bone resorption during periods of egg laying. Normally, medullary bone is mobilized during egg-laying as a labile source of calcium for shell formation, but resorption of cortical bone during egg-laying puts birds at high risk for fracture. This is especially prevalent in domesticated egg-laying hens as they lay year-round, unlike wild hens (Whitehead, 2004). Intensive production coupled with minimal opportunities for load-bearing exercise in conventional cages leads to a high incidence of osteoporosis and fractures. Studies evaluating management styles have determined that housing which allows hens and pullets opportunities for load-bearing and wing loading lead to better bone health compared to birds housed in conventional cages (Jendral et al., 2008; Regmi et al., 2015).

Similar to managing housing and exercise to optimize bone in animals, differing exercises have resorptive or formative responses in the skeletal system in human athletes. Experienced individuals who train and race regularly in road cycling have lower BMD than individuals who train and race regularly in mountain biking. Mountain biking exposes the skeletal system to many more dynamic strains than road cycling, comparable to those experienced by sprinting. Mountain cyclists spend more of their time with only their hands and feet in contact with the bike, and less time with the seat as a point of contact, compared to road cyclists (Warner et al., 2002). This difference in contacts as well as the difference in cycling terrain explains the greater BMD in mountain cyclists, further suggesting that dynamic loads, not static loads lead to osteogenic responses in bone.

Humans exposed to micro-gravity, such as that from exercise via swimming, have lower BMD compared to counter-parts involved in intensive weight-bearing activity or not exposed to exercise (Taaffe et al., 1995). Exposing juvenile athletes involved in swimming to dynamic loading can aid in counteracting lowered bone mass due to the lack of weight-bearing activity involved in swimming. Astronauts are the most common victims of bone resorption caused by lack-of-gravity. During flight, astronauts are not able to experience dynamic loads which illicit bone formation and as a result, individuals returning from space-missions do so with increased bone resorption. Even though exercise is closely monitored and mandated for space flights, it has failed at this point to cease or reverse bone resorption in space, calling to attention the necessity of dynamic loads for skeletal maintenance or improvement (Stein, 2013).

It is common practice in the horse industry that young horses are removed from pasture and kept in stalls during race training. While young horses are in the initial stages of race training they undergo walking, trotting, and cantering before speed is added to their exercise regimen. This schedule of slow exercise, coupled with the loss of free-choice exercise from pasture leads to decreased bone mineral content, and presumably strength (Nielsen et al., 1997; Hoekstra et al., 1999). The addition of dynamic loads to confinement is crucial in counteracting the loss of bone strength (Hiney et al., 2004a,b). Sprinting young horses' short distances 1, 3, or 5 d/wk, as done with the calves in this study, should lead to a subsequent increase in strength of the third metacarpal, potentially reducing the risk of catastrophic injury during their racing career.

CONCLUSION

Collectively, the results of this study support that animals sprinted the short distance of 71-m 1, 3, or 5 d/wk attained heightened dorsal CW and fracture force needed to bend bone to failure. Sprint exercise also influences bone formation evidenced by the fact that calves sprinted

5 d/wk had greater OC concentration. Calves sprinted 1 d/wk exhibited a 26% increase to fracture force compared to calves confined without sprinting. Over the 6-wk study, calves assigned to sprinting 1 d/wk only sprinted 426 m. This demonstrates the very few strides at speed needed to increase bone health, and that lack of dynamic loading for just 6-wk leads to deleterious effects on skeletal strength. On an implementation stand-point, sprint-exercising young animals 1 d/wk for 6 wks while young requires little extraneous time and funds of the owner while increasing the physical welfare of the young animal and potentially reducing the risk of a musculoskeletal injury during training and racing. Further research in this topic is needed to determine if sprinting animals 1 d/wk at a young age can maintain heightened bone strength into maturity.

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LITERATURE CITED

LITERATURE CITED

Allen, M. R., and D. B. Burr. 2014. Bone modeling and remodeling. In: D. B. Burr and M. R. Allen, editors, Basic and Applied Bone Biology. Academic Press, San Diego, CA. p.75–90.

American Horse Council Foundation. 2018. 2017 Economic Impact of the U.S. Horse Industry: Michigan Economic Impact Study. https://www.horsecouncil.org/product/state-breakout-economic-impact-studies/

American Quarter Horse Association. 2010. Annual Report. https://www.aqha.com/media/8793/2010_annualreport_web1.pdf (Accessed November 11 2018).

American Quarter Horse Association. 2017. Annual Report. https://www.aqha.com/media/24096/2340-18-64-2017-annual-report.pdf (Accessed November 11 2018).

ASABE. 2017. Shear and three-point bending test of animal bone. ASABE STANDARDS. ANSI/ASAE Standard S459. Amer. Soc. Agr. Bio. Eng. St. Joseph, MI.

Beisser, A. S. McClure, G. Rezabek, K. H. Soring, and C. Wang. 2014. Frequency of and risk factors associated with catastrophic musculoskeletal injuries in Quarter Horses at two Midwestern racetracks: 67 cases (2000-2011). J. Am. Vet. Med. Assoc. 245 (10): 1160-1168. doi:10.2460/javma.245.10.1160.

Donabedian, M., P. R. van Weeren, G. Perona, G. Fleurance, C. Robert, S. Leger, D. Bergero, O. Lepage, and W. Martin-Rosset. 2008. Early changes in biomarkers of skeletal metabolism and their association to the occurrence of osteochondroisis (OC) in the horse. Equine Vet. J. 40 (3): 253-259. doi:10.2746/042516408X273657.

Frisbie, D. D., F. Al-Sobayil, R. C. Billinghurst, C. E. Kawcak, and C. W. McIlwraith. 2008. Changes in synovial fluid and serum biomarkers with exercise and early osteoarthritis in horses. Osteoarthritis Cartilage. 16 (10): 1196-1204. doi:10.1016/J.JOCA.2008.03.008.

Green, L. E., V. J. Hedges, Y. H. Schukken, R. W. Blowey, and A. J. Packington. 2002. The impact of clinical lameness on the milk yield of dairy cows. J. Dairy Sci. 85: 2250-2256. doi:1-.3168/jds.S0022-0302(02)74304-X.

Hiney, K. M., B. D. Nielsen, D. Rosenstein, M. W. Orth, and B. P. Marks. 2004a. High-intensity exercise of short duration alters bovine bone density and shape. J. Anim. Sci. 82: 1612–1620. doi:/2004.8261612x.

Hiney, K. M., B. D. Nielsen, and D. Rosenstein. 2004b. Short-duration exercise and confinement alters bone mineral content and shape in weanling horses. J. Anim. Sci. 82: 2313–2320. doi:10.2527/2004.8282313x.

Hoekstra, K.E., B.D. Nielsen, M.W. Orth, D.S. Rosenstein, H.C. Schott, and J.E. Shelle. 1999. Comparison of bone mineral content and biochemical markers of bone metabolism in stall- vs. pasture-reared horses. Equine Vet. J. 30: 601–604. doi:10.1111/j.2042-3306.1999.tb05292.x.

Holtenius, K., and A. Ekelund. 2005. Biochemical markers of bone turnover in the dairy cow during lactation and the dry period. Res. Vet. Sci. 78 (1): 17-19.

doi:10.1016/J.RVSC.2004.05.002.

Inman, C. L., G. L. Warren, H. A. Hogan, and S. A. Bloomfield. 1999. Mechanical loading attenuates bone loss due to immobilization and calcium deficiency. J. Appl. Physiol. 87: 189-195. doi:10.1152/jappl.1999.87.1.189

Jendral, M. J., D. R. Korver, J. S. Church, and J. J. R. Feddes. 2008. Bone mineral density and breaking strength of while leghorns housed in conventional, modified, and commercially available colony battery cages. Poult. Sci. 87 (5): 828-837. doi:10.3382/ps.2007-00192

Lee., A. J., S. Hodges, and R. Eastell. 2000. Measurement of osteocalcin. Ann. Clin. Biochem. 37: 432-446. doi:10.1177/000456320003700402.

Matsuo, A., A. Togashi, K. Sasaki, B. Devkota, T. Hirata, and N. Yamagishi. 2014. Diurnal variation of plasma bone markers in Japanese black calves. J. Vet. Med. Sci. 76 (7): 1029-1032. doi:10.1292/jvms.14-0021.

Meyer, S. W., M. A. Weishaupt, and K. A. Nuss. 2007. Gait pattern of heifers before and after claw trimming: a high-speed cinematographic study on a treadmill. J. Dairy Sci. 90 (2): 670-676. doi:10.3168/jds.S0022-0302(07)71549-7.

Mujika, F. 2006. On the difference between flexural moduli obtained by three-point and four-point bending tests. Polym. Test. 25: 214-220. doi:10.1016/J.POLYMERTESTING.2005.10.006.

Nicholson, A. M., T. N. Trumble, K. A. Merritt, and M. P. Brown. 2010. Associations of horse age, joint type, and osteochondral injury with serum and synovial fluid concentrations of type II collagen biomarkers in Thoroughbreds. Am. J. Vet. Res. 71 (7): 741-749. doi:10.2460/avhr.71.7.741.

Nielsen, B.D., G.D. Potter, E.L. Morris, T.W. Odom, D.M. Senor, J.A. Reynolds, W.B. Smith, and M.T. Martin. 1997. Changes in the third metacarpal bone and frequency of bone injuries in young quarter horses during race training - Observations and theoretical considerations. J. Equine Vet. Sci. 17: 541–549. doi:10.1016/S0737-0806(97)80227-4.

NRC. 2001. Nutrient requirements of dairy cattle. 7th ed. Natl. Acad. Press, Washington, DC.

O'Connor-Robison, C. I., J. D. Spencer, and M. W. Orth. 2014. The impact of dietary long-chain polyunsaturated fatty acids on bone and cartilage in gilts and sows. J. Anim. Sci. 92: 4607-4615. doi:10.2527/jas2013-7028.

Pruyn M., B. J. Ewers III, and F. W. Telewski. 2000. Thigmomorphogenesis: changes in the morphology and mechanical properties of two *Populus* hybrids in response to mechanical perturbation. Tree Physiol. 20: 535-540. doi:10.1093/treephys/20.8.535.

Regmi, P., T. S. Deland, J. P. Steibel, C. I. Robison, R. C. Haut, M. W. Orth, and D. M. Karcher. 2015. Effect of rearing environment on bone growth of pullets. Poult. Sci. 94 (3): 502-511. doi:10.3382/ps/peu041.

Robison, C. I., and D. M. Karcher. (in press). Analytical bone calcium and bone ash from mature laying hens correlates to bone mineral content calculated from quantitative computed tomography scans. Poult. Sci. doi: 10.3382/ps/pez165.

Rogers, C.W., C. F. Bolwell, J. C. Tanner, and P. R. van Weeren. 2012. Early exercise in the horse. J. Vet. Behav. 7: 375–379. doi:10.1016/j.jveb.2012.01.003.

Ross, M. W., and S. J. Dyson. 2011. Diagnosis and management of lameness in the horse. 2nd ed. Elsevier/Saunders, St. Louis, MO.

Rubin, C. T., and L. E. Lanyon. 1984. Regulation of bone formation by applied dynamic loads. J. Bone Joint Surg. Am. 66: 397-402.

Skerry, T. M. 2008. The response of bone to mechanical loading and disuse: Fundamental principles and influences on osteoblast/osteocyte homeostasis. Arch. Biochem. Biophys. 473: 117–123. doi:10.1016/j.abb.2008.02.028.

Snow, C. M., D. P. Williams, J. LaRiviere, R. K. Fuchs, and T. L. Robinson. 2001. Bone gains and losses follow seasonal training and detraining in gymnasts. Calcified Tissue Int. 69: 7–12. doi:10.1007/s00223-001-0014-5.

Spooner, H. S., B. D. Nielsen, A. D. Woodward, D. S. Rosenstein, and P. A. Harris. 2008. Endurance training has little impact on mineral content of the third metacarpus in two-year-old Arabian horses. J. Equine Vet. Sci. 28 (6): 359-362. doi:10.1016/j.jevs.2008.04.012.

Stein, T.P. 2013. Weight, muscle and bone loss during space flight: another perspective. Eur J Appl Phsiol. 113: 2171-2181. doi:10.1007/s00421-012-2548-9.

Stover, S. M. 2003. The epidemiology of thoroughbred racehorse injuries. Clin. Tech. Equine Pract. 2: 312–322. doi:10.1053/j.ctep.2004.04.003.

Taaffe, D. R., C. Snow-Harter, D. A. Connolly, T. L. Robinson. M. D. Brown, and R. Marcus. 1995. Differential effects of swimming versus weight-bearing activity in bone mineral status of eumenorrheic athletes. J. Bone Miner. Res. 10 (4): 586-593 doi:10.1002/jbmr.5650100411

The Jockey Club. 2018. Supplemental tables of equine injury database statistics for Thoroughbreds (Equine Injury Database). http://jockeyclub.com/pdfs/eid_9_year_tables.pdf (Accessed March 7 2019).

The Jockey Club. 2019a. Size of field and starts per horse (Fact Book Index). http://jockeyclub.com/default.asp?section=FB&area=10 (Accessed March 7 2019).

The Jockey Club. 2019b. Gross purses (Fact Book Index). http://www.jockeyclub.com/default.asp?section=FB&area=7 (Accessed March 7 2019).

van Harreveld, P. D., J. D. Lillich, C. E. Kawcak, A. S. Turner, and R. W. Norrdin. 2002. Effects of immobilization followed by remobilization on mineral density, histomorphometric features, and formation of the bones of the metacarpophalangeal joint in horses. Am J Vet Res. 63: 276–281. doi:10.2460/ajvr.2002.63.282.

Warner, S.E., J.M. Shaw, and G.P. Dalaska. 2002. Bone mineral density of competitive male mountain and road cyclists. Bone. 30(1): 281-286. doi:10.1016/S8756-3282(01)00704-9.

Whitehead, C. C. 2004. Overview of bone biology in the egg-laying hen. Poult. Sci. 83: 193-199.

CHAPTER 3: Overall discussion and conclusions

This purpose of this study was to determine if sprint exercise allotted 1 or 3 d/wk provided similar benefits to bone as calves sprinted 5 d/wk. The results of this research proved that remarkably few strides are needed at speed to illicit benefits to bone health which aid to combat the loss of bone via stall confinement (Chapter 2). This research also demonstrated that dramatic negative effects to bone occur with just 6 wk of stall confinement.

While the results from this study are informative in terms of the response of bone to sprint exercise, there are adjustments to the methods of this study which could produce results further supporting the current conclusions. The largest point of concern in the methodology of this study is the uneven size of calf groups. Unfortunately, due to the slow rate of bull calf births at the MSU Dairy Teaching and Research Center, calf groups were not of even sizes. When a group of bull calves was born within 10 d of each other, they were grouped together and were selected to join the study. Group sizes ranged from 3 to 6 calves. An ideal situation would have been if calves entered the study in groups of multiples of 4, or if all 24 calves entered the study at the same time. The uneven number of calves in a group and treatments represented in a group accounts for the group effects displayed in the appendix. Group was not appropriate to include in the statistical model, as group effects were confounded with the uneven representation of treatments in each group. During the study, it was imperative that we take groups of any size greater than 2, as the MSU Dairy Teaching and Research Center endured a stretch of calf deaths as we were working to procure calves early in the study. We were willing to accept groups of uneven sizes so that the animal work spanned a reasonable amount of time. While we were diligent to accept all calves to our study whose birthdates were within 10 d of calves in a group,

the low number of bull calf births lead to the study still taking 6 months to complete the animal work.

While 6 months appears to be a relatively acceptable time span for the animal work, environmental conditions varied during the 6 months from study initiation to termination due to the region of study location. The first group of calves on the study was born in November 2017 and was euthanized in March 2018, while the last group of calves was born in March 2018 and euthanized in June 2018. Ambient temperatures were well below 0°C for much of the time group 1 was on study. However, by May and June of 2018, there were many days between 26 to 32°C with high humidity. Group effects in bone marker concentration (Appendix) may be impacted by the differing environmental impacts between groups. It is known that biomarkers of bone formation and resorption can be affected by seasonal variation (Thomsen et al., 1989; Vanderschueren et al., 1991). Should another study with similar design be performed, care should be taken that calves enter the study in groups of the same size relative to number of treatments, and that the animal work takes place over a shorter time period of similar environmental conditions.

At this point, it is not fully understood why there were no changes to cortical or cross-sectional area even though the dorsal CW was greater for calves exercised 1, 3, and 5 d/wk. Similarly, it is not known why the medullary cavity, measured as internal cortical diameter, was not smaller if the dorsal CW width was greater in sprinted calves. In terms of cortical area and cross-sectional area, the calves sprinted 1, 3, and 5 d/wk do have values that were suggestive that differences from the confined calves may have been found if larger numbers of subjects had been within each treatment. This study had a sample size of n=6 for all treatments, a power analysis determined that n=61 and n=21 would be needed for differences at $P \leq 0.05$ between treatments

in cortical area and cross-sectional area respectively. In the original calf study (Hiney et al., 2004), calves were maintained in tie stalls and could only lie-down and stand-up, while our calves could turn-around as well. Retrospectively, video analysis should have been performed or calves should have worn pedometers to determine how often calves moved in their stalls, and if there were differences between treatments. This additional opportunity for limb loading within stalls may be why there are minimal differences in bone morphology between treatments.

Overall, the results from this thesis are of great importance to the horse industry and have answered some critical questions in terms of the response of bone to exercise. Looking forward into future research, duration of increased bone health could be worthy of future investigation. If animals are exercised while young and exhibit increased bone health compared to confined animals, how long is the acquired bone health present when sprinted and non-sprinted animals enter the same training conditions? Similarly, the effects of sprinting on other tissues of juvenile animals should be evaluated, such as cartilage and tendon. This study answers questions that arose from the original calf study and sets up other questions as to the effects and longevity of sprint exercise. I have learned much about the execution of a research project from this study and look forward to being part of the design and execution of future research that may stem from this project and others.

APPENDIX

APPENDIX

This appendix contains figures and tables which represent some behaviors during sprinting as well group effects. It should be noted that figures and tables containing group effects are confounded with the uneven assignment of treatments to group, as well as environment effects of the period. This data is included to provide a well-rounded representation of how environment and treatment effects may have affected bio-markers and physical calf parameters. Means are represented as least-squares mean ± the standard error of the mean (SEM).

Table 10: Frequency of calves that ran straight during the middle 21 m of the 71-m sprints separated by week

Week	Frequency of calves that ran straight	Percent of calves that ran straight
1	11	61
2	12	67
3	15	83
4	15	83
5	14	78
6	13	72
Total	82	72

Table 11: Frequency of calves that turned around during the middle 21 m of the 71-m sprints separated by week

Week	Frequency of calves that turned around	Percent of calves that turned around
1	3	17
2	5	28
3	2	11
4	1	56
5	2	11
6	4	22
Total	18	16

Table 12: Frequency of calves that tripped during the middle 21 m of the 71-m sprints separated by week

Week	Frequency of calves that tripped	Percent of calves that tripped
1	1	6
2	0	0
3	2	11
4	0	0
5	0	0
6	2	11
Total	6	5

Table 13: Frequency of calves that stopped during the middle 21 m of the 71-m sprints separated by week

Week	Frequency of calves that stopped	Percent of calves that stopped
1	9	50
2	10	56
3	4	22
4	4	22
5	4	22
6	6	33
Total	41	36

Table 14: Frequency of calves that jumped or bucked during the middle 21 m of the 71-m sprints separated by week

Week	Frequency of calves that jumped or bucked	Percent of calves that jumped of bucked
1	4	22
2	4	22
3	5	28
4	4	22
5	6	33
6	7	39
Total	33	29

Table 15: Initial and final measurements of height, weight, and length of calves separated by group

Group	Initial height (cm)	Final height (cm)	Initial weight (kg)	Final weight (kg)	Initial length (cm)	Final length (cm)	ADG (kg/d)
1	90 ± 1	100 ± 2	89 ± 3	124 ± 8	66^{a} ± 1	80^{a} ± 2	0.8 ± 0.2
2	94 ± 1	103 ± 2	88 ± 3	118 ± 8	68^{ab} ± 1	79^{a} ± 2	0.7 ± 0.2
3	91 ± 2	100 ± 2	83 ± 4	126 ± 10	70^{b} ± 2	71^{b} ± 2	1.0 ± 0.2
4	92 ± 1	102 ± 2	86 ± 3	119 ± 7	69^{ab} ± 1	73^{b} ± 2	0.8 ± 0.2
5	90 ± 2	100 ± 2	78 ± 4	102 ± 10	65^{ac} ± 2	74^{ab} ± 2	0.6 ± 0.2
6	89 ± 2	98 ± 2	79 ± 4	106 ± 10	62^{c} ± 2	70^{b} ± 2	0.6 ± 0.2

^{a,b,c} Values lacking common superscripts within a column differ ($P < 0.05$)

Figure 8: Mean calf serum Osteocalcin concentration by group throughout the 6-wk study period
*Differences between groups ($P < 0.0001$) listed in Table 16 and interactions between week*group ($P < 0.0001$) listed in table 17*

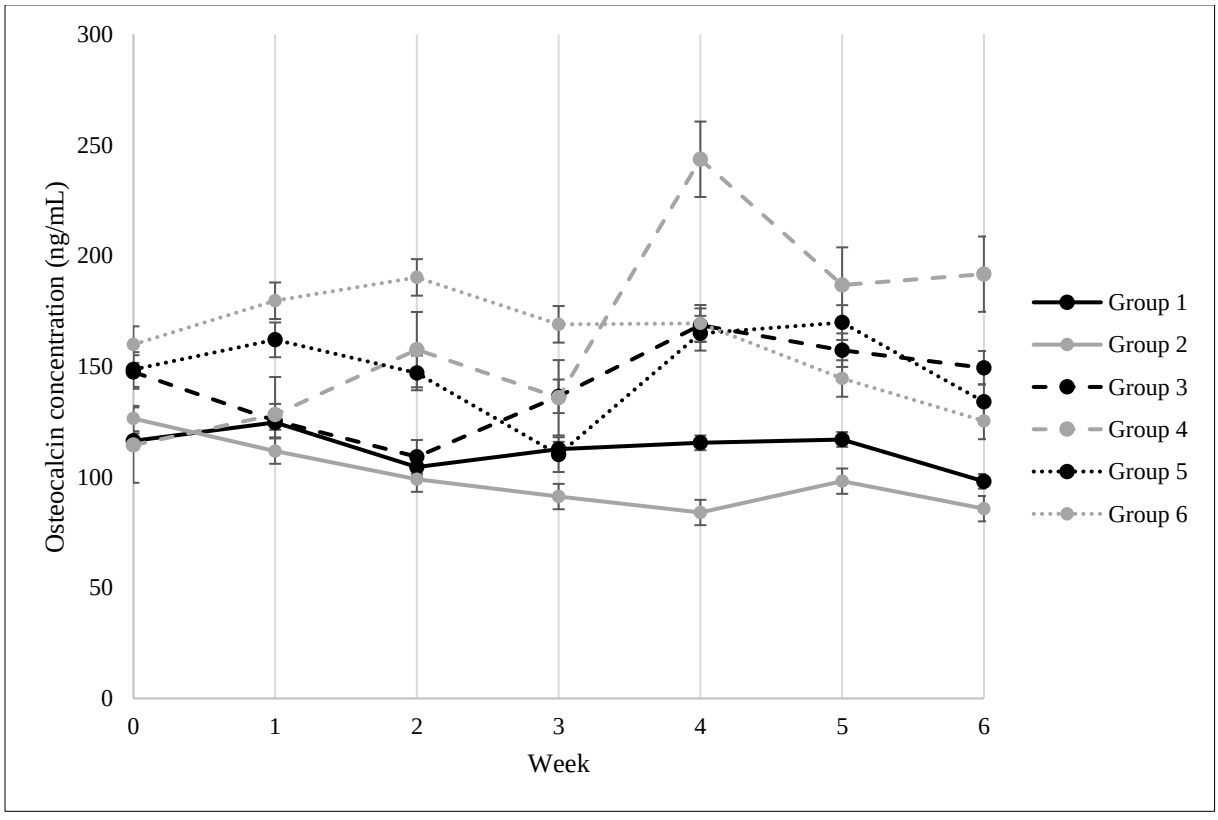

Table 16: Osteocalcin means separated by group

Group	Osteocalcin concentration (ng/mL)
1	$112.7^{a} \pm 6.2$
2	$99.5^{a} \pm 6.8$
3	$141.9^{bd} \pm 8.0$
4	$165.4^{c} \pm 5.6$
5	$149.1^{bcd} \pm 8.3$
6	$162.7^{cd} \pm 8.0$

^{a,b,c} Values lacking common superscripts differ ($P < 0.0001$)

Table 17: Interactions between week*group for Osteocalcin (OC, ng/mL)

Group	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	$116^{ab} \pm 14$	$125^{ab} \pm 14$	$104^{ab} \pm 14$	$113^{ab} \pm 14$	$115^{a} \pm 14$	$117^{ab} \pm 14$	$98^{a} \pm 14$
2	$126^{ab} \pm 15$	$112^{a} \pm 15$	$99^{ac} \pm 15$	$91^{b} \pm 15$	$84^{a} \pm 15$	$98^{a} \pm 15$	$86^{a} \pm 15$
3	$147^{ab} \pm 18$	$125^{ab} \pm 18$	$109^{ab} \pm 18$	$136^{abc} \pm 18$	$169^{b} \pm 18$	$157^{bc} \pm 18$	$149^{bc} \pm 18$
4	$114^{a} \pm 13$	$128^{ab} \pm 13$	$158^{cd} \pm 13$	$136^{ac} \pm 13$	$244^{c} \pm 13$	$187^{c} \pm 13$	$192^{b} \pm 13$
5	$149^{ab} \pm 18$	$162^{bc} \pm 18$	$147^{bd} \pm 18$	$110^{ab} \pm 18$	$165^{b} \pm 18$	$170^{c} \pm 18$	$134^{ac} \pm 22$
6	$160^{b} \pm 18$	$180^{c} \pm 18$	$190^{d} \pm 18$	$169^{c} \pm 18$	$169^{b} \pm 18$	$145^{bc} \pm 18$	$125^{ac} \pm 18$

^{a,b,c,d} Means that lack common superscripts within a column differ ($P < 0.0001$)

Figure 9: Mean C-terminal telopeptides of type 1 collagen (CTX-1) concentration in calf serum by group throughout the 6-wk study period *Differences between groups (P = 0.0005) listed in Table 18 and interactions between week*group (P = 0.0037) listed in table 19*

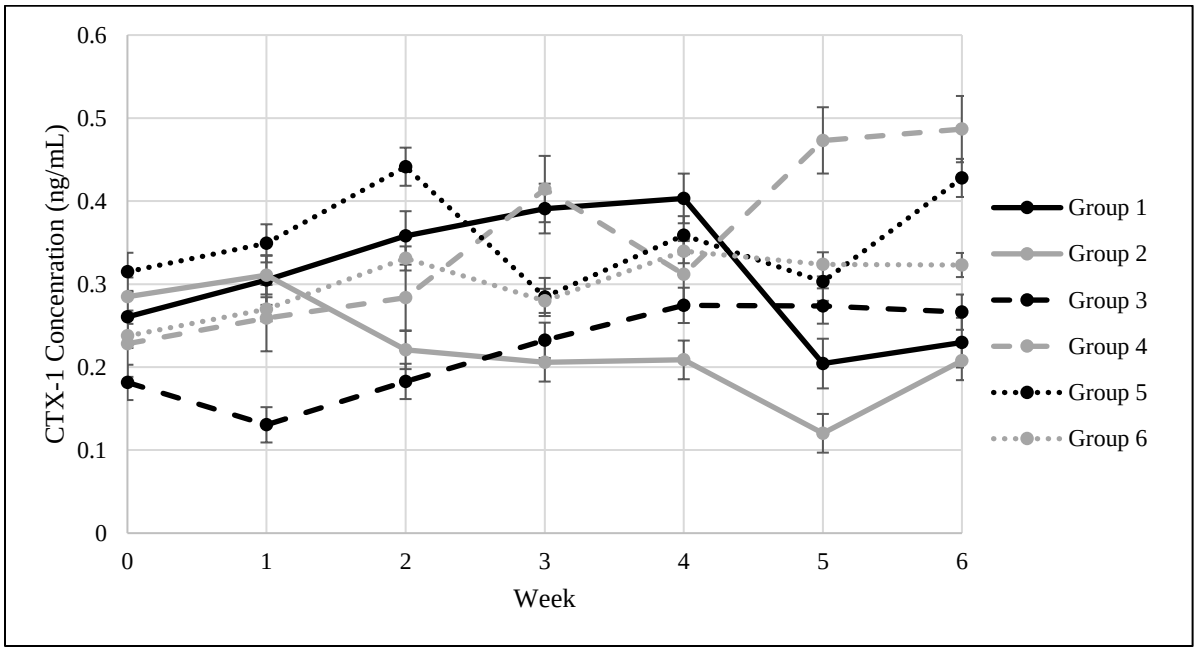

Table 18: C-terminal telopeptides of Type 1 collagen (CTX-1) concentration separated by groups

Group	CTX-1 Concentration (ng/mL)
1	0.31[ac]
2	0.23[b]
3	0.22[b]
4	0.35[a]
5	0.35[ac]
6	0.30[c]
SEM	0.02

[a,b,c] Values lacking similar subscripts differ (P = 0.0005)

Table 19: Interactions between week*group for C-terminal telopeptides of Type 1 Collagen (CTX-1, ng/mL)

Group	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	0.26 ± 0.05	0.30^{a} ± 0.05	0.36^{ac} ± 0.05	0.39^{a} ± 0.05	0.40^{a} ± 0.05	0.20^{ab} ± 0.05	0.23^{a} ± 0.05
2	0.28 ± 0.05	0.31^{a} ± 0.05	0.22^{ab}± 0.05	0.21^{b} ± 0.05	0.21^{b} ± 0.05	0.12^{a} ± 0.05	0.21^{a} ± 0.05
3	0.18 ± 0.06	0.13^{b} ± 0.06	0.18^{b} ± 0.06	0.23^{b} ± 0.06	0.27^{ab} ± 0.06	0.27^{ab} ± 0.06	0.27^{ac} ± 0.06
4	0.23 ± 0.04	0.26^{ab} ± 0.04	0.28^{ab} ± 0.04	0.41^{a} ± 0.04	0.31^{ab} ± 0.04	0.47^{c} ± 0.04	0.49^{b} ± 0.04
5	0.31 ± 0.06	0.35^{a} ± 0.06	0.44^{c} ± 0.06	0.28^{ab} ± 0.06	0.36^{ab} ± 0.06	0.30^{ab} ± 0.06	0.43^{bc} ± 0.06
6	0.24 ± 0.06	0.27^{ab} ± 0.06	0.33^{abc} ± 0.06	0.28^{ab} ± 0.06	0.34^{ab} ± 0.06	0.32^{bc} ± 0.06	0.32^{ac} ± 0.06

^{a,b,c} Means that lack common superscripts within a column differ (P = 0.0037)

Table 20: Inverse serum Collagen Type I and II Cleavage ELISA (C1,2C) concentrations separated by group

Group	1/C1,2C Concentration (µg/mL)
1	4.3^{ab} ± 0.5
2	2.9^{a} ± 0.5
3	5.1^{b} ± 0.6
4	4.1^{ab} ± 0.4
5	4.8^{b} ± 0.6
6	7.0^{c} ± 0.6

^{a,b,c} Values lacking common superscript tend to differ (P < 0.01)

Following mechanical testing via an Instron, fractures were measured by hand with a digital caliper. Upon observation, it was determined that fractures were located mainly in the distal portion of the bone, with some reaching the mid-point of the bone. The start of a fracture was recorded as the distance from the proximal end. The end of a fracture was also measured as the distance from the proximal end. There was one calf (1967) for which a fracture was not

visible with observation of the exterior of the MC III & IV. Two calves (1770 and 1782), did not exhibit a spread in the fracture, in stead the fracture formed a “band”.

Table 21: Length of left MC III & IV and location of fractures

Calf ID	Treatment	Length (cm)	Fracture start (mm)	Fracture end (mm)	Fracture length (mm)
1762	0	20.7	63.4	95.3	31.9
1763	1	21.0	54.5	80.3	25.8
1764	3	20.5	47.8	75.8	28.0
1765	5	20.2	68.7	77.7	9.0
1766	0	20.5	72.0	86.6	14.6
1767	5	22	N/A	N/A	N/A
1769	3	20.5	48.2	69.4	21.2
1770	1	21.1	87.13[1]	87.13	0
1771	0	20.0	69.1	80.8	11.7
1779	5	20.8	71.5	85.7	14.2
1781	1	20.6	50.9	80.9	30.0
1782	3	20.6	68.79[1]	68.79	0
1783	3	20.5	70.2	78.1	7.9
1785	5	20	58.7	85.2	26.5
1786	0	21.2	74.1	94.7	20.6
1788	1	20.2	75.2	84.8	9.6
1789	3	21.5	47.8	76.8	29
1790	1	20.7	45.7	61.5	15.8
1791	0	20.3	75.6	84.8	9.2
1794	1	20.9	82.5	89.9	7.4
1795	5	20	74.0	92.8	18.8
1797	3	21.1	79.1	89.8	10.7
1798	5	20.2	42.7	79.9	37.2
1799	0	20.5	70.7	96.1	25.4

[1]Fracture did not spread down towards the distal portion of the limb

LITERATURE CITED

LITERATURE CITED

Hiney, K. M., B. D. Nielsen, D. Rosenstein, M. W. Orth, and B. P. Marks. 2004. High-intensity exercise of short duration alters bovine bone density and shape. J. Anim. Sci. 82: 1612–1620. doi:/2004.8261612x.

Thomsen, K. E. F. Eriksen, J. C. R. Jørgensen, P. Charles, and L. Mosekilde. 1989. Seasonal variation of serum bone GLA protein. Scand. J. Clin. Lab. Invest. 49(7): 605-611. doi: 10.1080/00365518909091535.

Vanderschueren, D. G. Gevers, J. Dequeker, P. Guesens, J. Nijis, P. Devos, M. D. Roo, and R.Bouillon. 1991. Seasonal variation in bone metabolism in young healthy subjects. Calcif. Tissue Int. 49(2): 84-89.