EFFECT OF EXERCISE ON BONE DENSITY, GROWTH PARAMETERS AND BIOCHEMICAL MARKERS OF BONE METABOLISM IN YEARLING QUARTER HORSES

By

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CHAPTER I

INTRODUCTION

The development of an adequate skeletal support system is among the most important requirements of a potential racing, working or competition horse. Subsequent entry into training and competition exposes the bones of the lower limbs to remarkable mechanical stress (Glade et al., 1986). A major concern with young, growing horses is the incidence of skeletal problems induced by management practices. The practice of transferring young, growing horses from their normal habitat to stalls prior to yearling sales or training may indeed predispose them to skeletal complications. Research has demonstrated decreases in bone mineral content of the third metacarpal in young horses soon after the onset of race training, as well as a change in housing from pasture to stalls (Nielsen et al., 1997; Hoekstra et al., 1999). This decrease in bone mineral content could result from a decreased or increased strain on the bone due to exercise regimens, housing, or a combination of the two. Transferring young horses from pasture to stalls results in a slowdown in the rate of bone formation due to a decrease in physical activity (Maenpaa et al., 1988). This has been demonstrated in other species, which have also decreased bone strength due to confinement rearing (Knowles and Broom, 1990). It has been shown that housing in stalls and reducing exercise has a negative impact upon bone mineral content, however, a proper exercise regimen has not been determined to prevent loss of bone mineral content while housed in stalls. Thus, yearling horses housed in stalls with

limited free exercise may have a skeletal structure less prepared for forces applied upon them during training than those horses forced to exercise on a daily basis. Therefore, the objective of this study was to determine the effect of different exercise regimens (non-exercised vs. exercised) on bone metabolism. It is a common industry practice to transfer yearlings from pasture to stalls in the summer for 60 to 90 days prior to fall yearling sales. The primary objective was to reproduce these settings imposed by the industry to better define the proper level of exercise that will optimize skeletal development in the yearling Quarter Horse.

CHAPTER II

REVIEW OF LITERATURE

Bone Structure

The skeletal system is composed of cancellous and cortical bone. Cortical bone is characterized by its dense solid mass that forms the outside layer of bone, and makes up the majority of the total skeletal mass (Jee, 1998). Cancellous bone is characterized by its rods, plates, and arches which are individually known as trabeculae (Jee, 1988). A typical long bone consists of the diaphysis (shaft) and the epiphysis (spherical ends). The region that connects the epiphysis and diaphysis is known as the metaphysis. The diaphysis is primarily comprised of cortical bone, while the epiphysis and metaphysis are mainly composed of cancellous bone. In growing animals, the epiphyseal-metaphyseal complex, or growth plate, separates the epiphysis from the metaphysis. The region in which cancellous bone production and bone elongation occur is at the cancellous bone surrounding the metaphysis and the growth plate (Jee, 1988). The diaphysis is lined with a membranous layer, known as the endosteum, which contain the osteoblasts and osteoclasts. The periosteum is located on the outer surface of bones, which is lined with a layer of undifferentiated cells (mesenchymal stem cells). These cells can be recruited to increase bone growth or repair injured bone when stimulated.

The bone may exist in two forms, primary or secondary (Jee, 1988). Primary bone is characterized by collagen fibers that lack an ordered arrangement and is considered immature bone. Primary bone typically has a lower mineral content as compared to secondary bone. When bone tissue initially forms, it is composed mainly of primary bone. Secondary bone has a matrix consisting of collagen fibers with a high degree of orientation. Secondary bone, also has a higher mineral content and replaces primary bone when maturation occurs.

The main structural unit of cortical bone is the osteon or Haversian system (Jee, 1988). Each individual osteon is composed of a large Haversian canal surrounded by circular sheets of collagen fibers. The haversian canal contains blood vessels, nerves, and connective tissue (Jee, 1988). Nutrients are provided to the osteocytes by a network of Volkmann's canals. Osteocytes are located within the central canals and are the main cells of mature bone (Currie, 1998). However, cancellous bone does not contain Haversian systems. The cancellous bone contains trabeculae within its framework, which are functionally similar to the osteon. Lacunae are found in the cavities of both cortical and cancellous bone. They contain osteocytes and are connected by thin tubular channels, or canuliculi (Marks and Popoff, 1988). The cytoplasmic processes of the osteocytes are contained in the canuliculi. This allows for communication with other osteocytes through gap junctions and provide nutrients to the bone surface. In addition to osteocytes, other cells in the bone include osteoblasts. These cells, known as Howships lacunae, are usually found near the resorption pits located on the bone surface (Jee, 1988). Osteoblasts serve several functions regarding bone metabolism. They are bone-forming cells that regulate mineral homeostasis, participate in calcification of bone, and synthesize and

secrete unmineralized bone matrix. Osteoclasts are giant multinucleated cells that originate from bone marrow. The function of osteoclasts is primarily the resorption of bone, either internally or on the surface of the bone (Jaworski, 1984). Thin elongated cells line the bone and are found opposite to the inactive bone surfaces (Marks and Popoff, 1988). These bone-lining cells separate interstitial fluid from the bone fluids of the canulicular system. Jee (1988) suggests that these cells are involved in sensing the magnitude, distribution, and rate of mechanical strain placed upon the load-bearing limbs. During loading, this information is transmitted to the active bone cells, thus stimulating the resorption response. Due to their wide distribution throughout the organic matrix and their ability to communicate with each other, osteocytes are believed to be responsible for this occurrence (Lanyon, 1987).

Initiated by the proteolytic degradation of the organic matrix of the bone, the process of bone degradation by osteoclastic cells releases calcium and phosphorus into the extracellular fluid (Marks and Popoff, 1988). A clear zone surrounds the ruffled border, or the surface of an active osteoclast, and provides a seal in order to maintain a microenvironment for bone degradation (Jee, 1988). The Golgi stores acid hydrolase's in the cytoplasm of the osteoclast which are transported to the ruffled border region. Acid hydrolase's are then released into the extracellular space created between the bone and the cell by the clear zone (Marks and Popoff, 1988). The acidic proteases can then degrade the bone in a more favorable environment. Once the minerals are released, the organic matrix is resorbed (Jee, 1988).

Osteoblasts are believed to initiate osteoclastic resorption by their destruction of the tissue lining the bone matrix (Weryha and Leclere, 1995). Bone formation by

osteoblasts occurs at the boundary between osteoblastic cells and the tissue lining the bone matrix (Jee, 1988). Mineralization occurs at the interface between the tissue lining the bone matrix and the most recently mineralized bone. Jee (1988) suggests that the lag time between matrix formation and mineralization is approximately 10 days. In order to prepare for mineralization, the matrix undergoes a series of steps during this time. The process of mineralization lasts several months and is divided into two distinct phases (primary and secondary) (Jee, 1988). Approximately 70 percent of total mineralization occurs during the primary phase, which occurs over several days. This phase is thought to be regulated by osteoblasts and osteocytes within the lacunae of the tissue lining the bone matrix. The remaining 30 percent of mineralization occurs during the secondary phase over a period of months. The availability of calcium and phosphorus to the matrix may be the controlling factor during the secondary phase of mineralization. Weryha and Leclere (1995) indicate that various interleukins, prostaglandins, growth factors, and hormones have been associated with the regulation of osteoclast and osteoblast cell activity (Weryha and Leclere, 1995).

In the young actively growing animals, growth plates separate the epiphyses and metaphyses of the long bones. The epiphyseal-metaphyseal complex is the area in which the long bone grows (Currie, 1988). This region consists of five zones, known as the resting, proliferative, maturation, hypertrophic, and calcification zones (Jee, 1988). Each specific zone supplies a distinctive function in the proliferation of the long bone. The resting zone serves as a source of progenitor cells, which have the ability to undergo mitotic division and differentiation (Currie, 1988). The proliferative zone is composed of the resulting differentiated cells. This zone has the ability to synthesize and secrete

cartilage and sustain mitotic division. In the zone of maturation, synthesis and the preparation of the matrix for calcification occurs (Jee, 1988). Cells can be characterized by their enlargement and intracellular glycogen and calcium stores in the hypertrophic zone. The calcium is then removed from the cells and deposited in the cartilage matrix (Currie, 1988). This primary step in the maturation of cartilage to bone occurs in the calcification zone. Osteoblasts and blood vessels then enter the mineralized cartilage. To form calcified bone, osteoblasts then secrete an organic matrix containing lipids, proteins, phosphoproteins, and type I collagen, which then becomes mineralized by the deposition of calcium and phosphorus (Currie, 1988; Marks and Popoff, 1988). It can be considered that elongation of the bone occurs by the growth plate growing away from the diaphysis (Currie, 1988). Eventually, matrix calcification induces the death of chondrocytes due to a lack of available nutrients. The primary bone is absorbed and restored with secondary bone in the metaphysis of the long bone. The growth plate continues to grow until cells of the resting zone can no longer undergo mitotic division. When growth of the long bone ceases, the epiphysis and metaphysis fuse together and the cartilage is replaced with cancellous bone. After proliferation in the bone has ceased, a period elapses during which remodeling occurs as the bone undergoes maturation. After the bone assumes its mature form, the bone is composed primarily of secondary bone. In the horse, closure of the growth plate in the long bone will take place between 9 and 30 months of age, depending on the specific bone (Evans et al., 1990).

Findings would indicate that the metacarpal bone reaches maximum ash content, cortex area, and failure stress resistance at an age of 4 to 7 years (El Shorafa et al., 1979). The horse's skeletal system typically will not reach full maturity until approximately 4 yr

of age (El Shorafa et al., 1979). Utilizing Thoroughbred yearlings and 2 yr olds, Nunamaker et al., (1989) showed that the most significant changes in the cross-sectional area and inertial properties occur during this juvenile period. Previous research has demonstrated that BMC increases rapidly the first year of life, and slows down until approximately 7 yr of age when maximal BMC occurs (El Shorafa et al., 1979). Collectively, these studies indicate that the skeletal system of young juvenile horses are very sensitive at a time in which the initial stresses of the breaking and training procedures occur. During this period of growth, the manner in which the bone models and remodels will predetermine whether the juvenile horse will have an optimal skeletal structure when entering training and competition.

The diaphysis of the long bone develops by deposition and degradation of the bone (Currie, 1988). Bone formation occurs on the bone surface inside the periosteum, and resorption occurs on the inner surface of the shaft wall. The internal resorption of the diaphysis results in the development of a cavity for bone marrow. The cortical width of the long bone decreases as the diameter of the medullary cavity expands. Finally, bone formation on the surface increases the thickness and strength of the bone. The flared ends of the long bone occur by mineral deposition on the periosteal surface and osteoclastic resorption on the endosteal surface of the diaphysis. The opposite occurs in the metaphyseal region, in which mineral is deposited on the endosteal surface, while osteoclastic resorption occurs in the periosteal region (Jee, 1988). This therefore results in a long bone with a narrow shaft, flared epiphysis, greater diaphyseal diameter, and an enlarged marrow cavity.

Modification of Bone

The skeletal structure is determined genetically and primarily serves to protect the body's soft tissues, such as the skull and the ribs (Lanyon, 1987). The general shape and composition of load-bearing limbs are also determined genetically. Bones develop into their mature form in the absence of functional loading. An adaptive response to physical strain will enable the structural features of the bone to withstand repetitive strain, preventing damage from occurring. These structural features include girth, cross-sectional shape, cortical thickness, longitudinal curvature, and bone mineral content (Jee, 1988). A specific adaptation to a particular functional strain is the result of bone remodeling. Therefore, as the magnitude of the strain on the bone is reduced, structural damage to the bone will be prevented (Raub et al., 1989).

During the growth of bone, both modeling and remodeling occur simultaneously. Modeling determines the size and shape of bone in the adult animal's body (Jee, 1988). Modeling occurs by the resorption of bone on the bone surface. Previous work determined that drift (shifting of the midshaft), flaring of the ends of long bones, and increasing bone volume during growth are examples of modeling (Jaworksi, 1984). However, the replacement of primary bone by secondary bone, or replacement of old or damaged bone is considered remodeling (Jee, 1988). Approximately 50 percent of the replacement of immature bone with mature bone occurs by 3 years of age in the horse. However, remodeling continues throughout life, as secondary bone is continuously being damaged and subsequently replaced (Riggs and Evans, 1990). In remodeling, bone degradation and formation occur at the same skeletal location (Jee, 1998). No change in the shape or amount of bone deposited occurs during the remodeling process as a result

of the coupling of bone resorption and formation. In contrast, an increase or decrease in the amount of bone or a change in shape of bone will occur with modeling (Jaworski, 1984).

Norwood (1978) determined that during remodeling, bone endures the most stress during loading and sustains maximal strain. Three major phases make up the remodeling cycle, which include; osteoclastic resorption, the short reversal phase, and osteoblastic deposition. Typically, layers of bone are removed and then replaced from the surface of the bone. However, osteoclasts may tunnel their way through the inner cortex of bone, which results in a canal that is subsequently filled by osteoblastic cells, producing a secondary Haversian system (Riggs and Evans, 1990). The adaptive response of remodeling occurs by the recruitment of new osteoblasts and osteoclasts, rather than increasing the activity of established cells. Lanyon (1989) suggests that the specific mechanism linking the mechanical loading stimulus to the activation of the bone cells responsible for the remodeling process is unknown. The functional unit of the remodeling process is referred to as the "bone remodeling unit" (Jee, 1988). This unit includes the amount of bone involved, as well as the group of cells responsible for the amount of bone resorbed and replaced at a particular site.

As strain is applied to the bone, it deforms as a result of the strain. If the shape of the bone returns to its original form after being loaded, the bone is said to have been loaded within its elastic region (Lanyon, 1989). Typically, little modeling or remodeling will occur within this region of elasticity. If the bone responds to an increase in loading to the point in which the bone can no longer resist the change in shape and continues to deform, it has crossed a threshold beyond its elastic region. This is referred to as entering

the yield region, which occurs due to the level of strain being increased to a point beyond what the skeletal structure is accustomed. Continued strain within the yield region without allowing for skeletal adaptation to the more intense activity through remodeling will ultimately result in irreparable damage to the bone. It has been proposed that one remodeling cycle will take approximately 4 mo to complete in the horse (Norwood, 1978). The resorption phase is said to last approximately 1 mo, the reversal phase (preparation for formation) about 1 wk, and the bone deposition phase approximately 3 mo. The bone is most susceptible to injury due to its overall weakness and porosity during the period of time between the start of resorption and completion of bone deposition. The number of Haversian systems that are being actively remodeled will effect the porosity of the bone. As the number of Haversian systems undergoing remodeling increase, the greater the porosity of the bone. As a result, the strain applied to the bone during this period will increase in magnitude (Nunamaker, 1986). The risk of injury increases as the amount of time for the bone to heal and modify itself decreases before additional stress is placed upon the bone (Lanyon, 1984). Irreparable damage may also result from a single massive strain applied to the load-bearing limbs or from failure due to repetitive loading (Lanyon, 1987), such as juvenile racehorses breaking down on the track.

Skeletal Adaptation to Exercise

Wolff's Law indicates that as the biomechanical load on a bone changes, the internal structure of the load-bearing limb will modify in order to accommodate for the new stress placed upon it (Norwood, 1978). The general shape of bone is sufficient in

strength and rigidity to withstand repetitive loading without damage (Lanyon, 1987). The adaptation of the skeletal system in the young performance horse is in response to the strain experienced as a result of training. In order to ensure that the skeletal structure can withstand subsequent loading forces, exercise is necessary to stimulate bone formation (Price et al., 1995). This adaptive response determines the functional characteristics of bone, as well as the tendon and muscle strength needed for the physical activity encountered during training (Lanyon, 1989). The bone cells responsible for the adaptation to exercise recognize and appropriately match the skeletal system to the strain magnitude applied during training (Lanyon, 1987). When the bone is loaded beyond the point to which it was previously strained, the formation of bone will occur. After formation, the amount of strain placed on the load-bearing limbs will be reduced by simply increasing the amount of bone tissue present. Further modifications to the skeletal structure are unnecessary unless the magnitude, rate, or distribution of the strain applied during training changes. Therefore, subsequent loading in the same form will not elicit a greater adaptive response (Lanyon, 1984)

Previous research has determined the effects of a prolonged, moderate intensity exercise program on the composition, mechanical properties, and structural properties of the femur of immature swine (Woo et al., 1981). The study included a non-exercised control group and an exercised group, which experienced 12 mo of conditioning on a treadmill at a level of 65 to 80 percent of maximum heart rate. It was found that non-exercised and exercised groups did not differ in their mechanical properties, overall bone density, and biochemical contents of the femur at the end of the trial. In contrast, the exercised group had a significantly higher cortical thickness, cross-sectional area, total

volume, ash, and calcium content. Therefore, it could be ascertained that the skeletal differences between the groups were caused by an increase in bone quantity, however there was no affect on bone quality. Animals responded to the mechanical loading by increasing the amount of bone tissue present and thus reducing the strain encountered during exercise.

Similar to the results demonstrated by Woo et al. (1981) and McCarthy and Jeffcott (1992) evaluated the effects of treadmill exercise on the equine third metacarpal bone. The structural properties and cellular activity of cortical bone in the third metacarpal of two groups of horses were compared in this study. One group was kept in restricted housing, while the exercised group endured treadmill exercise at near maximal speeds. Investigators reported that exercised horses underwent little cortical bone remodeling. However, an increase in the thickness of the dorsal cortex occurred, indicating that bone formation took place. This data would indicate that exercised horses did not enter the yield region as to cause damage to the bone and induce bone remodeling, however, the strain encountered was high enough to stimulate bone formation in an attempt to adapt to the new strain placed upon the bone.

Buckingham and Jeffcott (1991) evaluated the effects of a long-term submaximal exercise program on the bone mass of yearling Standardbreds compared to non-exercised horses. Although statistically insignificant, bone density increased in the exercised group and decreased in the non-exercised group. Data from this study would indicate that exercise could possibly initiate a trend towards elevating bone strength. Even though the low intensity exercise did not elicit an adaptive response to functional loading, it should be noted that exercise was not detrimental to the skeletal system.

Skeletal Adaptation to Limited Exercise

Mechanical loading must be performed on a routine basis in order to develop an optimal skeletal system (Lanyon, 1989). When strain applied to the bone falls below what is necessary to sustain an optimal skeletal structure, bone formation will slow and bone resorption will occur until the skeletal environment matches the functional load applied to the bone (Rubin, 1984). Bell et al. (1999) concluded that the lack of loading may produce bone that is not prepared for the stress of training and may predispose horses to bone-related injuries. Maenpaa et al. (1988) demonstrated a decrease in serum osteocalcin concentrations when transferring young horses from pasture to stalls, indicating a reduced rate of bone formation associated with decreased physical activity. Bell et al. (1999) concluded that stalling weanlings without exercise prevents normal mineral deposition in the third metacarpal. If the strain on the load-bearing limbs is abrubtly reduced, the skeletal environment will respond by reducing bone mass to a more appropriate level induced by the new strain (Lanyon, 1984). Therefore, examples such as these support the existence of an adaptive response between mechanical loading and the skeletal structure.

Previous research has demonstrated that the body's response to complete skeletal disuse is primarily due to bone resorption and reduced bone mineral content (Rubin and Lanyon, 1984). When the natural strain was removed from an intact rooster ulna by isolation, it resulted in a 12 percent decrease in bone mineral content over a 6 wk period. However, only 4 strain cycles per day were necessary to prevent the resorption mechanism and maintain a consistent level of bone mass. Although statistically insignificant, new bone formation did occur, which results from the coupling of

resorption and formation in the remodeling process. LeBlanc et al. (1990) demonstrated a 10.4 percent reduction in bone mineral content of the calcaneous bone in clinically healthy adult men who were subjected to 17 wk of bed rest. Investigators decreased bone mineral content by .45 percent per wk in a 4 mo deconditioning period in highly conditioned Arabians (Porr et al., 1998). It can be concluded that an optimal skeletal environment for the load-bearing limbs can by maintained only if strain applied to the bone occurs on a constant basis.

Placing yearling horses in stalls in preparation for sales or the commencement of training without any controlled exercise regimen is common in the horse industry. Few studies have examined the skeletal effects of stalling yearlings in combination with different exercise regimens. Concern about the effects of stalling on bone growth in horses was initiated from various research studies evaluating the effects of confinement housing on other livestock species. Knowles and Broom (1990) found that laying hens housed in battery cages were found to have less humeri strength than birds that were kept in a perchery. Also, breaking strength and radiographic density of the humeri from battery caged laying hens were 40 to 50 percent lower than birds housed in two different perchery systems (Fleming et al., 1994). Norgaard-Nielsen (1990) found a similar decrease in humerus breaking strength of 45 percent in caged birds. Likewise, sows housed in stalls were found to have only two-thirds of the humeri and femur breaking strength of group-housed sows (Marchant and Broom, 1996).

Research suggests that when a young, growing horse during growth is limited in the amount of exercise it receives, the bone will respond by remodeling, thus resulting in negative consequences. These consequences may induce a skeletal environment that is

less than optimal and therefore unprepared for the stresses of training and subsequent performance. Obviously, as young horses enter training, their skeletal structure should be prepared for mechanical loading. If functional loading of the limbs is elevated in the young horse, cortical bone will be increased by the modeling mechanisms (Frost, 1997). Additionally, as functional loading decreases, cortical deposition will decrease. Since the modeling response primarily occurs in the young, growing animal, adapting the skeletal structure to stress while the animal is capable of altering bone structure would be appropriate. This practice could aid in the prevention of bone-related injuries. However, if young performance horses are subjected to stalling, thus decreasing bone mineral content, their competitive career could be shortened or even terminated due to bone-related injuries.

Indicators of Skeletal Change

One of the difficulties in detecting changes in bone lies in the lack of accurate, non-invasive tests that can be performed practically on a large number of animals. Current technology allows for the comparison of bone density and relative mineral content by radiography, as described by Meakim et al. (1981). This technology, however, may not be sensitive enough to detect small changes in bone metabolism that may be of physiological significance to the animal (Hiney et al., 2000). Although biochemical markers of bone metabolism may not be able to pinpoint localized changes in a particular bone, they may be useful in monitoring overall physiological changes resulting from exercise (Hiney et al., 2000). Due to the recent increase in investigations involving human osteoporosis and bone loss, non-invasive methods to determine the activity of

osteoclasts and osteoblasts have been developed, which may prove to be of great value in equine research. Current research is being conducted to assess the capabilities of biochemical markers of bone turnover in horses as a supplement to other noninvasive methods (radiographic photometry, photon absorptiometry, and ultrasonography) of assessing bone mineral content. Development of such biochemical markers would provide a complimentary and perhaps more sensitive means of identifying equine athletes at risk of injury (Hiney et al., 2000). Development of sensitive bone assays may aid in detecting periods in which the skeletal system is actively involved in bone resorption and thus the risk of bone-related injury is highest. Assays for biochemical indicators of bone turnover in serum and urine already exist in human medicine for the clinical investigation of osteoporosis and other metabolic bone diseases (Delmas, 1993). These assays are now being utilized for equine research.

There are several relatively new markers of bone activity based on the metabolism of type I collagen, the sole collagen present in the organic matrix of bone. Type I collagen is the most abundant collagen type in the body and the only collagen type found in bones and tendons. It accounts for more than 90% of the organic matrix of bone. In addition, type I collagen is found in loose connective tissues together with other collagen types such as III, V, and VI. In these locations the proportion of type I collagen is the largest. These markers are more specific to distinct events in collagen metabolism and may therefore be of greater applicability than osteocalcin (BGP). Type I collagen originates from a large molecule, type I procollagen, which contains both amino-terminal and carboxy-terminal trimeric extension domains referred to as propeptides (Hiney et al., 2000). The portion of the protein removed from the carboxy-terminal end of the molecule

during post-translation modification is known as the carboxy-terminal propeptide of type I procollagen (PICP). The amount of PICP fragments released into the blood is believed to exist in a stoichiometric relationship to the molecules of type I collagen formed. Concentrations of PICP in the blood should therefore increase during bone formation, as synthesis of type I collagen increases (Hiney et al., 2000). Serum PICP has been correlated with bone formation rate in diseases of increased or decreased bone turnover. Changes in PICP concentrations may reflect different stages of osteoblastic activity. Osteocalcin is more likely involved only with the latter stages of mineralization and not the synthesis of organic matrix. As matrix formation precedes mineralization, increased serum PICP concentrations may signal bone formation long before serum BGP increases (Hiney et al., 2000).

A similar assay based on detection of the cross-linked carboxy-terminal telopeptide of type I collagen (ICTP) has been developed to detect changes in bone resorption (Hiney et al., 2000). ICTP is the carboxyterminal telopeptide region of type I collagen, cross-linked by pyridinoline cross-links and liberated during the degradation of type I collagen. As bone is resorbed and the collagen matrix degraded, these ICTP peptides are liberated into the circulation where they are resistant to further degradation. Concentrations of ICTP have been shown to increase when localized degradation of bone is occurring (Hiney et al., 2000). The application of these biochemical markers of bone formation and resorption in the horse have the possibility of determining normal bone growth patterns in the healthy horse and identifying abnormal bone deterioration.

In addition to the use of biochemical markers of bone metabolism, various noninvasive methods of evaluating bone are available. Radiographs are commonly used

for qualitative evaluation of bone, particularly when a disorder has been encountered, however, they can also be used for quantitative estimates of changes in bone mineral content (Nielsen et al., 1997). Using dorsal and palmar radiographs, Meakim et al. (1981) developed a method to evaluate radiographic bone aluminum equivalence, a measurement of bone mineral content. In that study, it was determined that radiographic bone aluminum equivalences are highly correlated with bone mass. Williams et al. (1991) found a correlation coefficient (r) between bone mineral content and radiographic photometry of .967 ($P<.0001$) in the third metacarpal of cattle. This value was shown to be higher than r-values between bone mineral content and two other noninvasive methods evaluated, photon absorptiometry (.908, $P<.0001$) and ultrasonography (.406, $P<.0001$). Research has demonstrated that radiographic bone aluminum equivalence of the third metacarpal is an appropriate method of evaluating the changes in bone mineral content and skeletal architecture that occur during normal bone growth (Meakim et al., 1981; Frey et al., 1992) and exercise (Nielsen et al., 1997).

Single photon absorptiometry has been shown to be effective in determining bone mineral content of the third metacarpal (Jeffcott et al., 1987). However, when utilizing this method, preparation of the horse in combination with the procedure requires approximately 30 min per horse. Also, horses must be sedated to facilitate the most accurate bone scan possible. Therefore, this method is considered impractical in the research setting, as large numbers of animals are utilized.

A method for determining cross-sectional area of the third metacarpal using ultrasonography has been reported (McCartney and Jeffcott, 1987). In comparison to single photon absorptiometry, ultrasonography is considered to be a relatively simple and

consistent technique for evaluating changes in the skeletal architecture. However, ultrasonography measures are volumetric in nature, thereby limiting its use. Previous research would suggest that the use of radiographic photometry to evaluate changes in the skeletal architecture and estimate bone mineral content of the third metacarpal in the horse may be the most efficient and effective technique in the research setting.

The force plate can measure a wide range of effects in the horse. The same instrument can record forces from more than a ton in the galloping animal to 25 g associated with the action of the heart (Pratt et al., 1976). Force plate measurements can be taken at the walk, trot, canter, and even at rest. Measurements on the horse at rest reveals the forces associated with the beating of the heart, which propagate through the legs to the plate. If a differential measurement is made (e.g. force on one front leg is subtracted from the force on the other front leg), feedback forces by which the animal stays erect can be observed (Pratt et al., 1976). Ground reaction force data measures specific aspects of limb behavior. The net joint moment describes the torque produced primarily by the soft tissues such as muscles, ligaments, and tendons. The joint power is a measure of the mechanical work performed in the horse.

Since the early force plate evaluation of equine locomotion reported by Pratt et al., (1976), other researchers have used force plate analysis to quantify normal and abnormal gait patterns in the horse (Merkens et al., 1985; Schamhardt and Merkens, 1987; Merkens and Schamhardt, 1988a; Morris and Seeherman, 1987; Merkens et al., 1993). Previous studies of horses have identified abnormalities of movement by consideration of individual parameters on ground reaction force. Merkens and Schamhardt (1988b) used asymmetry of forelimb peak vertical forces to quantify the

degree of lameness induced in an experimental situation (Williams et al., 1999). Obviously, human judgment of performance is primarily subjective, which prevents a quantitative evaluation. A force plate can be used to quantify objectively the loading pattern of each of the four limbs (Merkens et al., 1988). Researchers have shown that changes in the peak vertical ground reaction forces were proportional to the severity of arthritic conditions. Silver et al. (1983) used the force plate to evaluate a model of tendonitis and monitored recovery after a variety of treatments using multivariate statistics. These studies enabled the detection of subtle differences in the loading pattern of the lame limb, which were not evident using conventional clinical observations (Merkens et al., 1988). The equine limb is a highly complex structure, and historically, often the only way of perceiving that the horse was suffering injury or disease was by subjective clinical opinion (Williams et al., 1999). Mean peak vertical force was evaluated in the current study in order to possibly observe any subclinical lameness that might occur before becoming visually apparent.

CHAPTER III

MATERIALS AND METHODS

Management of Animals

Sixteen Quarter Horse yearlings were utilized in a 90-d experiment to evaluate the effects of different exercise protocols on bone density, parameters of growth, and serum markers of bone metabolism. Horses had a mean age of 14 mo with a range of 13 to 15 mo. Horses were paired by age and weight and randomly allotted to two treatment groups, exercised (EX) and non-exercised (NEX). Both groups were housed in 3.6 X 3.6 m box stalls throughout the experimental period. Horses were fed at 2.5% of body weight (BW) per day, which was divided into two equal feedings (7am and 7pm). Each pair's daily feed intake was adjusted according to the highest body weight. The diet consisted of 50% concentrate, 30% prairie grass hay, and 20% alfalfa cubes. Diets were formulated to meet or exceed NRC requirements for yearling horses during moderate growth. Daily refusals of concentrate, hay, and cubes were weighed back 12 hr after each feeding and recorded to calculate daily intakes. Prior to starting the trial, horses were allowed a 45 d acclimation period on native pasture and received the concentrate portion of the experimental diet along with free-choice prairie grass hay. During this time, they received an initial vaccination and 30 day booster of rhinopneumonitis, tetanus, influenza, encephalomyelitis, and streptococcus equi. In addition, they were dewormed, trimmed, and introduced to the routine feeding schedule.

Exercise Protocol

During the 45 d acclimation period, all horses were introduced to the 18 m diameter round pens and taught to longe. During this stage, horses were asked to walk, trot, and canter in both directions for approximately 10-15 min depending upon the progress of the horse. Following the acclimation period, horses were paired by weight and age and then randomly allotted to either the EX or NEX treatment (8 horses per treatment). One horse from the EX group was removed after the first 45 days of the trial due to severe lameness caused by epiphysitis.

During the first 45 days of the trial, horses in the EX group were longed in round pens for 15 min prior to the morning feeding, six days per week. Horses were exercised at the trot and canter in both directions for an equal amount of time. The NEX group was turned out into 30.5 X 30.5 m dry lots after the morning feeding for 3 hrs per day, six days per week. On off days, all horses were maintained on the normal feeding schedule. During the second 45 days of the trial, horses in the EX group were increased from 15 min of longeing to 20 min per day as horses were becoming increasingly fit. Horses continued to be exercised at the trot and canter in both directions for an equal amount of time.

Sample Collection

Dorsal-palmar and lateral-medial radiographs of each horse's left front leg were taken to determine radiographic bone aluminum equivalence (RBAE) of the lateral, medial, dorsal, and palmar cortices of the third metacarpal (MCIII). Blood samples were taken via jugular venipuncture every 14 d to determine serum ICTP and PICP

concentrations. Seventy-two-hr fecal collections were taken at the beginning of periods I (d 0), II (d 45), and III (d 90). Peak vertical force was measured at the beginning of each period. Peak vertical force was determined by subjecting horses to the force plate in which the average of 6 strikes was calculated. Body weight, wither, hip, hock, knee, and shoulder height and heartgirth circumference were recorded for each horse every 7 d.

Fecal Collections

Seventy-two hr fecal collections were conducted at the beginning of periods I, II, and III. Samples were collected every 30 min throughout the 72-hr period and the total fecal collection was weighed every 24-hrs. At that time, grab samples were taken in duplicate and frozen for subsequent analysis. Samples were unthawed and placed in a drying oven at 60°C for 72-hrs. During the drying period, samples were mixed thoroughly every 12-hrs to prevent scorching. Samples were then ground, stored, and subsequently analyzed for calcium and phosphorus concentrations. Horses in the EX group were exercised as determined by protocol during the 72-hr collection period. If the horse defecated during exercise, the sample was collected and included in the total fecal collection, but was not included in the grab samples for analysis. Horses in the NEX group were walked on a mechanical walker for 20 min per d during the 72-hr collection period. If the horse defecated during exercise, the walker was immediately turned off and the sample was collected. The sample was included in the total fecal collection, but was not included in the grab samples intended for analysis. For feed (CP, DE, Ca, P, Mg, K, Na, and Cl) and fecal (Ca and P) mineral analysis, 1 gram of the composited sample was weighed out into pre-dried beakers, dried at 60° C for 24 hr and then weighed again to

determine a final dry weight. Samples were ashed in a muffle furnace at 500° C for 4 hr then 3 ml of 6N HCL was added to the ash residue and evaporated to dryness at 100°-200°. Minerals were extracted with an acid solution (1.5N HNO_3+0.5N HCL) and determined using Inductively Coupled Plasma Spectroscopy (ICAP 61, Thermo Jarrell Ash).

Blood Collection and Analysis

Blood samples were collected every 14-d and analyzed for serum ICTP and PICP concentrations. Samples were collected via jugular venipuncture in plain glass Vacutainers™. The blood was allowed to clot at room temperature, and then centrifuged at 0°C for 15 min. Serum was then aspirated from the top of the sample and frozen at –20°C for subsequent analysis. Commercial radioimmunoassays were used to quantitatively determine ICTP and PICP concentrations in the serum (Diasorin, Stillwater, MN). This kit had not been previously validated for use in horses. However, our lab was successful in executing recovery of mass and parallelism by assaying equine serum. Recovery of equine PICP and ICTP standard was linear. Also, our lab documented dilutional parallelism by assaying equine serum at 1:5 and correcting the mean result for dilution.

Radiographs

A radiographic photometric technique for estimating bone mineral content (BMC) of the third metacarpal (MCIII) was utilized in this study (Meakim et al., 1981). Dorsal-palmar and lateral-medial radiographs of the left third metacarpal were taken to

determine RBAE values as described by Meakim et al. (1981). An aluminum stepwedge penetrometer was exposed simultaneously with each radiograph for use as a standard of reference. This provided the necessary standard for comparison of radiographs. Dorsal-palmar views were taken with the cassette parallel to the front of the leg and centered midway between the proximal and distal ends of the third metacarpal. Lateral-medial views were taken with the cassette perpendicular to the front of the leg and centered midway between the proximal and distal ends of the third metacarpal.

A Bio-Rad Model GS-650 imaging densitometer (Bio-Rad Laboratories, Hercules, CA) was used to scan the radiographs at the nutrient foramen of the third metacarpal. A logarithmic regression was developed by using the thickness of the steps on the stepwedge, which provided a means to estimate bone mineral content in RBAE at the maximum optical density reading to the dorsal, palmar, lateral, and medial cortices (Meakim et al., 1981).

The scans of the dorsal-palmar and lateral-medial radiographs of MCIII and the aluminum stepwedge penetrometer were used to measure total RBAE in mm^2 Al. The area under the stepwedge curve corresponding to the steps with thicknesses of 26, 23, 20, 17, and 14 mm Al and the total area under the curve of the third metacarpal was determined. The total area under steps 4-8 from the stepwedge scan was 1270 mm^2. The total RBAE was then determined by multiplying the total area under the curve of MCIII by 1270 mm^2 Al and then dividing by the area under the stepwedge curve.

Force Plate

Utilizing a piezoelectric force plate measuring 900 X 1200 mm (Kistler Instrumente AG, Winterhur, Switzerland), ground reaction force (GRF) patterns were recorded for the left forelimb at a set velocity of 2.1-2.5 m/s. The force plate was mounted centrally in a 20 m runway covered with high-density rubber matting. An experienced handler led the horses at a trot, maintaining a consistent velocity through the data collection area. In a successful pass, the left fore hoof landed entirely on the force platform, without any other hoof being in contact simultaneously, and with the horse moving straight and consistently. Six successful passes were recorded for each horse in order to remove any individual variation among passes (Clayton et al., 1998; Merkens et al., 1988). Mean peak vertical force (MPVF) was then calculated by averaging the peak vertical force for each successful pass. Data for MPVF were expressed as a percent of body weight (BW) in order for comparison between treatments.

Feed and Growth Data

I. Wither height- the vertical distance from the ground to the highest protruding thoracic vertebrae in centimeters (cm).

II. Hip height- the vertical distance from the coronary band on the posterior side of the hoof to the furthest protruding point of the buttocks in cm.

III. Hock height- the vertical distance from the coronary band on the posterior side of the hoof to the point of the hock in cm.

IV. Shoulder height- the vertical distance from the coronary band on the anterior side of the hoof to the point of the shoulder in cm.

V. Knee height- the vertical distance from the coronary band on the anterior side of the hoof to the end of the distal radius in cm.

VI. Body weight- weight determined at a single weighing, 6 hours after the morning feeding, and recorded to the nearest pound.

VII. Heartgirth- the circumference of the thorax immediately posterior to the front leg in cm.

Table I. Composition of diet, as fed basis	
Ingredient (%)	
Cracked Corn	14.15
Wheat Midds	14.15
Alfalfa Meal	12.50
Soybean Meal	8.45
Limestone	0.13
Trace Mineral Salt	0.25
Dicalcium Phosphate	0.37
Prairie Grass Hay	30.00
Alfalfa Cubes	20.00
Nutrient	
DE, Mcal/ kg	2.60
CP, %	16.40
Ca, %	0.84
P, %	0.32
Nutrient: Calorie Ratio (g/ Mcal DE)	
CP/ Mcal DE	60:1
Ca/ Mcal DE	3.2:1
P/ Mcal DE	1.2:1

Statistical Analysis

Data for biochemical bone markers, radiographs, force plate measurements, blood, growth parameters, dry matter digestibilities, and mineral balance were analyzed as repeated measures using PROC MIXED analysis (SAS, 1996). Physiological differences were analyzed for effects of treatment, period, and treatment by period interactions according to the mixed method of SAS. Differences between treatments were detected using least significant differences. Also, differences between treatments at ($P<.05$) were considered significant, while differences at ($P<.10$) were noted and discussed as trends. In order to determine changes from initial RBAE values, data were normalized by subtracting d 0 values from all other subsequent days. Results in tables are plotted with the SEM indicated for each mean.

CHAPTER IV

RESULTS & DISCUSSION

Feed and Mineral Intakes, Fecal Output, and Digestibilities

Data for changes in dry matter intake (DMI), fecal output (FO), and dry matter digestibility (DMD) in response to exercise are shown in table 2. No overall treatment differences were observed in DMI, FO, or DMD within periods I, II, or III. Data for changes in calcium intake, output, and digestibility in response to exercise are shown in table 3. Calcium intake, output, and digestibility did not differ ($P>.05$) between treatments during periods I, II, or III. Data for changes in phosphorus intake (PI), phosphorus output (PO), and phosphorus digestibility (PD) in response to exercise are shown in table 4. No differences in PI, PO, or PD were found in periods I and II. However, PD was higher ($P<.05$) for NEX horses as compared to EX horses in period III. This can be attributed to the numerically higher PI and lower PO of NEX horses. Cooper et al., (1998) also reported a low phosphorus digestibility (16%) in horses at 330 days of age. It was also reported that phosphorus digestion and retention decreased significantly with increasing age (Cooper et al., 1998). Furthermore, Wall et al., (1997) found that growing horses sampled at 240, 300, and 360 days of age were found to have extremely low phosphorus digestibilities, ranging from 7 to 17%.

Table II. Effect of exercise on dry matter intake (DMI), fecal output (FO), and dry matter digestibility (DMD)[ab]			
Period I	EX	NEX	SEM[c]
DMI, kg/d	8.66	8.78	.20
FO, kg/d	2.68	2.76	.16
DMD %	68.97	68.31	1.74
Period II			
DMI, kg/d	9.50	9.65	.20
FO, kg/d	2.59	2.71	.16
DM Dig, %	72.64	71.89	1.74
Period III			
DMI, kg/d	9.98	10.42	.20
FO, kg/d	2.98	2.89	.16
DM Dig, %	70.30	72.15	1.74

[a]Values are least squares means.

[b]Means within a row do not differ (P>.05).

[c]Values are average standard errors.

Table III. Effect of exercise on Ca intake (CaI), Ca output (CaO), and Ca digestibility (CaD)[ab]			
Period I	EX	NEX	SEM[c]
CaI, g/d	72.69	73.69	1.72
CaO, g/d	33.02	33.54	2.48
CaD %	54.66	54.47	2.83
Period II			
CaI, g/d	79.70	80.92	1.72
CaO, g/d	34.95	35.38	2.48
CaD %	56.36	56.41	2.83
Period III			
CaI, g/d	83.78	87.43	1.72
CaO, g/d	39.41	35.72	2.48
CaD %	53.35	59.02	2.83

[a]Values are least squares means.

[b]Means within a row do not differ (P>.05).

[c]Values are average standard errors.

Table IV. Effect of exercise on phosphorus intake (PI), phosphorus output (PO), and phosphorus digestibility (PD)[a]			
Period I	EX	NEX	SEM[d]
PI, g/d	27.69	28.07	.65
PO, g/d	20.64	20.64	1.22
PD %	25.65	26.51	3.75
Period II			
PI, g/d	30.36	30.83	.65
PO, g/d	21.74	23.21	1.22
PD %	28.25	24.82	3.75
Period III			
PI, g/d	31.92	33.30	.65
PO, g/d	25.55	22.17	1.22
PD %	20.25[b]	32.38[c]	3.75

[a]Values are least squares means.

[bc]Means within a row with different superscripts differ (P<.05).

[d]Values are average standard errors.

Growth Parameters

Changes in growth parameters in response to exercise are shown in table 5. Throughout the trial, there were no significant differences in average daily gain (ADG), wither height, hock height, shoulder height, or heartgirth circumference. However, horses in the EX group had a significantly lower change in body weight (57.84 vs. 78.52 kg) and a larger (P<.05) change in hip height (8.13 vs. 5.78 cm) as compared to NEX horses. Additionally, final knee height was greater (P<.05) at the end of the trial for the NEX horses versus the EX. Moffett et al., (2002) reported similar results in yearling Quarter

Horses for ADG (.71 kg/d). In the present study, the significantly larger change in body weight seen in NEX horses may be explained by the fact that these horses were becoming fatter due to the lack of exercise at an equal energy intake.

Table V. Changes in growth parameters in response to exercise[a]			
BW (kg)	**EX**	**NEX**	**SEM[d]**
Initial	352.39	348.46	12.36
Final	410.23	426.99	11.92
Change	57.84[b]	78.52[c]	7.10
ADG (kg)	.73	.86	.09
Wither Height (cm)			
Initial	136.56	137.58	1.13
Final	143.28	143.59	1.13
Change	6.72	6.02	.49
Hip Height (cm)			
Initial	138.28	141.09	1.46
Final	146.41	146.88	1.17
Change	8.13[b]	5.78[c]	.67
Hock Height (cm)			
Initial	53.13	54.69	.81
Final	55.47	56.41	.61
Change	2.34	1.72	.63
Knee Height (cm)			
Initial	37.97	38.91	.81
Final	40.47[b]	42.66[c]	.63
Change	2.50	3.75	.58

Shoulder Height (cm)			
Initial	94.69	95.00	1.07
Final	98.75	100.94	.98
Change	4.06	5.94	.72
Heartgirth (cm)			
Initial	159.38	157.34	1.94
Final	168.91	169.69	2.04
Change	9.53	12.34	1.09

[a]Values are least squares means.

[b]Means within a row with different superscripts differ (P<.05).

[d]Values are average standard errors.

Force Plate

Data for changes in mean peak vertical force (MPVF) in response to exercise are shown in table 6. No differences (P>.05) between treatments were found in MPVF within periods I and III. However, the NEX horses had a higher (P<.05) MPVF during period II as compared to the EX group. Data for changes in MPVF over time are shown in figure 1. No differences (P>.05) were found between periods II and III in the EX treatment. However, EX horses differed (P<.05) between period I versus II and III. No differences (P>.05) were found between periods I and II in the NEX treatment. However, NEX horses differed (P<.05) between periods I and II versus III.

Mean peak vertical force was evaluated in order to possibly detect any subclinical lameness that might occur before becoming visually apparent. These data demonstrated that MPVF decreased at a faster rate in the EX versus NEX horses, which may indicate an adaptive response to exercise. Although subjective, it was noted that, following period

II, EX horses tended to be somewhat sore when exercised. Furthermore, one horse was removed from the EX treatment after period II (d 45) due to clinical physitis.

Table VI. Changes in mean peak vertical force (MPVF) in response to exercise[a]

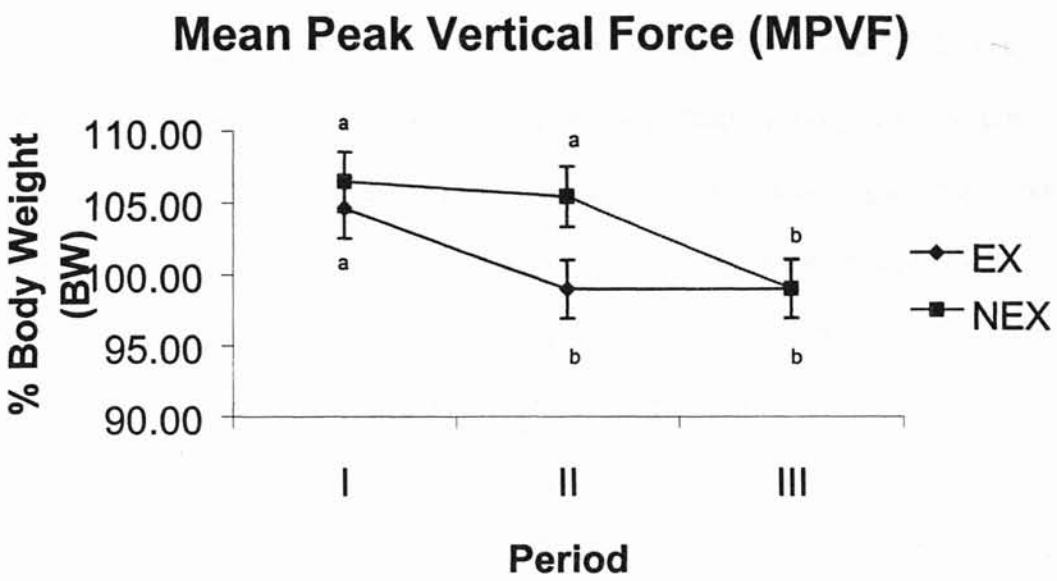
	EX	NEX	SEM[d]
Period I	104.65	106.47	2.074
Period II	98.95[b]	105.43[c]	2.074
Period III	99.00	99.03	2.074

[a]Values are least squares means.

[bc]Means within a row with different superscripts differ (P<.05).

[d]Values are average standard errors.

Figure I. Changes in mean peak vertical force (MPVF) over time.



[a,b]Means with different superscripts differ (P<.05) between periods.

Serum ICTP and PICP

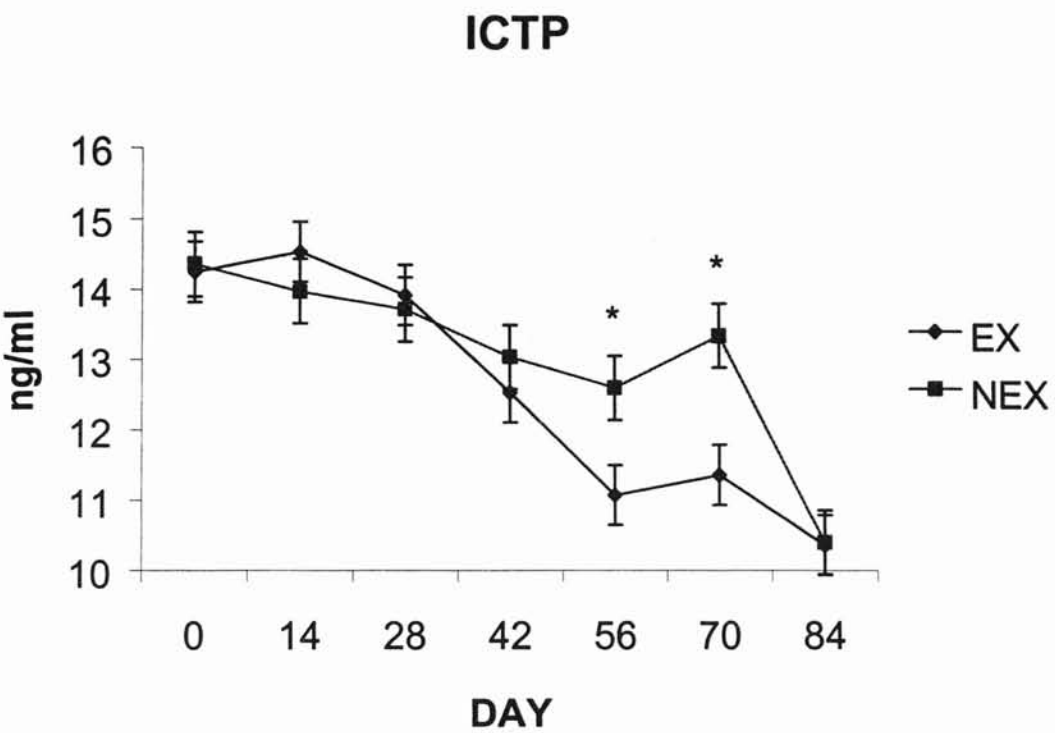
Data for changes in serum ICTP and PICP in response to exercise are shown in figures 2 and 3, respectively. Both EX and NEX horses experienced a decrease (P<.0001) in serum ICTP concentration with day of trial. The concentration of ICTP in the serum was similar (P>.05) between treatments during the first half (d 0-42) of the trial and at the end (d 84). However, serum ICTP concentration in the NEX horses was higher (P<.05) on days 56 and 70 as compared to EX horses. Serum PICP decreased (P<.05) between d 56 and d 84 in both treatments. The serum concentration of PICP was not different (P>.05) between the EX and NEX horses on d 0, 14, 28, 42, and 84. Yet, PICP for NEX horses was higher (P<.05) on d 56 as compared to EX horses.

Although biochemical markers of bone metabolism may not be able to pinpoint localized changes in a particular bone, they may be useful in monitoring overall physiological changes resulting from exercise (Hiney et al., 2000). Serum ICTP is a biochemical marker of bone resorption, indicating osteoclastic activity. Thus, serum concentrations of ICTP have been shown to increase when localized degradation of bone occurs (Hiney et al., 2000). In the present study, ICTP values in both treatments decreased significantly across time. Hiney et al., (2000) reported similar results in both exercised and non-exercised yearling horses in which serum ICTP concentrations dropped significantly by d 98 of the trial. Michael et al., (2001) further demonstrated that concentrations of ICTP were lower during the second half (d 60-128) as compared to the first 60 d of the trial in both groups of horses. In the current study, increased concentrations of ICTP in NEX horses on d 56 and d 70 indicate a higher rate of bone loss as compared to EX horses. These data may indicate that exercise minimizes the effects of stalling on bone loss. In contrast, Mathison-Kochan et al., (2001) demonstrated a significant increase in serum ICTP due to onset of race training thus, indicating an increased rate of bone resorption and remodeling. This difference may be explained by the fact that horses in the present study were not exercised at the same level of intensity or duration as horses in the previously mentioned trial.

Serum PICP is a biochemical marker of bone formation, indicating osteoblastic activity. Concentrations of PICP in the blood should therefore increase during bone formation as synthesis of collagen increases. In the present study, the significant decrease in serum PICP concentrations between d 56 and d 84 would indicate a decreased rate of bone deposition in both treatments. These data agree with Hiney et al., (2000) who found

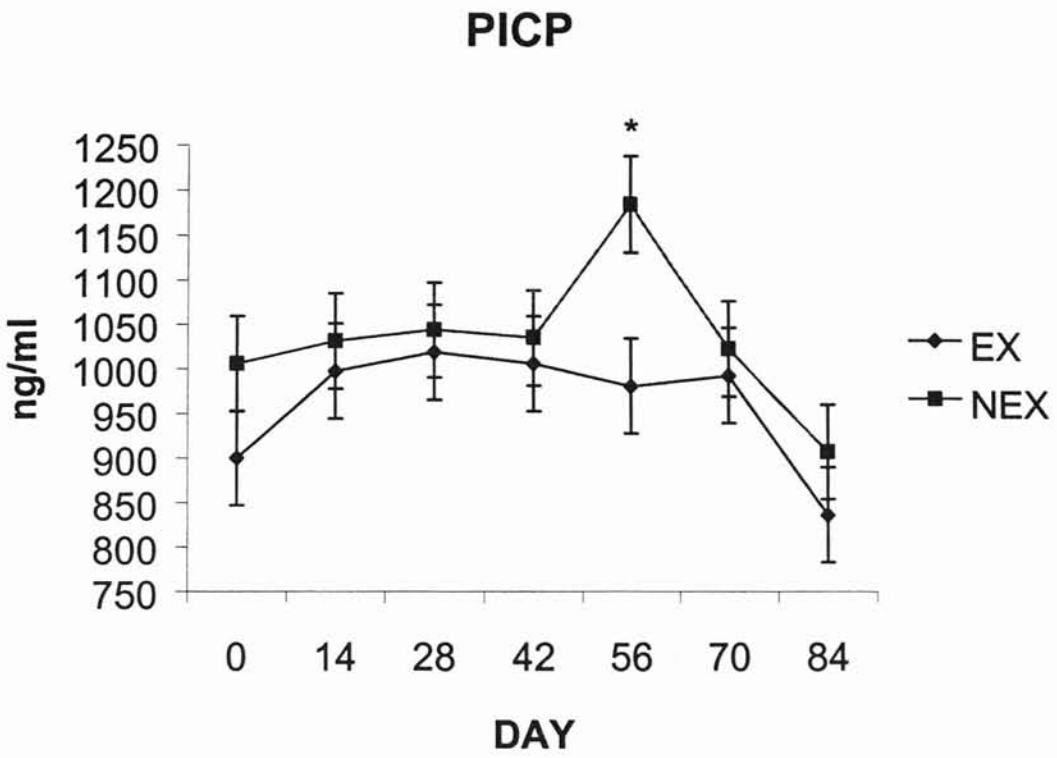
that PICP decreased significantly between d 56 and d 98 for both exercised and non-exercised horses. These findings would therefore suggest a slowdown in the rate of bone formation due to the effect of stalling over time. Similar to ICTP, serum concentrations of PICP in NEX horses were significantly higher than EX horses on d 56. In contrast, Hiney et al., (2000) found no difference in PICP from d 0 to d 98 between exercised and non-exercised yearling horses. The decrease in ICTP between d 0 and d 84 and PICP between d 56 and d 84 would indicate an overall slowing of bone turnover. However, the enhanced values of these serum markers observed in NEX horses may indicate a higher rate of bone turnover when compared to EX horses. Consequently, exercised horses may have a more stable rate of bone metabolism when confinement housing is utilized.

Figure II. Changes in ICTP concentration in response to exercise.



*Means within a day differ (P<.05) between treatments.

Figure III. Changes in PICP concentration in response to exercise.



*Means within a day differ (P<.05) between treatments.

Radiographic Bone Aluminum Equivalence (RBAE)

Changes in RBAE in response to exercise are shown in table 7. Within periods I, II, and III, no significant difference in RBAE was observed in the lateral, medial, dorsal, or palmar cortices. Data for changes in lateral, medial, dorsal, and palmar RBAE's over time are shown in figures 4, 5, 6 and 7, respectively. In order to determine changes from initial RBAE values, data were normalized by subtracting d 0 values from all other subsequent days. No difference between periods I, II, or III occurred in the lateral or medial cortices. However, the RBAE values of the dorsal cortex was found to be higher ($P<.05$) in period I than period III in the NEX treatment. The RBAE values between periods II and III tended ($P<.10$) to decrease for both groups in the dorsal cortex. In the palmar cortex, RBAE values for NEX horses were higher ($P<.05$) in period I as compared to period III. Furthermore, both treatments tended ($P<.10$) to decrease from period II to period III.

Although statistically insignificant, numerically higher RBAE values would suggest that EX horses may have a greater BMC as compared to NEX horses in periods II and III. Previous research has demonstrated an increase in RBAE in the dorsal and palmar cortices following 90 d of exercise (Nielsen et al., 1997). Furthermore, Hiney et al., (2001) demonstrated a greater overall bone density in exercised calves as compared to group housed or confined calves throughout the duration of the trial. While overall bone geometry and density were the least optimal in the confined calves, they were not statistically different from the group-housed calves, with the exception of the percentage of cortical area of the bone. However, running these calves 50 m down a concrete runway, 5 d per wk, appeared to positively influence both bone mass and structure even

beyond the calves allowed free exercise. These data would suggest that only short periods of high-intensity exercise are needed to cause an adaptation in the bones of young, growing animals. In the present study, no differences between treatments were found in RBAE within periods, however, changes in RBAE across periods demonstrated a tendency for decreased bone density in both the EX and NEX horses between periods II and III. Bone mineral content has been shown to decrease significantly following approximately 60 d of training (Nielsen et al., 1997; Nielsen et al., 1998). Also, significant decreases in BMC have been observed in horses following 28-56 d of stalling when compared to pasture reared horses (Hoekstra et al., 1999). This decrease may indicate that stalling horses for 90 d results in a decreased BMC, which could predispose horses to subsequent injury. Furthermore, the significant decrease in bone density observed in the NEX horses throughout the duration of the trial would indicate that stalling and lack of exercise may result in a greater loss of BMC as compared to the forced exercise horses.

Table VII. Changes in radiographic bone aluminum equivalence (RBAE mm^2 Al) in response to exercise[a]			
Period I	EX	NEX	SEM[b]
Lateral	23.01	21.97	0.88
Medial	22.22	20.21	0.93
Dorsal	20.80	21.15	1.03
Palmar	20.13	20.49	1.09
Period II			
Lateral	24.34	23.46	0.88
Medial	22.35	21.27	0.93
Dorsal	22.15	20.00	1.03
Palmar	21.61	19.24	1.09
Period III			
Lateral	23.18	21.09	0.88
Medial	20.99	20.08	0.93
Dorsal	19.66	17.53	1.03
Palmar	18.89	16.82	1.09

[a]Values are least squares means.

[b]Values are average standard errors.

Figure IV. Change in lateral RBAE over time.

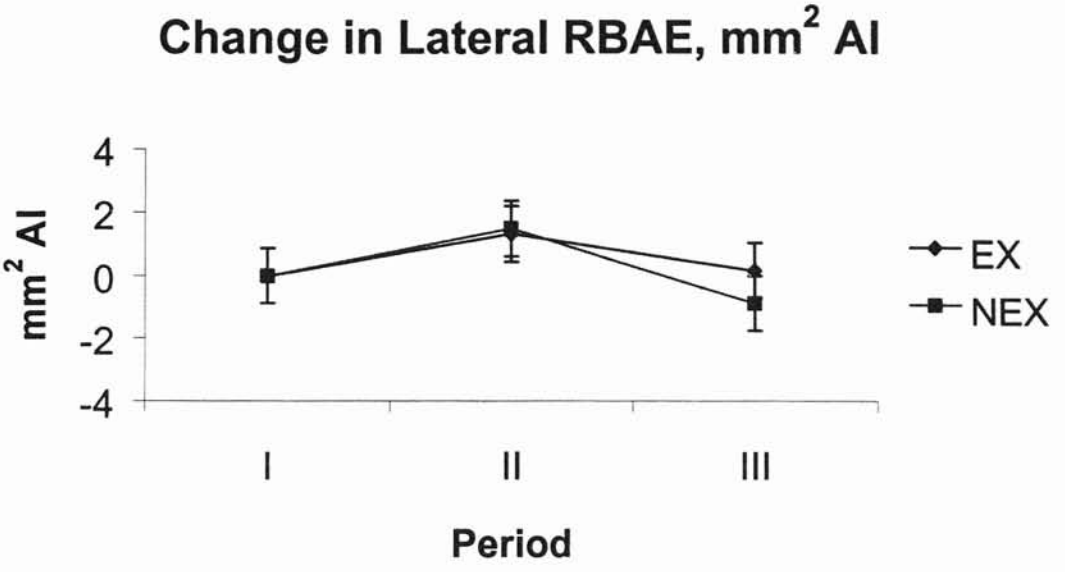


Figure V. Change in medial RBAE over time.

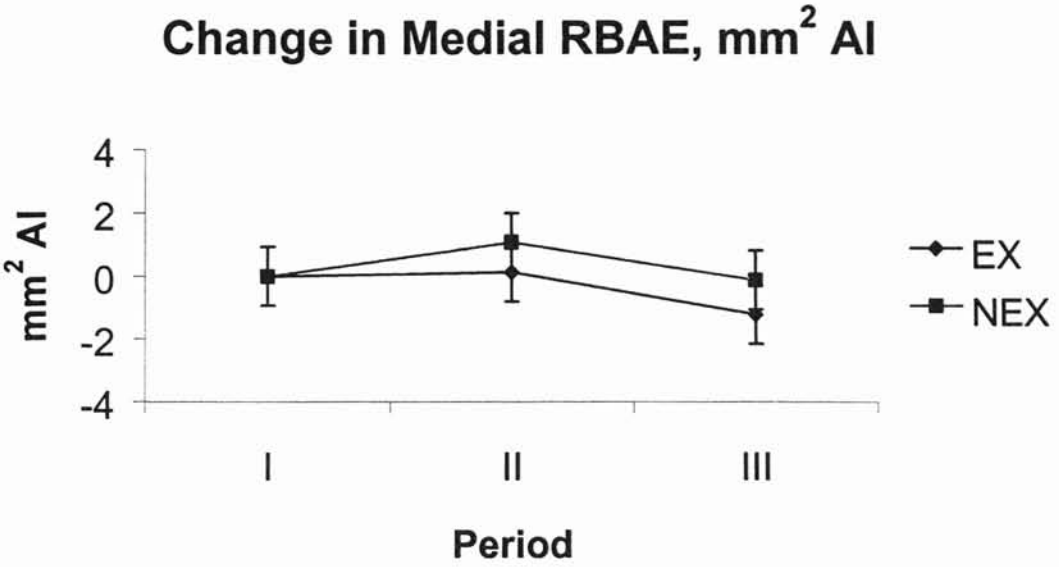
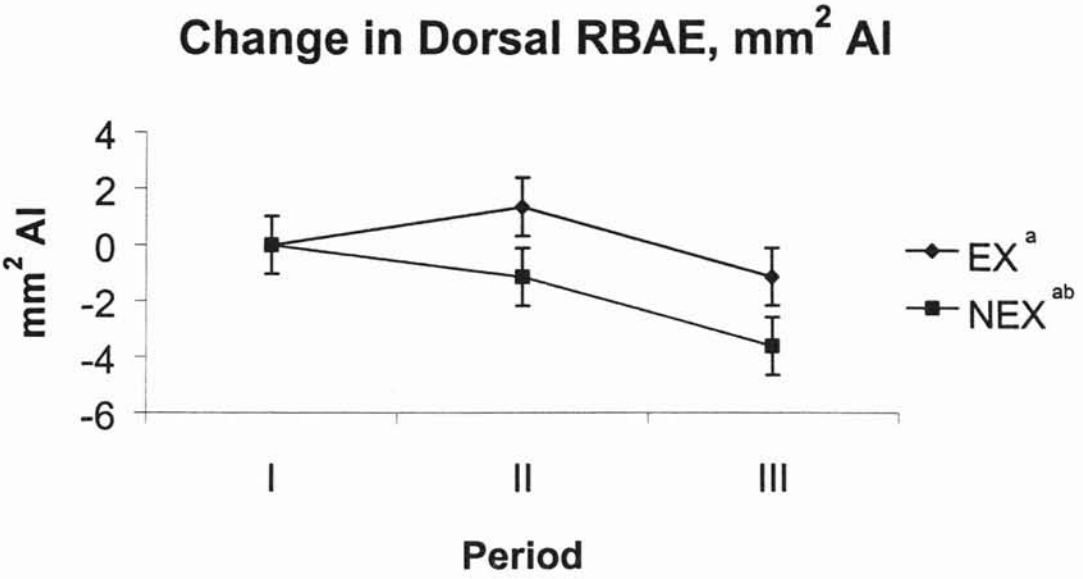


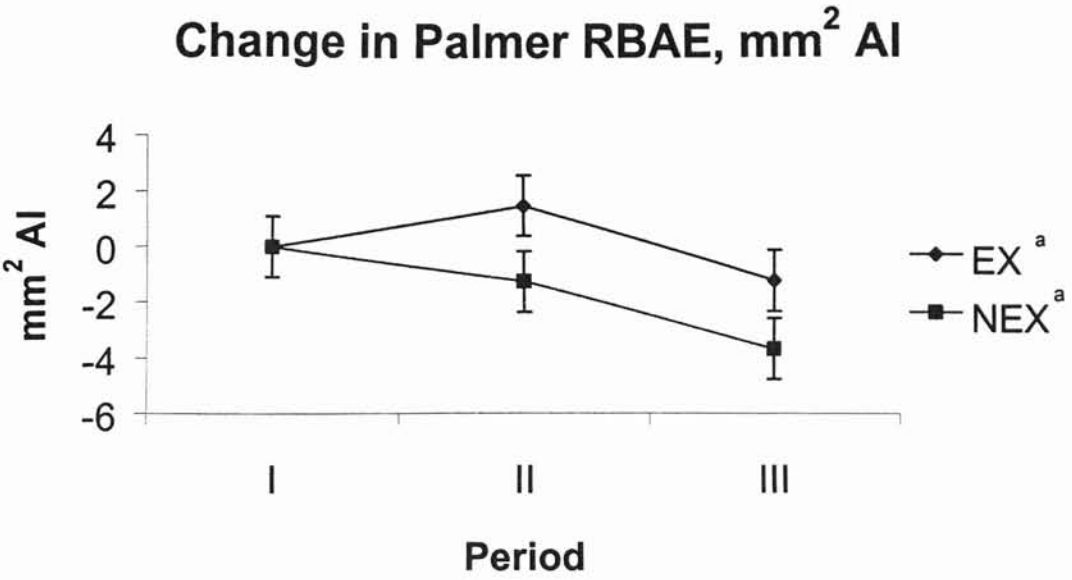
Figure VI. Change in dorsal RBAE over time.



[a]Means tended (P<.10) to be different between periods II and III.

[b]Means differ (P<.05) between periods I and III.

Figure VII. Change in palmar RBAE over time.



[a]Means tended (P<.10) to be different between periods II and III.

[b]Means differ (P<.05) between periods I and III.

CHAPTER V

CONCLUSIONS

Young, growing horses are routinely housed in stalls in preparation for the onset of training or yearling sales without exercise more intense than mechanical walking. Results of the current study suggest that housing yearling horses in box stalls with limited, free exercise may negatively affect bone metabolism compared to that experienced by horses forced to exercise on a daily basis.

Burr et al., (1989) concluded that limiting a horse's access to exercise may result in a reduced strain applied to the load-bearing limb, thereby stimulating reduction in BMC as part of the bone's adaptive response. Implications to the industry involve not only yearlings housed in stalls, but also young performance horses confined to stalls following an injury and subsequently returned to training. In order to prevent reoccurring or additional injuries, Moyer and Fisher (1991) suggest that training should resume at the same level at the time in which the injury occurred to account for the loss of bone associated with the reduction in physical activity. After evaluating prolonged bed rest in healthy male subjects, Bloomfield (1997) reported the loss in bone mass was not recovered until after 6 mo of normal weight bearing activity. Weinreb et al., (1997) indicated that the rate of bone loss was much faster than the rate of bone mass recovery in young, growing rats. Researchers found that after only 9 d of unloading the hind limb by external fixation, recovery of bone mass did not occur until approximately 2 to 3 weeks.

Allowing free access to exercise for young, growing horses confined in stalls, may not elicit the strain on load-bearing limbs necessary to allow adequate skeletal development. Further research is necessary to determine what velocity and duration of exercise is necessary to establish a skeletal system that is better developed to handle the strain during training or performance. Providing the young, growing horse with an environment more beneficial to bone development may result in a skeletal system that is capable of withstanding the strains induced during training. However, if a form of forced exercise is not an option or is out of the manager's control, training must be adapted to the horse accordingly to account for the possibility of bone loss experienced as a result of lack of exercise. Ordidge (1985) suggests that the younger the horse, the greater the possibility for bone-related injuries as well as bone formation, thus conditioning programs must be evaluated carefully.

The overall results from this trial indicate that stalling may adversely affect bone metabolism. Furthermore, horses with limited, free exercise may experience a higher rate of bone resorption and consequently a lower BMC. Thus, horses confined to stalls may benefit from a forced exercise regimen and therefore be better prepared for future mechanical stress.

CHAPTER VI

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