DIETARY CALCIUM AND CATION-ANION DIFFERENCE INFLUENCES CALCIUM STATUS AND BONE REMODLING IN EXERCISING AND SEDENTARY ARABIAN HORSES

by

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(ABSTRACT)

Diet and exercise are two manageable factors that affect bone remodeling. Experiments were designed to test the hypothesis that bone density and calcium status would be affected by conditioning and deconditioning, and by dietary Ca and cation-anion difference (DCAD). In experiment one, 12 Arabian horses were conditioned for 12 wk on a high speed equine treadmill. Diets were designated LH, which contained low Ca (.35%)-high Cl (.6%), LL, low Ca-low Cl (no added Cl), HH, high Ca (.7%)-high Cl, and HL, high Ca-low Cl. Data were collected while horses were at rest every 21 d. Serum and plasma were analyzed for total and ionized Ca, P, Mg, Cl, total protein, albumin, parathyroid hormone (PTH), osteocalcin, and hydroxyproline. Radiographs of the left third metacarpal bone were taken. An aluminum step wedge exposed simultaneously was used as a reference standard for estimating bone mineral content (BMC) with an image analysis program. Bone measurements, including BMC,

bone and medullary width, and cortical area, were taken in the proximal diaphyseal and proximal metaphyseal area. Bone variables increased with training and with high Ca diets as compared to horses fed low Ca diets. Serum Ca decreased and serum PTH increased with training. Hydroxyproline was unchanged in horses fed the low Ca diets and decreased in horses fed the high Ca diets. Serum osteocalcin increased then decreased with training. The high Ca diet appeared to facilitate bone remodeling in response to training, but dietary Cl, hence DCAD, had no effect. In experiment two, 11 conditioned Arabian horses were taken out of training and placed in stalls for 12 wk. They were walked on a mechanical walker in two 30 min sessions 7 d/wk. Diets were designated LC (.35% Ca) and HC (.7% Ca). Data collection and analysis were identical to those in experiment one. Bone variables decreased with deconditioning but were unaffected by dietary Ca. Serum Ca increased with deconditioning and was greater in horses fed the HC diet, but PTH did not change. Horses fed the HC diet had greater serum osteocalcin, which decreased with deconditioning. Dietary Ca influenced bone remodeling in response to training, but did not have an effect on bone response to deconditioning. Loss of BMC during 12 wk of stall confinement may predispose horses to skeletal injuries when training is resumed.

(Key words: remodeling, calcium, exercise, cation-anion, horses)

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Introduction

The equine athlete requires a sound skeletal system that can withstand the intense strains associated with daily training and frequent competition. Skeletal breakdowns occur for many reasons. The cause could be a sudden trauma, such as a fracture from stepping in a hole, or from repeated stresses that eventually result in fatigue-related fractures. As a physiological system, bone is slower to respond to training than muscles, resulting in a delay in its ability to handle exercise that the muscular and cardiovascular systems handle more quickly.

Bone development and exercise performance are known to be affected by genetics, conformation, nutrition, and exercise. Two of these, nutrition and exercise, readily lend themselves to management.

Bone responds to stress, such as that associated with training, by remodeling. In this process, stressed bone is first resorbed, then replaced with new stronger bone. This physiologically slow process temporarily increases the overall bone porosity and decreases the overall bone strength (Nunamaker, 1986; Martin, 1991; Jeffcott, 1992).

Recently, attention has been focused on the role of dietary cations and anions and their role in Ca balance and bone development. The dietary cation-anion difference (DCAD), commonly expressed as (Na + K) - Cl in mEq/kg DM, affects the incidence of milk fever in dairy cattle by affecting the Ca status of the animal (Goff et al., 1991; Beede, 1992; Beede et al., 1992). A low DCAD increases bone resorption in cattle

(Beighle et al., 1990), and increases the urinary excretion of Ca in horses (Baker et al., 1993). A low DCAD combined with a diet low in Ca could lead to increased urinary Ca losses and a negative Ca balance in exercising horses (Wall et al., 1992). A negative Ca balance could impact bone development and remodeling. The DCAD has additional effects. Horses on a highly anionic diet experience a nutrition-induced metabolic acidosis that may impair athletic performance (Baker et al., 1992; Stutz et al., 1992; Popplewell et al., 1993).

Skeletal unloading, which is experienced when a limb is placed in a cast or an animal is restricted from normal movement, is associated with a loss of bone mass due to continued resorption and decreased bone formation (Sessions et al., 1989; Machwate et al., 1994). This also occurs when animals that in training are placed in stalls in order to recover from an injury. The decrease in bone mass could then affect the animals performance when training is resumed, predisposing them to a bone related injury. Knowledge of how quickly bone loss occurs when a conditioned animal is forced into inactivity could be used to develop retraining programs that include a skeletal strengthening component.

The following studies were conducted to evaluate the effect of differing dietary Ca and Cl levels on bone development and Ca balance in exercising and sedentary horses.

Bone Formation

Bone is a dynamic tissue, changing constantly throughout life to maintain optimal strength and adapt to exterior forces. Several cell types, including osteoclasts, osteoblasts, and osteocytes, are responsible for enabling bone to adapt to strains encountered throughout the life of the animal. Bone matrix, also known as osteoid, is the organic fraction of bone tissue. Bone mineral, the inorganic fraction of bone, forms the major storage site for calcium, phosphorus, and other important elements.

Osteoclasts. Osteoclasts are bone cells that originate in the bone marrow from pluripotential cells and are responsible for the removal of damaged, old, or dead bone. Osteoclasts function for three to four weeks, digesting the bone surface and forming erosion cavities. Resorption is carried out by a limited specialized surface of the osteoclast known as the ruffled border. The sealing zone, located at the edge of the ruffled border, adheres tightly to the bone and seals the resorption space. The pH within the resorption space is approximately 4 and lysosomal enzymes degrade the matrix (Rodan, 1992). In terms of bone processing, an osteoclast is 10 to 50 times more efficient at removing bone than an osteoblast is at forming bone.

Osteoblasts. Osteoblasts originate in mesenchymal stem cells, or osteogenic precursor cells. These cells have a forming period of approximately 100 days. They are responsible for producing and mineralizing bone matrix and for forming new bone

to fill in the cavities left by the osteoclasts. Osteoblasts are rich in alkaline phosphatase which participates in mineralization. About 10% of osteoblasts become buried in the matrix and are called osteocytes while others become flattened on the bone surface and are called lining cells (Rodan, 1992).

Osteocytes. Osteocytes are formed almost entirely by burial of osteoblasts in matrix. These cells are located throughout bone tissue. As the osteoblasts become embedded in the matrix they change shape and generate long processes which make contact with neighboring cells, establishing gap junctions between them which form lines of communication. A number of roles have been proposed, including strain-related adaptive modeling/remodeling, repair of microdamage, revitalization of dead tissue, and mineral exchange. Mechanically adaptive control of bone architecture requires feedback concerning the relationship between current loading and existing architecture, feedback which is most likely derived from the strain in the matrix. The osteocyte network seems suited to detect strain in the matrix and influence adaptive modeling and remodeling in a strain related manner (Lanyon, 1993).

Mechanical Properties of Bone

Stress is a measure of the force applied to a bone. When a bone is placed under stress, it will deform. This deformation can be measured and is termed strain, a dimensionless variable used to quantify deformation of a bone under stress and defined as:

$$\text{strain} = \frac{L - L_o}{L_o}$$

where L is the length of (part of) the structure when loaded and L_o is the length when not loaded. Microstrain is more often used as a unit, being a strain of 10^{-6} (Van Den Bogert, 1994).

Stress and strain are related, and the relationship can be plotted (Figure 1). Initially, the curve is linear and the bone is referred to as behaving in an elastic manner. In this case, stress is proportional to strain and Young's modulus of elasticity, a measure of elasticity, can be calculated as the stress divided by the strain. When behaving in an elastic manner, the bone returns to it's original shape when the load is removed. Bone made of relatively less stiff material would need to have thicker cortices to resist bending (Riggs and Evans, 1990; Markel, 1996).

Above a certain stress, termed the yield point, the relationship between stress and strain is no longer directly proportional, and the bone enters a region of inelastic deformation. A bone stressed beyond the yield point will not return fully to it's original shape when the load is removed. The ultimate tensile strength of the bone refers to the stress applied to the bone that causes it to fracture (Riggs and Evans, 1990; Markel, 1996).

Biomechanical Behavior of Bone

When bone is mechanically deformed, it becomes electrically charged with "stress generated potentials" as a result of piezoelectric forces. One possible mechanism for

this is termed "streaming potentials", where the deformation of bone causes a separation between the walls of the channels within bone and the fluid contained in those channels. The separation creates a pressure difference which causes the fluid to flow in a particular direction, producing an electrical current within the bone (Walsh and Guzelsu, 1991). The voltage waveforms produced with physiologic loading have been shown to affect the function of bone cells, whether they arise from endogenous or exogenous fields. The cells do not seem to make a distinction between sources of the field (McLeod and Rubin, 1990).

Loading mode, including tension, compression, bending, shear, and torsion, will cause the bone to respond differently. A bone under tensile loading lengthens and becomes more narrow, while a bone under compressive loading the bone shortens and becomes wider. Bending loads cause the bone to bend around an axis, and is a combination of tension on one side and compression on the other side of the axis. Torsion causes a bone to twist around an axis. During normal daily activity, combined loading of several modes occurs (Markel, 1996).

Fatigue occurs as a consequence of cyclical loading and represents a progressive loss of strength and stiffness before failure. This process occurs at loads well below those that would cause a fracture as a single event. The number of cycles to failure is related to the deformation associated with the cycle, with greater deformation shortening the fatigue life of the bone (Riggs and Evans, 1990; Markel, 1996). The fatigue life of bone is affected tremendously by load bearing (Pratt, 1982) and is partly

dependent upon bone density. Using equations that take into account the load per unit area and the yield stress of the bone, the number of strides to failure of the McIII can be computed (Norwood, 1978). An evenly distributed load increases the cortical area over which forces are applied and will result in a longer fatigue life for bone (i.e., number of strides). Loads applied unevenly decrease the load bearing surface and result in decreased strides to failure.

Bone Modeling and Remodeling

The functions of bone include mechanical support of the body, levers for muscle action, hematopoiesis, and mineral storage (Rodan, 1992). When functioning as mechanical support, bone is modeled or remodeled to assume the optimal structure for minimal material to maintain a balance between strength, flexibility, and weight (Martin, 1991; Turner, 1991). Bone responds to strain through modeling of immature bone and remodeling of mature bone. Modeling and remodeling differ in nature, function, and minimum effective strain (MES) ranges that switch them on and off. Minimum effective strain represents the lowest level of recurring strain necessary to initiate new bone formation (Frost, 1990a; Turner, 1991). Furthermore, the sequence of bone formation is different for each. When bone is being modeled, formation precedes resorption, whereas during remodeling, resorption precedes formation.

Bone Modeling. Modeling refers to the shaping and forming of bone during the growth phase of the animal. It helps determine the ultimate form of mature bone (Pool, 1987) and is responsible for providing adaptation to overloading strains by

molding the bone architecture, resulting in lower strains at a given workload (Frost, 1990a; Turner, 1991). Alteration of bone alignment occurs during growth in order to affect bone architecture for such things as shape, size, content, and distribution of bone tissue. Peak strains below the MES have no effect on modeling, while repeated strains above the MES activate modeling drifts. Resorption and formation change the bone architecture to lower the strains, and the bone is considered biomechanically adapted when the change is accomplished (Frost, 1990a).

Quarter Horse (QH) and Thoroughbred (TB) weanlings placed in an exercise program, consisting of trotting on an automatic walker at a medium pace for 20 min/d 5 d/wk, demonstrated an improvement in bone density and stress-bearing characteristics of the third metacarpal (McIII) as compared to a non-exercised group (Raub et al., 1989) . These responses may enable the exercised group to better withstand the stresses imposed on the skeletal system during race or exercise training.

Bone Remodeling. The change from modeling to remodeling is gradual and occurs in different bones at different times. Remodeling is the process by which bone adapts to underloading or disuse and to increased use after skeletal maturity (Millis, 1983; Frost, 1990b). It includes regeneration through the removal of small amounts of bone tissue and replacement with new bone (Frost, 1964; Pool, 1987). This allows for the replacement of immature bone with mature bone, resorption and replacement of damaged or dead bone, and the formation and maintenance of metabolically active and biomechanically sound bone structure (Pool, 1987). While modeling ceases with

skeletal maturity, remodeling is a continuous process (Frost, 1990b).

The signals that stimulate bone remodeling have been identified but not quantified in regard to training. However, it is known that strain rate, magnitude, and distribution play important roles in the remodeling response (Pratt, 1982; Rubin and Lanyon, 1987), all of which can be altered by the training program. Strain rate and magnitude imparted to the radius and ulna of exercising sheep increased linearly with the speed of the treadmill. An artificial loading regime devised to simulate the same strains as seen during locomotion on the treadmill showed that sheep subjected to high strain rates increased BMC, moderate strain rates resulted in slight increases in BMC, and low strain rates resulted in a loss of BMC (O'Conner and Lanyon, 1982). The artificially loaded ulnas of male turkeys were subjected to differing magnitudes of strain, with a similar rate and number of cycles. Results showed that a threshold existed, above which the magnitude of strain affected bone formation (Rubin and Lanyon, 1985). The distribution of strain on the surface of the bone was also shown to provide a stimulus for bone formation (O'Conner and Lanyon, 1982; Rubin and Lanyon, 1985). Intermittent loading to the ulna of mature roosters revealed a minimum threshold for the number of cycles at a given rate and magnitude in order to prevent bone resorption. A greater number of cycles at that rate and magnitude did not result in further bone formation (Rubin and Lanyon, 1984).

Strain is a fundamental stimulation for bone remodeling (Rubin, 1984). Peak strains occur in the swing phase at the walk, due to tendon tension, and in the stance

phase at higher speeds due to ground reaction forces (Woo et al., 1981; Pratt, 1982; Rubin and Lanyon, 1987). In vivo strain gauges have allowed the relationship between mechanical strain and remodeling response to be estimated. The response is an increase in bone mass in relation to strain.

Bone responds to strain in two ways: by increasing density through inhibition of intracortical bone remodeling, which minimizes cortical bone porosity, and by increasing bone mass and altering the distribution of this mass to provide more bone at high strain locations. Sites that remodel are sites under greater strain, usually along lines of maximum load transmission (Norwood, 1978). To remodel, stressed bone is resorbed before it is replaced with new bone, creating a temporary increase in bone porosity, which affects the mechanical properties of bone. An increase in bone porosity results in a decreased bone strength (Nunamaker, 1986; Martin, 1991).

Bone is remodeled both internally and on the surface (Frost, 1964). Surface remodeling occurs on periosteal and endosteal surfaces. It can contribute to the development of osteoporosis and osteomalacia. Internal remodeling occurs within the space encompassed by the periosteum and endosteum, and participates in the senescence-deterring effect of remodeling as well as the physical and histological quality of bone. It can also contribute to osteomalacia when a mineralization defect occurs. Internal remodeling can also occur due to altered vascular relationships affecting the supply of nutrients to bone cells, the mobilization of bone to compensate for a dietary deficiency, biomechanical stress, or in response to immobilization or

disuse (Lawrence, 1986). Equilibrium of bone mass depends on equal amounts of resorption and formation, both of which can be affected by activation rate of osteoclasts and osteoblasts. Variations in activation frequency can add or remove bone mass from the skeleton even if resorption and formation amounts are approximately equal (Martin, 1991). Remodeling rates decrease with increasing age, resulting in a slow loss of bone mass, i.e. increased porosity, over time.

Bone remodeling is sensitive to different types of physical activity (Millis, 1983). Weight-bearing exercise, particularly resistance exercise, has been shown to be effective for increasing bone development in animals and humans. Female body builders had a greater bone mineral content (BMC) as compared to runners, swimmers, and non-athletic controls (Heinrich et al., 1990).

Skeletal Unloading

When an animal is placed in confinement, skeletal unloading occurs and is associated with a loss of bone mass due to increased bone resorption and decreased bone formation (Sessions et al., 1989; Machwate et al., 1994). The decrease in bone could then affect the animals performance when training is resumed, predisposing them to a bone related injury. Placing one forelimb in a cast for eight weeks resulted in a decrease in BMC in both forelimbs of Standardbred (SB) horses (Buckingham and Jeffcott, 1991). Foals placed in stalls for the winter months showed decreased serum osteocalcin and alkaline phosphatase (ALP) concentrations, which was interpreted to indicate decreased bone formation (Mäenpää et al., 1988).

Dorsal Metacarpal Complex

A common injury associated with young TB racehorses is dorsal metacarpal complex, or bucked shins. This injury tends to occur during the late training or early racing period, and is characterized by tenderness or soreness of the left or both front metacarpal bones. A prospective study of 48 two-year old TB horses in race training revealed that 65% of the animals demonstrated clinical evidence of bucked shins, with a 91% occurrence in those horses in uninterrupted training (Stover et al., 1988).

Bucked shins are thought to be caused either by the accumulation of microfractures to the cannon bone sustained through repeated stresses, e.g. training, (Norwood, 1978; Stover et al., 1988) or by the rapid formation of periosteal (superficial) bone, which is more porous and therefore weaker, over the stressed area (Nunamaker, 1986; Nunamaker et al., 1990). Lamellar bone, which is more dense than periosteal bone, is produced more slowly. The development of microdamage causes progressive weakening of the bone (Stover et al., 1988) and bucked shins may occur when the stressed bone does not have adequate time to rebuild. With the formation of periosteal bone, the periosteum becomes inflamed and results in bucked shins. Horses that have had bucked shins are more susceptible to saucer fractures later in their career (Nunamaker, 1986).

Bucked shins are common in TB racehorses, an injury which is not common in SB racehorses. Both breeds begin training during adolescence and may race as youngsters. In vitro fatigue testing of the McIII from adult TB and SB racehorses

revealed no difference in mechanical properties of bone between the two breeds (Nunamaker et al., 1989), but differences did exist during the growth stage (Nunamaker et al., 1991). The effect of remodeling due to training during growth in TB racehorses may be related to the incidence of bucked shins. Differences in gait and speed used for racing and training influence the deformation of the McIII. The TB racing gait, a gallop, is faster than the SB trot or pace, and the TB propels itself over a single lead forelimb, while the SB will have two feet on the ground during the support phase of the trot or pace. The TB racing gait develops more strain in the McIII than the SB pace or trot, and the strain is rotated approximately 40° when speeds are increased from the trot to the gallop (Nunamaker et al., 1990). Also, SB racehorses train for long distances at racing speeds. Thoroughbred racehorses train infrequently over relatively short distances at racing speeds. The McIII of the TB remodels in response to the slower, conditioning speeds used during training, and may not be prepared to withstand the stresses associated with racing speeds.

The high strains of training and racing on the immature skeleton of the young horse can result in an increased risk of bucked shins. Studies on 18 month old QH (Nielsen, 1992) and TB (Porr, 1993) in race training demonstrated a decrease in BMC of the McIII early in training, most likely due to the remodeling response to exercise. Both groups of horses began training with long, slow distance workouts designed to condition the muscular and cardiovascular systems. Approximately 8 wk into training, both groups began doing short distance, high speed workouts, the QH every 2 weeks

and the TB every 5 days. This high speed work was required when BMC was lowest and the horses were most susceptible to skeletal injury.

Biological Factors

A variety of biological factors help control the metabolism of bone cells. Hormones (including PTH, calcitonin, vitamin D_3 metabolites, estrogens, growth hormone, and thyroid hormones) and autocrine and paracrine factors (prostaglandins and insulin-like growth factors (IGF)) orchestrate bone metabolism.

Insulin-like Growth Factors. Insulin-like growth factors (IGF) are important in the formation and maintenance of bone mass. In addition to other tissues in the body, IGF are produced by osteoblasts, osteocytes, and bone marrow stromal cells. Systemic hormones such as growth hormone, estrogens, and PTH modify the IGF regulatory system which regulates bone remodeling. The IGF stimulate osteoblast proliferation and differentiation, and may also activate osteoclasts. Variations in IGF activity may be due to differences in receptor types, modification of IGF binding proteins (IGFBP), or influence of IGFBP-specific protease action on IGFBP (Rosen et al., 1994).

Parathyroid Hormone. Blood PTH concentration is inversely related to blood Ca concentration below a threshold. A decrease in blood Ca concentrations will trigger the release of PTH, which will act to increase blood Ca concentrations via several mechanisms: 1) direct mobilization from bone stores, potentially resulting in reduced bone mass, 2) stimulation of Ca reabsorption in the kidney, and 3) indirect increase of the rate of intestinal reabsorption in the intestine. Parathyroid hormone increases

the synthesis of $1,25(OH)_2D_3$ in the kidney, which increases Ca absorption from the intestinal tract.

Recent evidence indicates anabolic effects of intermittent, subcutaneous infusion of PTH on bone. Intermittent, low doses of PTH stimulated de novo bone synthesis whereas continuous PTH administration prevented type 1 collagen synthesis in fetal rats (Canalis et al., 1991). In chick osteoblasts, IGF-I increased thymidine incorporation while PTH alone had no effect. When the two were combined, the effects of IGF-I were potentiated (Spencer et al., 1989).

Vitamin D. There are several metabolites of vitamin D_3 in the body, including $25(OH)D_3$, $1,25(OH)_2D_3$, and $24,25(OH)_2D_3$. The $1,25(OH)_2D_3$ is a regulator of Ca and P metabolism and increases bone resorption (Turner, 1991; Rodan, 1992). However, administration of $24,25(OH)_2D_3$ reduces bone resorption and increases bone volume with a decrease in osteoclast number (Matsumoto et al., 1992). It was postulated that $24,25(OH)_2D_3$ stimulated degradation of $1,25(OH)_2D_3$ and may have a unique inhibitory effect on the formation and function of osteoclasts. The inhibitory effect of $24,25(OH)_2D_3$ on osteoclastic bone resorption may play a role in the local modulation of the actions of osteotropic hormones.

Calcitonin. Calcitonin (CT), which is released from the thyroid gland, initiates events which decrease blood Ca concentrations above a threshold. In bone, CT acts directly on osteoclast receptors, inhibiting bone resorption. Blood CT and Ca concentrations are positively and linearly correlated, suggesting a proportional control

(Kronfeld et al., 1976).

Estrogens. The primary effect of estrogen on bone remodeling is to slow bone resorption. Human fetal bone-derived cells cultured with estrogen stimulated osteoblasts to produce growth factors, including IGF-I, IGF-II, and transforming growth factor ß1 (Slater et al., 1994). These factors participate in the regulation of cell proliferation and differentiation.

Prostaglandins. Arachidonic acid is the primary substrate for prostaglandin production. It is present in the phospholipid membrane of osteoblasts and chondrocytes. Systemic and localized factors can cause the release of arachidonate from the membrane for biosynthesis of prostaglandin E_2 (PGE_2), which can then exert its effects on bone tissue. Prostaglandin E_2 has biphasic effects on bone formation and resorption; at low concentrations bone formation is stimulated, at high concentrations bone formation is inhibited (Raisz, 1993). It is the primary prostaglandin affecting bone metabolism. The anabolic action of PGE_2 may involve IGF because PGE_2 is known to induce biosynthesis of IGF-I and IGF-I binding proteins in osteoblast cell cultures.

Measuring Changes in Bone and Calcium Status

Changes in bone formation or resorption can be evaluated by invasive procedures both in vivo and in vitro, or by non invasive procedures in vivo. Changes in hormones and other blood borne substances that may also reflect changes in bone and Ca status.

Invasive Procedures

Invasive techniques for measuring bone density include biopsy (in vivo), strain gauges (in vivo), breaking strength (in vitro), and composition (in vitro).

Biopsy. Bone biopsy requires surgical removal of a portion of the bone. This can be performed on an anesthetized horse. Interpretation of biopsy results can be difficult, and may be impossible without precise history and details of the surgical site. The sample can be evaluated histologically

Composition. Determining the ash composition of the bone for a direct measurement of BMC requires either that a biopsy be performed or that the bone be removed from the body.

Strain gauges. The use of in vivo strain gauges allows manipulation of stress and cyclic history to study their effects on bone strain in a live animal. Gauges have been used to measure surface bone strain (Turner et al., 1975; Nunamaker et al., 1990; Van den Bogert, 1994) and principal strains and direction (Nunamaker et al., 1990).

Breaking load and strength. The determination of breaking load or strength requires that the bone of interest be removed from the body. The force required to cause fracture of the bone is called the breaking load, and is directly proportional to the BMC. Bone breaking strength, which is defined as the maximum stress bone can withstand before failure, is not linearly related to BMC, rather it is logarithmic. The strength of the bone can be determined by repetitive loading of the bone or by placement of an increasing load until failure occurs (Riggs and Evans, 1990; Williams

et al., 1991b; Lawrence et al., 1994).

In vitro means do not always indicate the realistic repetitive loading seen in real life (Turner et al., 1975). Also, some of these procedures are not practical if the purpose of a study is to monitor ongoing bone development in young horses, remodeling in mature animals, or if the animals cannot be euthanised.

Non-invasive Procedures

Some non-invasive techniques for measuring changes in bone density include radiographic photometry and densitometry, photon absorptiometry, and ultrasound.

Radiographic photometry and densitometry. Radiographic photometry and densitometry can provide an accurate estimate of BMC and is relatively inexpensive and nondestructive. It can also provide morphometric measurements of cortical bone. Since cortical bone makes up approximately 80% of the bone material, this should give a fairly accurate reflection of overall skeletal status. An aluminum (Al) step wedge is used as the standard comparison to provide radiographic bone aluminum equivalents (RBAE) in mm Al using image analysis. A high correlation between BMC and RBAE suggests that the aluminum step wedge provides an accurate method of comparison (Meakim et al., 1981; Ott et al., 1987; Kälebo and Strid, 1989).

Some advantages of radiographic photometry and densitometry include the increased sample size due to the ability to radiograph the entire McIII, the short exposure time of only 1/15 to 1/60 of a second, and the ability to determine cortical area and bone geometry. Disadvantages include sources of error inherent in the use of

x-ray, such as variation in energies of x-ray beams which cause nonuniformity in film exposure and radiation scattering. If uncontrolled, errors in BMC estimation may exceed 10% (Lawrence and Ott, 1985). Ultrasound, compared in-vivo and post mortem, showed significant differences in bone density between exercised yearling horses and unexercised controls, but no difference was seen using radiographic photodensitometry (McCarthy and Jeffcott, 1992).

Photon absorptiometry. Photon absorptiometry provides the most accurate prediction of BMC by measuring it directly (Lawrence and Ott, 1985; Lawrence, 1986). An advantage of photon absorption over radiographic techniques is the use of a monochromatic photon source as opposed to multiple energy levels of x-ray. The photon source is connected to a detector which allows the direct measurement of photon energy absorbed by bone (Lawrence and Ott, 1985). Lawrence (1986) compared photon absorption, ultrasound transmission, and radiographic photometry and showed that photon absorption is slightly more accurate in estimating BMC than radiographic photometry. It is much less satisfactory for scanning live horses, however, primarily due to the long scan time which requires anesthetizing the animal.

Ultrasound transmission. The principle of ultrasound measurements is that the speed of sound through bone is determined by bone density as well as physical properties which are correlated with bone strength. Theoretically, ultrasound bone measurements should provide more information about bone strength and structure than densitometric techniques (Hans et al., 1993). It is highly correlated to the modulus of

elasticity (stiffness) of the bone than other non-invasive techniques. It has been shown to be slightly less accurate than either photon absorption or radiographic photometry in the estimation of BMC (Lawrence, 1986: Williams et al., 1991b). Transducers that send and receive soundwaves are placed on either side of the bone to be scanned. The time it takes for the soundwaves to go from one transducer to the other is divided into the width of the cannon bone, as measured by vernier calipers, giving the apparent velocity (Lawrence, 1986). This is assuming that the path of the beam through the bone is a straight line. Owing to the cylindrical cross section of the metacarpal bone, the fastest path for the sound wave may be via the cortex, i.e., around the medullary cavity (Buckingham and Jeffcott, 1991; Buckingham et al., 1992; McCarthy et al., 1992). However, Langton et al. (1991) demonstrated that the ultrasound wave followed a path straight through the bone with a time lag in the medullary cavity due to the slower transmission velocity of the marrow.

Ultrasonic waves pass through the bone and can be converted to a numerical reading of velocity in millimeters per second. A higher velocity is related to thicker bone and low velocities can indicate swelling of soft tissue or microdamage to bone. Comparison of a series of readings allows the determination of progress, with an increase in velocity indicating new bone development and a decrease in velocity indicating a loss of bone mineral (Lawrence, 1986).

Ultrasound could potentially reveal problems before they are visible to the naked eye, which would allow for a change in training before the problem becomes severe.

A decrease in ultrasound velocity in both front limbs of horses with one limb immobilized in a cast for eight weeks has been demonstrated (Buckingham and Jeffcott, 1991). All areas and properties of the bone measured tended to recover after the removal of the cast and subsequent turnout of the horses. Horses completing a 12 week training program demonstrated an increased ultrasound velocity of the McIII as compared to unexercised controls, which experienced a decrease in ultrasound velocity. Measurements performed post mortem confirmed the same difference recorded in vivo (McCarthy and Jeffcott, 1992).

Several blood and urinary markers may be used to estimate bone activity and Ca status. These include certain minerals, $1,25(OH)_2D_3$, PTH, calcitonin, and other possible markers of bone turnover. Alkaline phosphatase, type I collagen peptides, and osteocalcin are biochemical markers of bone formation. Hydroxyproline, plasma tartrate-resistant acid phosphatase, and collagen pyridinium crosslinks are biochemical markers of bone resorption. A summary of biochemical markers of bone metabolism is shown in Table 1.

Blood minerals. Minerals associated with bone resorption or development include Ca, P, and Mg. Blood concentrations of these minerals are not, however, good indeces of bone activity. Homeostasis maintains blood Ca concentrations within a narrow margin under most circumstances, so blood Ca concentration is not usually regarded as a sensitive index of bone remodeling or calcium status. Serum inorganic phosphate reflects dietary P intake and renal function and tends to decrease in response

to several dietary conditions. Serum total and ionized Ca have been shown to decrease after an acute bout of exercise in humans and horses (Ljunghall et al., 1986; Nishiyama et al., 1988; Geiser et al., 1995), but recovery to baseline was rapid. In horses, conditioning has led to a prolonged decrease in serum Ca (Nielsen, 1992; Porr, 1993) which could inhibit bone remodeling. The varied results reported for Mg concentrations in response to exercise have not been explained. The results are not consistent, nor have they been proven to be in direct response to bone activity. Therefore, Mg concentrations should not be considered an accurate measurement of bone activity (Lawrence, 1986).

Parathyroid hormone. Increases in blood PTH concentration in response to a low blood Ca is associated with increased bone resorption. A commercially available immunoradiometric assay (IMRA) designed to measure human PTH has been validated for use with equine serum (Wilson, 1991). The primary objective was to validate the two-site IMRA for use with equine serum. It was concluded that the IMRA was useful for evaluating PTH concentrations in equine serum. A secondary objective was to measure PTH concentrations in young growing horses using the same method. Concentrations were found to be highly variable over a 16 wk period.

Vitamin D_3. Parathyroid hormone is involved in promoting the conversion of $25(OH)D_3$ to $1,25(OH)_2D_3$ in the kidney. The $1,25(OH)_2D_3$ is a regulator of Ca and P metabolism (Hintz and Schryver, 1976), increasing intestinal absorption of Ca and bone resorption (Turner, 1991; Rodan, 1992). Blood concentration of $1,25(OH)_2D_3$

could be used as an indirect indicator of bone activity, with increased concentrations of $1,25(OH)_2D_3$ indicating increased bone resorption.

Alkaline phosphatase. Major sources of ALP are bone, liver, kidney, and intestine, and serum concentrations may be elevated in response to disease. Skeletal ALP is localized in the osteoblasts, and may be involved in catalyzing the hydrolysis of pyrophosphate, a potent inhibitor of Ca deposition (Fraher, 1993; Risteli and Risteli, 1993). Total ALP, which is easier to measure than skeletal ALP, is considered an insensitive marker of bone formation due to the large number of sources (Ljunghall and Lindh, 1989). However, a bone-specific ALP immunoradiometric assay has been developed recently for use with human bone ALP and has led to more specific measurements (Fraher, 1993).

Osteocalcin. Bone Gla-protein, also known as osteocalcin, is a vitamin K-dependent bone protein synthesized by osteoblasts, and a marker for bone formation (Ljunghall and Lindh, 1989). The precise function of osteocalcin remains unknown, but the protein contains gamma-carboxyglutamate residues which may bind Ca (Tohmé et al., 1991; Risteli and Risteli, 1993). Its expression may be controlled by $1,25(OH)_2D_3$ (Lian and Gundberg, 1988; Risteli and Risteli, 1993). It correlates well with ALP. Plasma osteocalcin concentrations have been found to correlate well with actual bone formation rates when measured by invasive methods (Fraher, 1993).

Serum osteocalcin follows a circadian rhythmicity in SB horses that is biphasic in nature (Lepage et al., 1991), but this pattern was not seen in Arabian horses (Hope et

al., 1993). Serum osteocalcin concentrations in SB were constant during daytime and underwent significant variations during the dark period. The suggested optimal sampling time would be daytime before exercise, since the effect of exercise on osteocalcin in horses is still not known.

Although associated with new bone formation, serum osteocalcin increased in a group of unexercised controls as compared to a group of exercised horses (McCarthy and Jeffcott, 1992). Unconditioned yearlings were placed in either a control or an exercise group at the start of the study. Both groups had similar serum osteocalcin concentrations. The exercise group had lower serum osteocalcin concentrations at the end of the study despite an increase in BMC. In humans, basal serum osteocalcin was higher in athletes than in nonathletes (Nishiyama et al., 1988).

Serum osteocalcin decreased with increasing age in normal, female SB horses (Lepage et al., 1990). This would indicate a decrease in new bone formation in mature as compared to young horses. Sex has also been reported to affect serum osteocalcin concentrations, with female humans having a higher concentration than males in the same age group (Epstein et al., 1984).

Type I procollagen propeptides. Type I collagen is synthesized as a larger procollagen molecule. There are extra peptide sequences at either end of the molecule which prevent premature association of the molecules into collagen. When procollagen is secreted into extracellular spaces the extra peptides are cleaved off to form propeptides (Tohmé et al., 1991; Fraher, 1993; Risteli and Risteli, 1993). There

are two types of type I procollagen propeptides; carboxyterminal (PICP) and aminoterminal (PINP). A molecule of PICP is larger than PINP; molecular weights are 100,000 and 35,000, respectively. In most situations, serum PICP concentration is related to the rate of histomorphometrically assessed bone matrix formation (Delmas, 1993; Risteli and Risteli, 1993). If bone matrix formation and mineralization are synchronized, as they normally are, there is a correlation between serum PICP and mineralization rate (Fraher, 1993; Risteli and Risteli, 1993). Serum PICP was correlated with bone ALP, providing further evidence that PICP reflects the rate of bone fomation in horses (Price et al., 1995).

Hydroxyproline. Hydroxyproline is found almost exclusively in collagen (Dull and Henneman, 1963; Fujii et al., 1981; Ljunghall and Lindh, 1989). Urinary hydroxyproline concentration was shown to be an indicator of bone disorders (Dull and Henneman, 1963; Millis, 1983). This lead to the conclusion that bone modeling and remodeling were associated with hydroxyproline concentrations (Fujii et al., 1981; Horowitz et al., 1984).

Creatinine is excreted in the urine at a fairly constant rate and is used to evaluate the excretion of other metabolites. Urinary hydroxyproline:creatinine ratios are commonly used to measure bone activity. Urinary total hydroxyproline represents approximately 10% of total collagen catabolism and may be poorly associated with bone resorption (Delmas, 1993). The total pool of urinary hydroxyproline is derived from both endogenous sources as well as from collagenous proteins in the diet. Free

plasma hydroxyproline correlates fairly well with total urinary hydroxyproline, and blood is more convenient for sample collection in horses (Fujii et al., 1981; Millis, 1983; Lawrence, 1986).

Tartrate-resistant acid phosphatase. The greatest concentrations of acid phosphatase are found in the liver, spleen, erythrocytes, platelets, bone, and prostate gland, with the latter contributing up to one-half of the circulating acid phosphatase. Bone acid phosphatase, an enzyme released into the plamsm by osteoclasts, is tartrate resistant (TRAP) and can be used as an indicator of osteoclastic activity (Millis, 1983; Lawrence, 1986; Tohmé et al., 1991). Plasma TRAP is increased in a variety of metabolic bone disorders with increased bone turnover but it is not clear whether this marker is more specific than hydroxyproline which is a non-specific marker. The lack of specificity of plasma TRAP for the osteoclast, its instability in frozen samples, and the presence of enzyme inhibitors in serum are drawbacks at present, but the recent development of an immunoassay should be valuable in assessing the value of this marker in detecting bone metabolism (Delmas, 1993).

Collagen pyridinium cross-links. The pattern of collagen cross-linking is tissue specific (Tohmé et al., 1991). Pyridinoline cross-links have been found in fibrillar collagens from several tissues but are particularly characteristic of type II collagen in cartilage and type I collagen of bone (Tohmé et al., 1991; Risteli and Risteli, 1993). Deoxy-pyridinoline is located exclusively in bone and dentin (Tohmé et al., 1991; Delmas, 1993). A major advantage of these cross-links is that they are specific for

fibrillar collagen and cannot be derived from the degradation of recently synthesized collagen. An immunoassay has been developed which detects free pyridinoline. In general, there is a good correlation between accelerated bone turnover and the urinary excretion of the cross-links (Risteli and Risteli, 1993).

Nutrient Effects

The NRC has published recommended minimum nutrient requirements for horses (NRC, 1989). There is a question as to how these minimums relate to optimal ranges. An optimal diet or ration has been defined as one that provides energy and nutrients in optimal ranges and proportions that ensure maximum performance (Kleiber, 1961). Upper and lower limits of tolerable range may be set by undesired indicators, such as clinical signs or lesions pertaining to deficiency or toxicity (Kronfeld et al., 1994).

Much research has been done on the effects of nutrition on exercise performance, and nutrition and exercise on bone development, as nutrition and exercise fall most obviously under direct control of management. Energy, protein, vitamins, and minerals have all been shown to play a role in bone development. A creep feeding program providing NRC-recommended levels of all nutrients for foals begun even while the mare is at peak lactation increases growth rate with little or no decrease in bone quality (Thompson et al., 1988).

Energy. Dietary energy in traditional equine diets comes from a combination of forages, usually more than 35% fiber, and concentrates. High starch diets increase the risk of colic and laminitis and may contribute to the incidence of developmental

orthopedic disease (Kronfeld et al., 1990).

Protein. Protein is needed for production of organic bone matrix and synthesis of enzymes necessary for the transformation of osteoblasts to osteocytes (Hintz and Schryver, 1976). Protein deficiency horses can result in insufficient growth and underdevelopment in young horses.

Vitamins. Vitamins play roles as co-factors in several physiological processes involving bone development and remodeling. Vitamin A affects growth and development of epiphyseal cartilage cells (Hintz and Schryver, 1976), and may to be associated with a modification of Vitamin D metabolism (Anonymous, 1988). Vitamin C is required for collagen synthesis. The demand for Vitamin C is increased during stress. Blood ascorbate was depleted in horses with severe infections, long bone fractures, and overtraining (Jaeschke and Keller, 1978; Jaeschke, 1984). Vitamin D is a regulator of Ca and P metabolism (Hintz and Schryver, 1976), increasing intestinal absorption of Ca and bone resorption (Turner, 1991; Rodan, 1992). Vitamin K has recently been shown to have effects on bone metabolism, most likely through the carboxylation of glutamate residues in osteoblastic bone proteins (Vermeer et al., 1996) such as osteocalcin. Pyridoxine-deficiency in rapidly growing chicks resulted in tibias of reduced dry weight and cortical thickness. Less collagen was present which suggested that collagen maturation was not achieved, indicating that pyridoxine is an essential nutrient for connective tissue in poultry (Massé et al., 1994).

Microminerals. Several of the microminerals have been shown to influence proteoglycan and collagen synthesis, namely Cu, Zn, and Mn. Copper deficiency results in bone formation disorders due to a failure of normal osteoblast function and skeletal changes presumably caused by the production of biomechanically inferior bone collagen due to a decreased synthesis of the Cu dependent enzyme lysyl oxidase (Rucker and Murry, 1978). Zinc is also required for the synthesis of ALP. Zinc deficiency can cause a decrease in the synthesis of this enzyme (Ciancaglini et al., 1990). Manganese is an important constituent of cartilage and bone matrix. The effects of Mn on bone growth are most likely related to synthesis of chondroitin sulfate in developing cartilage (Leach, 1971).

Macrominerals

Calcium. Calcium is the most abundant mineral found in the body, with 99% of Ca being found in the bones and the remaining 1% being located in the soft tissues and blood (Haymes, 1991). Calcium is required in bone for the formation of hydroxyapatite, in plasma for blood clotting, in muscle fibers for muscle contraction, and at synaptic clefts for the release of neurotransmittors (Haymes, 1991). Plasma Ca concentration is highly regulated, being influenced by the hormones PTH, calcitonin, $1,25(OH)_2D_3$, estrogen, and cortisol as well as Ca intake and bone Ca turnover (Kronfeld et al., 1976; Weaver, 1990). A decreased serum Ca concentration results in an increased serum PTH concentration, increasing bone resorption and stimulating kidney production of $1,25(OH)_2D_3$, which increases gastrointestinal absorption of

available Ca. An increased serum Ca concentration triggers calcitonin release from the thyroid, decreasing Ca resorption from the bone and increasing Ca excretion (Rodan, 1992).

Horses tend to absorb most dietary Ca and P, regulating the excretion of excesses via the kidney (Schryver et al., 1970, 1974), whereas ruminants tend to regulate Ca and P through the digestive tract (Kronfeld et al., 1976). The effect of type of diet, roughage versus concentrate, has an effect on the absorption of dietary Ca and Ca excretion in horses. Calcium was more readily absorbed when horses were fed a diet of alfalfa hay as compared to a diet of concentrate when the Ca intake was nearly equal. Horses fed the alfalfa diet had a higher plasma Ca concentration and urinary Ca excretion when compared to horses fed a commercially pelleted concentrate (Meyer et al., 1992).

High dietary Ca has been associated with increased risk of bone disease in dogs (Slater et al., 1992) and cattle (Kronfeld et al., 1976), and both high and low dietary Ca have been associated with increased risk of bone disease in horses (Jordan et al., 1973; Kronfeld et al., 1990). High dietary oxalates have been shown to inhibit Ca absorption in ponies (Hintz and Schryver, 1976). High dietary Al did not affect Ca absorption, but did result in an increased Ca excretion in the urine of ponies (Schryver et al., 1986). The increased urinary Ca loss may have been due to an inability by the ponies to utilize absorbed Ca for the formation of hydroxyapatite due to a lack of P.

A decreased urinary excretion and increased retention of Ca has been found in exercising horses (Schryver et al., 1978). Estimates of Ca loss in sweat, approximately 1 mg of Ca/hour of work/kg of body weight, suggest that long-term exercise may increase dietary Ca requirements.

Chelated or Proteinated Minerals. Feeding minerals in organic forms can enhance their bioavailability (Wedekind et al., 1992) as compared to non-chelated sources in some cases, but not all. Rats receiving Ca citrate-malate (CCM) in the diet had a higher rate of trabecular bone deposition than animals receiving Ca carbonate (Kochanowski, 1990). At adequate levels of Ca no differences were expected, but the animals receiving CCM had a 44-47% increase in bone deposition over animals receiving Ca carbonate.

Phosphorus. Phosphorus is the second most abundant mineral found in the body, and approximately 85% is found in bones. Phosphorus is necessary for the formation of hydroxyapatite. It plays a critical role in energy production as part of the high energy phosphate compounds ATP and creatine phosphate, cyclic AMP, and provides phosphate ions involved in glycolysis (Haymes, 1991). Low P diets in cattle have been associated with decreased bone mechanical properties of the McIII as compared to animals on an adequate P diet (Williams et al., 1991a). High Al intake inhibits P absorption in ponies, presumably due to the formation of insoluble, non-absorbable aluminum-phosphate complexes in the intestinal tract (Schryver et al., 1986).

Skeletal abnormalities from Ca and P deficiencies have been identified in poultry, swine, and cattle. The results include decreased osteoblastic activity and chondrogenesis, limb deformities, lameness, and enlarged joints. Similar problems have been reported in horses (Knight et al., 1985). A deficiency of either Ca or P will result in decreased bone mineralization, causing weak, porous bones (Hintz and Schryver, 1976; Anonymous, 1988). A diet containing insufficient Ca or excessive P can result in nutritional secondary hyperparathyroidism, or "big-head" disease in horses (Hintz and Schryver, 1976).

Calcium to Phosphorus Ratio. The ratio of Ca to P (Ca:P) in the diet is important, especially for horses. Shetland ponies were fed diets with a 2:1 or a 6:1 ratio for three years (Jordan et al., 1973). Radiographic evaluation of metacarpal bones of the high Ca:P group showed an enlarged medullary region and smaller cortical area when compared to a group fed a diet consisting of a 2:1 ratio. The 6:1 Ca:P group also showed decreased bone mineral in the bone cortex.

Magnesium. Bone contains over 50% of the Mg in the body and muscle approximately 25% (Haymes, 1991). It is required for the formation of alkaline phosphatase (ALP).

Dietary Cation-Anion Difference

The dietary cation and anion difference (DCAD) may be important for bone development. Dietary cations include Ca, Mg, Na, and K, while anions include Cl, S, and P. The DCAD (mEq/kg dietary DM) is calculated as:

$$[(Ca^{2+} + Mg^{2+} + Na + K) - (P + Cl + S)]$$

Mongin (1981) reduced the formula to:

$$(Na + K) - (Cl)$$

for use with chickens, given that the dietary requirements for Ca, Mg, P, and S are well established and fixed in the diet. Researchers began using the Mongin formula for cattle (Block, 1984), goats (Fredeen et al., 1988), swine (Patience et al., 1989), and horses (Topliff et al., 1989). Its application to other species has been less successful (Beighle et al., 1990; Popplewell et al., 1993), and has been followed by the use of the following formula:

$$(Na + K) - (Cl + S)$$

The DCAD has been shown to affect the Ca status and incidence of parturient hypocalcemia in dairy cattle (Block, 1984; Gaynor et al., 1989) and to have an impact on the acid-base status of goats (Fredeen et al., 1988), cattle (Beede et al., 1992; Block, 1994), swine (Patience et al., 1987), and horses (Stutz et al., 1992; Baker et al., 1992; Popplewell et al., 1993). It also affects gain and feed intake in finishing beef cattle (Ross et al., 1994). Dairy cows fed a highly cationic diet were less responsive to PTH than those fed a highly anionic diet (Goff et al., 1991).

Radiographic observation of the femoral head of rapidly growing dogs fed various DCAD levels revealed that dogs receiving a low DCAD had less incidence of subluxation (Kealy et al., 1993). Differences in DCAD did not result in differences in weight gain; therefore, the reduction in subluxations was unrelated to weight gain.

However, a low DCAD has increased the incidence of tibial dyschondroplasia in young chickens (Halley et al., 1987; Ruíz-López et al., 1993) and was associated with lesions of dyschondroplasia in young horses (Savage et al., 1993). A low DCAD increases bone resorption in goats and cattle (Fredeen et al., 1988; Beighle et al., 1990), and increased the urinary excretion of Ca and Cl in horses (Topliff et al., 1989; Wall et al. 1992; Baker et al., 1993). Depending on the level of dietary Ca, a low DCAD could lead to a negative Ca balance in exercising horses resulting in a deleterious effect on bone remodeling.

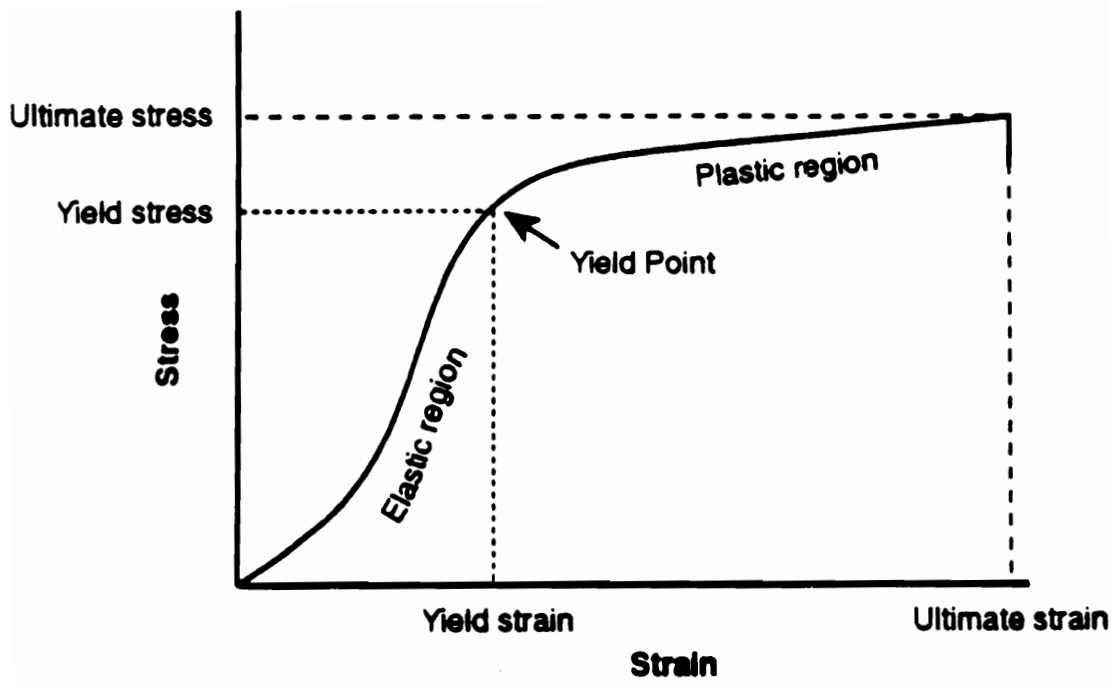

Figure 1. Stress-strain curve of a bone sample.

Table 1. Biochemical markers of bone turnover

Marker	Indicator of	Comment
Serum Ca	bone activity	lacks sensitivity
Parathyroid Hormone	resorption	highly variable in equines[a]
Alkaline Phosphatase	formation	lacks specificity[b]
Osteocalcin	formation	
Type I Procollagen Propeptides	formation	correlated with alkaline phosphatase[c]
Hydroxyproline	resorption	lacks specificity[d]
Tartrate-resistant Acid Phosphatase	resorption	lacks specificity[d]
Collagen Cross-links	resorption	

[a] Wilson, 1991; Martin et al., 1996
[b] Ljunghall and Lindh, 1989
[c] Fraher, 1993; Risteli and Risteli, 1993
[d] Delmas, 1993

Dietary calcium and cation-anion difference influence calcium status and bone remodeling in exercising horses

Introduction

Bone modeling and remodeling are affected by conditioning (Nunamaker et al., 1990; Nielsen, 1992; Porr, 1993) and diet (Thompson et al., 1988; Williams et al., 1991a). Remodeling is a biphasic process where stressed bone is first resorbed, then replaced with new stronger bone. The initial process temporarily increases the overall bone porosity and decreases the overall bone strength (Nunamaker, 1986; Martin, 1991; Jeffcott, 1992).

Recently, attention has been focused on the role of dietary cations and anions and their role in Ca balance and bone development. The dietary cation-anion difference (DCAD), commonly expressed as (Na + K) - Cl in mEq/kg DM, affects the Ca status of animals. A low DCAD increases bone resorption in cattle (Beighle et al., 1990), and increases the urinary excretion of Ca in horses (Baker et al., 1993). A low DCAD combined with a diet low in Ca could lead to increased urinary Ca losses and a negative Ca balance in exercising horses (Wall et al., 1992). A negative Ca balance, especially when combined with the stresses of training, could impact bone development and remodeling. Dietary Ca affects bone directly through it's incorporation into hydroxyapatite and through DCAD, as a cation. Dietary Cl would affect bone through DCAD, as an anion.

Objective

The objectives of this study were to evaluate the effects of high and low levels of dietary calcium and chloride and conditioning by treadmill exercise for 12 wk on bone remodeling and calcium status in Arabian horses.

Materials and Methods

Animals

Twelve Arabian horses, seven geldings and five mares, were assigned to one of four treatment groups. The horses ranged from three to seven years of age. Animals were placed in groups using stratified random sampling based on sex to ensure that at least one mare was selected into each group. All horses had undergone no forced exercise for at least six mo prior to beginning the study.

Experimental Procedure

Housing. Horses were divided into three groups and kept in three dry lots, two 1920 m^2 lots with 3 horses each and one 7080 m^2 lot with 6 horses. The animals were placed in 2 x 4 m box stalls twice per d for feeding.

Exercise Protocol. An education period was conducted for three wk to introduce inexperienced animals (n = 5) and reintroduce experienced animals (n = 7) to a high speed treadmill (Mustang 2200, Kagra International, Fahrwangen, Switzerland). Care was taken not to induce exercise related changes on bone during this time by only exercising them a short time at slow speeds 1 to 2 d/wk. The exercise protocol for weeks 1 to 3 is shown in Table 2. Additional 3 min gallops at 8 m/s and 9 m/s, with

a 3 min period of trotting between each, were added during weeks 3 to 6 and 6 to 12, respectively. Horses were exercised on the treadmill and cooled out on a mechanical walker 2 d/wk, and were exercised for 1 h/d at 1.1 m/s on the mechanical walker another 2 d/wk.

Diet. Horses were fed at 0630 and 1500 h and allowed three hr to eat before being turned out. Before the start of the experiment and during the education period, all horses were fed mixed orchardgrass hay and a mineral mix (Southern States SCC-317805, Livestock Mineral 2:1) free choice. Two animals were supplemented with barley due to low body condition. At the start of exercise, horses were assigned to and fed one of four diets in a 2 x 2 factorial design (Table 3). Animals were initially fed 2 % of their body weight, then adjustments were made to maintain body weight. Hay was chopped and combined with the rest of the dietary ingredients, creating a complete mixed diet. A trace mineral mix was designed to meet 1.6 to 2.0 times 1989 NRC recommendations for exercising horses. It was based on analysis of the hay and typical values (NRC, 1989) of the concentrate (Table 4). Diets were designated LH, which contained low Ca (.35% Ca-1989 NRC recommended level)-high Cl (.6% Cl from 1.0% added NaCl), LL, which contained low Ca-low Cl (1.44% added $NaHCO_3$ to provide equivalent amounts of Na), HH, which contained high Ca (.7% Ca from a 63% dicalcium phosphate-37% limestone mix)-high Cl, and HL, which contained high Ca-high Cl. The P level was adjusted to maintain Ca:P at 1.4:1 (NRC, 1989).

Feed samples. Feed samples were collected four times during the experiment and

analyzed for DM, crude fiber, and CP using AOAC methods (1991), and Ca, P, Mg, Na, and K, using Induced Coupled Plasma Spectrometry (ICAP 9000, Thermo Jarrell-Ash, Franklin, MA). Samples for Cl analysis were sent to the DHIA laboratory, Ithaca, NY. Nutrient analysis of the diets is shown in Table 5. Water was analyzed for Ca, Na, Cl, and S using the same methods (Table 6). The DCAD, expressed as mEq/kg DM for each diet are shown in Table 7. Included are totals for:

$[(Ca^{2+} + Mg^{2+} + Na + K) - (P + Cl + S)$

$[(Na + K) - (Cl)]$

$[(Na + K) - (Cl + S)]$

Dietary intake data, averaged over the experiment, are shown in Table 8.

Measurements

Animals were weighed weekly on an electronic scale (Dyco, Incorporated, Model 1200, Scottsdale, AZ). Blood and urine samples, and dorsopalmar (DP) radiographs of the left metacarpus were taken at 21 d intervals.

Blood. Serum and plasma samples were collected from all horses by jugular venipuncture (Vacutainer, Becton Dickinson, Rutherford, NJ). An arterial sample also was taken in a lithium heparin blood-gas syringe from a carotid loop under the skin. The loop was translocated surgically at least 3 mo prior to the start of the experiment. All samples were collected between 0730 and 0900 h and spun in a refrigerated centrifuge (1660 x g for 12 min at 4°C). Serum or plasma were stored at -20°C within 2 h, unless otherwise indicated.

Unless otherwise stated, blood analysis was by colorimetric assay using diagnostic kits (Sigma Diagnostics, St. Louis, MO). One blood tube was allowed to clot at room temperature before being centrifuged to collect serum. Serum was used for the assay of creatinine (Procedure 557), Ca (Procedure 587), inorganic P (Procedure 360-UV), Mg (Procedure 596), total protein (Procedure 541), and albumin (Procedure 631). One blood tube was kept on ice until centrifuged to collect plasma. Plasma was used for hydroxyproline analysis by the method of Fujii et al. (1981). One blood tube was kept on ice until centrifuged to collect serum. Serum was used for osteocalcin analysis (Osteocalcin Radioimmunoassay Kit, INCSTAR Corporation, Stillwater, MN). One blood tube was allowed to cold clot (5°C) for 12-24 h before being centrifuged to collect serum, then stored at -80°C. This serum was used for parathyroid hormone (PTH) analysis using a two-site immunochemiluminometric assay (Allegro Intact PTH, Nichols Institute Diagnostic, San Juan Capistrano, CA) (Wilson et al., 1991). Plasma was used for Na, K, and Cl analysis (Clinical Chemistry Slide, Kodak Ektachem Clinical Products, Eastman Kodak Company, Rochester, NY).

Arterial blood was analyzed within 2 h for ionized Ca and pH using a potentiometric method (Ciba Corning 288 Blood Gas System, Ciba Corning Diagnostic Corp., Medfield, MA).

Urine. Mares were cleaned (Operand Iodine Scrub, Redi Products, Prichard, WV) and catheterized, and a sample was obtained from the mid-stream. Urine was frozen and stored at -20°C within 2 h.

Unless otherwise stated, urine analysis was by colorimetric assay using diagnostic kits (Sigma Diagnostics, St. Louis, MO). Urine was analyzed for Ca, P, Mg, and creatinine excretion.

At the end of the experiment, a series of collections were made to validate the method used. Urine was collected from the early stream, at an estimated mid-stream, and end of stream (remnant in the catheter) when it appeared the bladder was empty. The mares were then placed in stalls for one h, at the end of which time another collection was made. Urine samples were analyzed within 24 h. An aliquot of each sample was frozen for analysis at 2 and 4 mo post-collection.

Radiographs. Dorsopalmar radiographs (Kodak film, Kodak cassette with lanex screen) of the left front third metacarpal bone (McIII) were taken (80 kv, 20 ma, .04 s, 75 cm focal distance) with a portable x-ray machine (MinXRay 803, MinXRay Inc., Northbrook, IL) every 21 d. An aluminum step wedge was exposed simultaneously with the McIII and used as a reference standard, providing radiographic bone aluminum equivalents (RBAE) for estimation of bone mineral content (BMC) using an image analyzer (Southern Micro Instruments, Inc., Atlanta, GA) as described by Meakim et al. (1981). Three sites on the medial and lateral aspects of the McIII, at the nutrient foramen and one cm above and one cm below the nutrient foramen, were used to measure RBAE, bone width (BW), and medullary width (MW). Medial and lateral BMC were estimated using the equations described by Ott et al. (1987):

$$\text{medial BMC} = .87(\text{RBAE}) - 2.35 \quad (r^2 = .92)$$

lateral BMC = .93(RBAE) - 2.86 (r^2 = .93)

The three values were averaged to provide BMC for the proximal diaphyseal medial and lateral cortices. Values for BW and MW were each averaged and used for the calculation of cortical area (CA) based on circular bone geometry (Ott et al., 1987):

$CA = (BW)^2 - (MW)^2$ (r^2 = .88)

Medial and lateral BMC, BW, MW, and CA were also measured at one site in the proximal metaphyseal region of the McIII.

Although stratified random sampling was used to select horses into their respective groups, there was a difference in body weight between groups. Due to this difference, data collected by radiographic photometry was compared on a per unit body weight basis (per kg BW) to eliminate the effect of body weight on bone response.

Statistical Analysis

The experiment used a 2 x 2 factorial design. Data were analyzed using the GLM procedures of SAS (1991) and repeated measures analysis of variance, evaluating the effects of dietary Ca, Cl, sex, age, and interactions. Data from wk 0 was subtracted from subsequent data points, and these differences were also evaluated using the GLM procedures of SAS (1991) and repeated measures analysis of variance. Variables that were not significant were dropped from the model. Data for diets and nutrient intakes were reported as means ± standard errors, while data for serum, plasma, urine, and radiographs were reported as least squares means ± standard errors. Regressions were also done. Significance was inferred at $P < .05$, and a trend at $P < .10$.

Table 2. Exercise protocol for 12 wk of conditioning on a high speed equine treadmill

Speed, m/min	Gait	Slope %	Conditioning Time, min	Week Started
72	walk	0	2.5	0
72	walk	6	2.5	0
192	trot	6	4	0
420	gallop	6	3	0
192	trot	6	3	0
480	gallop	6	3	3
192	trot	6	3	3
540	gallop	6	3	6
192	trot	6	3	6
72	walk	0	2	0

Table 3. Feed composition as a percentage of dietary DM

Ingredient	HH	HL	LH	LL
Orchardgrass Hay	57.2	56.7	58.5	58
Cracked corn	20	20	20	20
SBM, 44% CP	10	10	10	10
Molasses	10	10	10	10
Trace mineral mix[a]	.5	.5	.5	.5
NaCl	1.0	---	1.0	---
$NaHCO_3$	---	1.44	---	1.44
Calcium mix[b]	1.3	1.3	---	---

[a] See Table 4
[b] Dicalcium phosphate - 63%, Limestone - 37%

Table 4. Trace mineral mix

Mineral Salt	g salt/100 lb premix[a]
$FeSO_4$,7 H_2O	2082.57
$ZnSO_4$	565.42
$CuSO_4$,5 H_2O	262.15
$MnSO_4$,1 H_2O	1788.06
Na_2SeO_3	0.20
KI	2.37

[a] soybean meal used as a carrier

Table 5. Nutrient analysis of diets[a, b, c]

Nutrient, %	HH	HL	LH	LL
DM	90.5 ± .4	90.8 ± .2	91.5 ± .9	90.9 ± .3
CP	12.0 ± 1.0	11.8 ± .4	12.5 ± .7	11.6 ± .6
ADF	25.7 ± 1.5	27.6 ± 2.2	26.1 ± .5	29.1 ± 1.7
Ca	.73 ± .07	.65 ± .04	.39 ± .04	.36 ± .03
P	.68 ± .1	.6 ± .1	.47 ± .1	.41 ± .08
Mg	.25 ± .01	.24 ± .01	.24 ± .01	.24 ± .01
S	.25 ± .03	.23 ± .01	.25 ± .02	.22 ± .02
Na	.43 ± .06	.39 ± .02	.44 ± .05	.38 ± .04
Cl	1.6 ± .2	.9 ± .05	1.7 ± .08	.83 ± .01
K	1.7 ± .08	1.8 ± .06	1.8 ± .08	1.6 ± .08

[a] dry matter basis
[b] means ± SE
[c] average of four samples for each diet

Table 6. Mineral analysis of water

Nutrient, mg/L	Lot 1 and 2	Lot 3	Barn
Ca	5.5	5.6	5.9
S	7.0	6.6	6.7
Na	9.4	9.7	9.1
Cl	40	40	45

Table 7. Calculated dietary cation-anion difference expressed as mEq/kg DM

Diet	(Na+K)-Cl	(Na+K)-(Cl+S)	$(Ca^{2+}+Mg^{2+}+Na+K)-(P+Cl+S)$
HH	167	88	440
HL	370	298	623
LH	164	86	324
LL	340	271	510

Table 8. Dietary intake data

Horse	Diet	Body weight (kg)[a]	Intake/d (kg)[a]	Intake as percent of body weight
3	HH	427.1	7.5	1.8
5	HH	487.4	9.4	1.9
9	HH	460.1	9.4	2.0
2	HL	484.1	9.4	1.9
6	HL	513.5	10.3	2.0
7	HL	506.6	10.3	2.0
1	LH	423.6	8.0	1.9
10	LH	484.4	9.8	2.0
11	LH	391.8	8.9	2.3
4	LL	406.6	7.1	1.8
8	LL	488.5	908	2.0
12	LL	442.5	10.3	2.3

[a] data are averaged over the 12 wk of the experiment

Results

All horses participating in the study completed the protocol in good health. During wk 4, one gelding was removed from exercise for 7 d due to lameness caused by a subsolar abscess. Following routine treatment, the gelding was fitted with an angle-bar shoe and exercise was resumed. The horse was sound for the remainder of the study.

Body Weight

Body weight was from 402 and 483 kg. There was no effect of training or diet.

Radiographic Photometry

Bone Mineral Content. Values for estimated proximal diaphyseal cortical bone mineral content (DBMC) was between 18.9 and 21.4 g/2 cm section on the medial aspect and between 17.6 and 20.8 g/2 cm section on the lateral aspect. Proximal metaphyseal cortical bone mineral content (MBMC) was between 17.1 and 20.1 g/2 cm section on the medial aspect and between 17.3 and 19 g/2 cm section on the lateral aspect. These were similar to values reported previously (Nielsen, 1992; Porr, 1993).

Medial DBMC showed a trend for an increase with training ($P = .083$). Horses fed high Ca diets showed a trend for an increase in medial DBMC ($P = .076$) as compared to a decrease seen between wk 9 and 12 in horses fed low Ca diets (Figure 2). Changes from wk 0 showed that horses fed the LH diet tended to have a decreased medial DBMC throughout the study ($P = .099$). There was no effect of training or diet on lateral DBMC.

Bone Mineral Content Per Kilogram Body Weight. Medial DBMC per kg BW showed an increase with training (P = .001). Changes from wk 0 showed this change to be affected by diet (P =.019), with horses fed the LH diet showing a decreased medial DBMC per kg BW. Changes from wk 0 showed a trend for horses fed high Ca diets to have a greater increase in medial DBMC per kg BW (P = .089) as compared to horses fed low Ca diets (Figure 3).

Lateral DBMC per kg BW showed a trend for an increase with training (P = .095). Three-year olds had a greater lateral MBMC per kg BW as compared to six-year olds (P = .042), but there was no difference when changes from wk 0 were analyzed.

Medullary and Bone Width. Proximal diaphyseal medullary width (DMW) was between 13.1 and 16.4 mm, and proximal diaphyseal bone width (DBW) was between 33.2 and 37.2 mm. Proximal metaphyseal medullary width (MMW) was between 12.8 and 18 mm, and proximal metaphyseal bone width (MBW) was between 39.4 and 42.8 mm. There were no effects of training or diet on DMW or MMW.

There was an increase in DBW with training (P = .009) (Figure 4). Changes from wk 0 showed that horses fed the LH diet showed the smallest increase in DBW, while horses fed the HL and HH diets showed the greatest increase in DBW (P = .032). Changes from wk 0 showed that six-year olds had a greater DBW as compared to three-year olds (P = .037). Changes from wk 0 showed a trend for an increase in MBW in horses fed high Ca diets as compared to horses fed low Ca diets (P = .091) (Figure 5). Six-year olds had a tendency for a greater MBW as compared to three-

year olds (P = .077), but there was no difference for changes from wk 0.

Medullary and Bone Width per Kilogram Body Weight. There was a trend for an increase in DMW per kg BW with training (P = .055). This increase was greatest for horses fed the LL diet as compared to the other diets (P = .055). There was a trend for an increase in MMW per kg BW with training (P = .052). Three-year olds tended to have a greater MMW per kg BW as compared to six-year olds (P = .094), but there was no difference when changes from wk 0 were analyzed.

Proximal DBW per kg BW increased with training (P = .002). Changes from wk 0 showed three-year olds to have a lesser increase in DBW per kg BW (P = .038). Three-year olds tended to have a greater MBW per kg BW as compared to six-year olds (P = .07), but there was no difference when changes from wk 0 were considered.

Cortical Area. Estimated proximal diaphyseal cortical area (DCA) was between 890 and 1166 mm^2, and estimated proximal metaphyseal cortical area (MCA) was between 1292 and 1611 mm^2.

The DCA increased with training (P = .011) (Figure 6). Changes from wk 0 showed that horses fed the HL diet showed a greater increase in DCA between wk 0 and 3 (P = .008), but there was no difference at wk 12. Changes from wk 0 showed that six-year olds had a greater increase in DCA (P = .037) between wk 0 and 9 as compared to three-year olds. There was no difference at wk 12 (Figure 7).

Horses fed low Cl diets had a greater estimated proximal metaphyseal cortical area (MCA) (P = .028) as compared to horses fed high Cl diets, but there was no difference

when changes from wk 0 were analyzed.

Cortical Area per Kg Body Weight. Estimated DCA per kg BW decreased with training (P = .006). Horses fed the HL diet showed the greatest increase in DCA per kg BW (P = .014) between wk 0 and 3, but there were no differences at wk 12.

Estimated MCA per kg BW tended to increase with training (P = .073). Horses fed the HH diet tended to show a greater increase in MCA per kg BW between wk 9 and 12 (P = .069) as compared to horses fed the other diets, while horses fed the LH diet showed a tendancy for a decrease in MCA per kg BW during the experiment (Figure 8).

Serum and Plasma Analysis

Serum Calcium. Serum Ca was between 2.4 and 3.2 mmol/L (9.4 and 12.7 mg/dl). Changes from wk 0 showed a decrease with training (P = .001), showing a linear relationship (Figure 9):

$$[Ca] = .211 - .136(T)$$

$$r = .94 \quad P = .017,$$

where T is time. Three-year olds showed a greater decrease in serum Ca concentration from wk 0 to 12 as compared to six-year olds (P = .001). The decrease in the three-year olds showed a linear relationship with time (Figure 10):

$$[Ca] = .286 - .21(T)$$

$$r = .94 \quad P = .018$$

Serum Phosphorus. Serum P was between .82 and 1.5 mmol/L (2.6 and 4.5 mg/dl). There was an increase in serum P with training (P = .001), reaching a peak at wk 3 and declining thereafter (Figure 11). This increase was not affected by diet.

Serum Magnesium. Serum Mg was between .9 and 1 mmol/L (1.7 and 2 mEq/L). Changes from wk 0 showed a decrease with training (P = .016). There was a linear decrease with time (Figure 12):

$$[Mg] = .038 - .025(T)$$

$$r = .9 \quad P = .037$$

There was no effect of diet.

Plasma Chloride. Plasma Cl was between 96.7 and 101.1 mmol/L. Changes from wk 0 showed a decrease with training (P = .007). This decrease was linear over time (Figure 13):

$$[Cl] = .743 - .583(T)$$

$$r = .94 \quad P = .018$$

Three-year olds showed a greater decrease between wk 9 and 12 as compared to six-year olds (P = .005), who showed an increase in plasma Cl during that time. There was no effect of diet.

Blood pH. Blood pH was between 7.38 and 7.42. Blood pH tended to be lower for horses fed the high Cl diet (P = .058) but there were no differences when changes from wk 0 were analyzed. There was no effect of training.

Serum Total Protein. Serum total protein was between 5.9 and 7.4 g/dL. Changes from wk 0 showed a decrease with training (P = .001) (Figure 14). This decrease tended to be affected by diet (P = .093), with horses fed the HH diet tending to have the lowest serum total protein. Three-year olds showed a greater decrease between wk 0 and 3 and tended to have a lower total protein concentration as compared to six-year olds (P = .088), but there was no difference at wk 12.

Serum Albumin. Serum albumin was between 3.1 and 3.7 g/dL. Changes from wk 0 showed a decrease with training (P = .003). Only three-year olds had a decrease in serum albumin and had lower concentrations throughout the study (P = .023) (Figure 15). There was no effect of diet.

Serum Parathyroid Hormone. Serum PTH concentrations were highly variable and ranged between 6 and 294 pg/mL. Changes from wk 0 showed an increase with training (P = .006) between wk 0 and 3 (Figure 16). A regression of serum PTH on serum Ca showed a slight linear relationship (Figure 17):

$$[PTH] = 501.7 - 131.9(Ca)$$

$$r = .66 \quad P = .225$$

Serum Osteocalcin. Serum osteocalcin concentrations varied from 7.2 to 14.5 ng/mL. Changes from wk 0 showed an initial increase followed by a decrease with training (P = .001). This decrease was affected by dietary Ca (P = .003), with horses fed the low Ca diets showing higher concentrations as compared to horses fed the high Ca diets. This decrease showed a linear relationship with time (Figure 18):

[Osteocalcin, high Ca diet] = .582 - 1.39(T)

r = .995 P = .005

[Osteocalcin, low Ca diet] = 4.531 - .843(T)

r = .9951 P = .004

Plasma Hydroxyproline. Plasma hydroxyproline concentrations were between 7.1 and 12.6 μmol/L. Changes from wk 0 showed a trend for a decrease in response to dietary Ca (P = .081) in the high Ca group (Figure 19).

Urinary Fractional Excretions (Creatinine Ratios)

Urinary FE was compared only among mares. The HH diet was represented by 2 mares, the other three diets by 1 mare each. The only evaluation made was on the effect of training on urinary FE. The highest values, seen at wk 0, were 28.9% for Ca, .9% for P, and 42.5% for Mg. Urinary FE of Ca (P = .001), P (P = .001), and Mg (P = .002) showed a significant decrease in FE between wk 0 and 3, and then remained constant for the remainder of the experiment (Figure 20).

Evaluation of Urine Collection Method

There was no effect of time of collection (i.e. early, mid, or late stream) on urinary FE of Ca, P, or Mg, nor was there an effect of storage time when samples were frozen at -20 C.

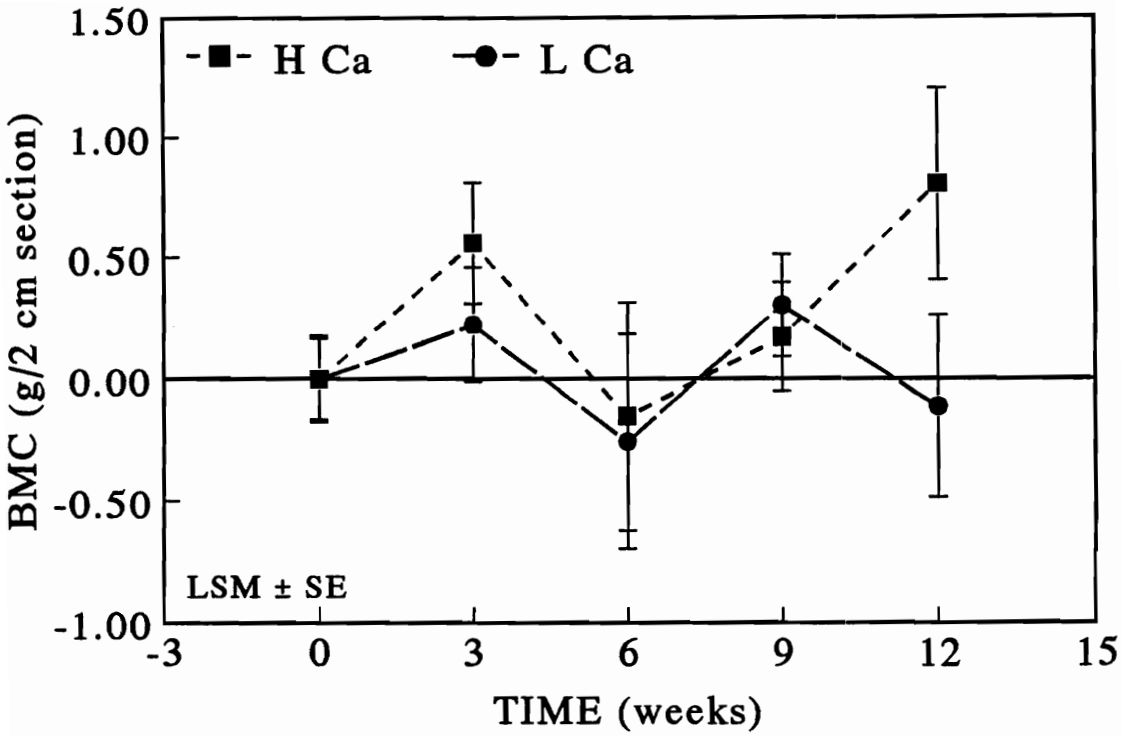

Figure 2. Dietary calcium effect (P = .076) on change in estimated bone mineral content of the medial cortex of the proximal diaphysis of the equine third metacarpal bone.

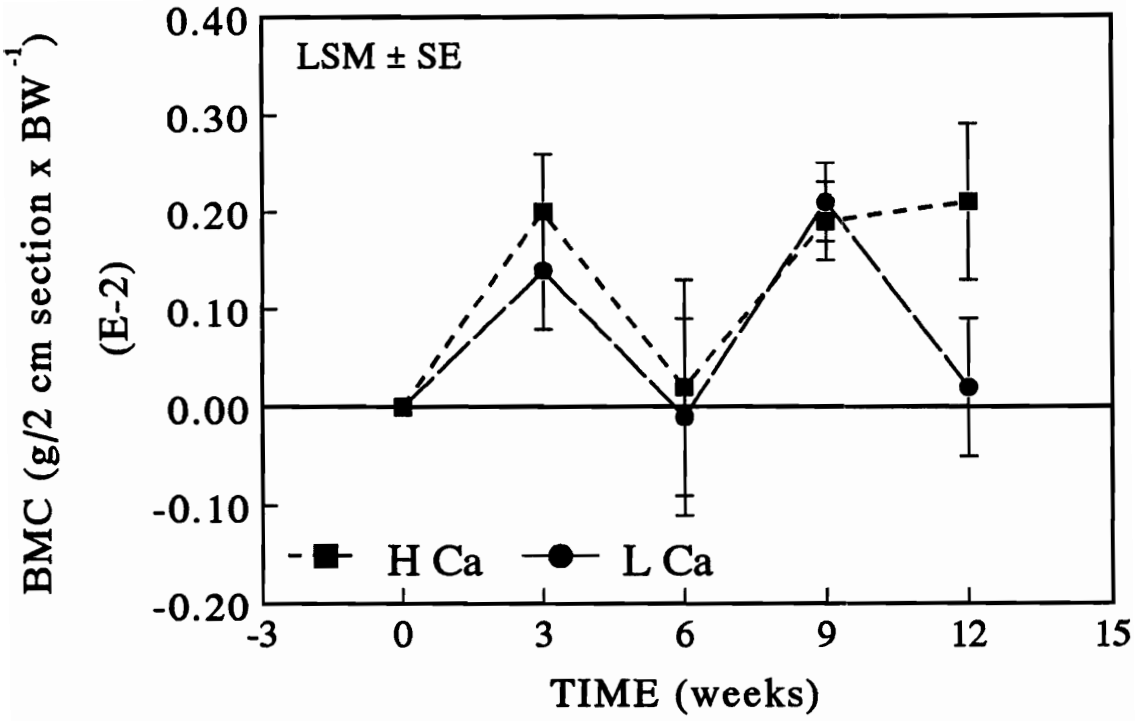

Figure 3. Dietary calcium effect (P = .089) on change in estimated bone mineral content per kilogram body weight of the medial cortex of the proximal diaphysis of the equine third metacarpal bone.

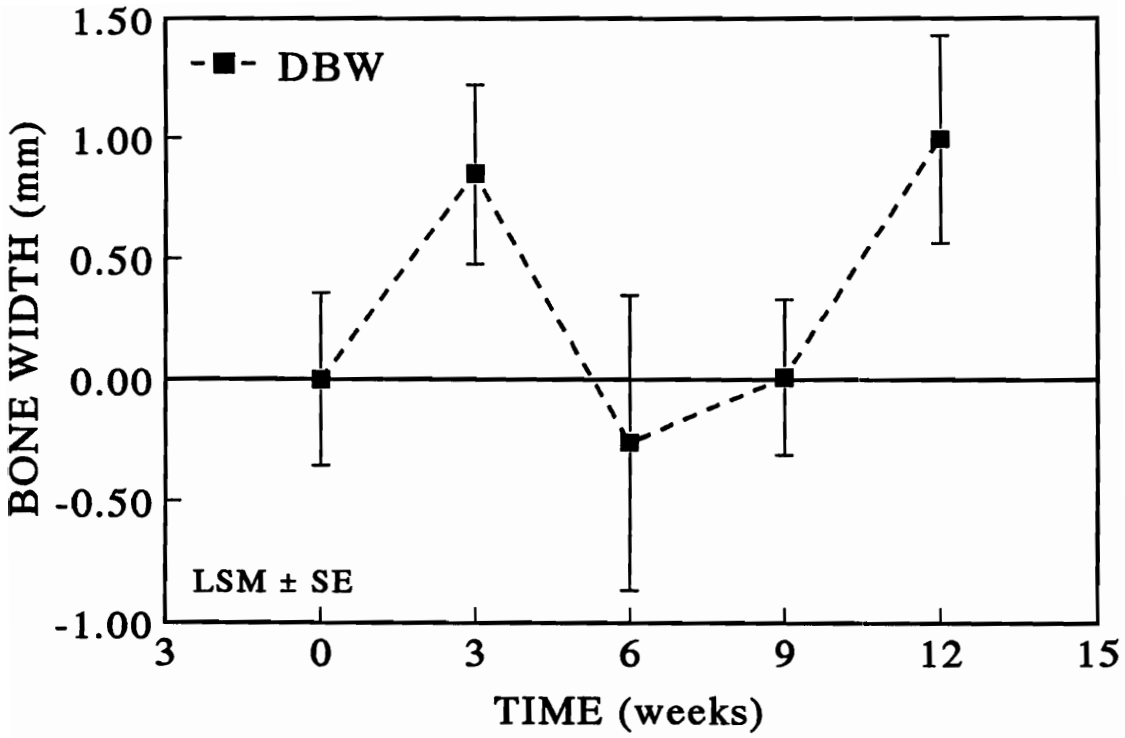

Figure 4. Training effect (P = .009) on change in bone width of the proximal diaphysis of the equine third metacarpal bone.

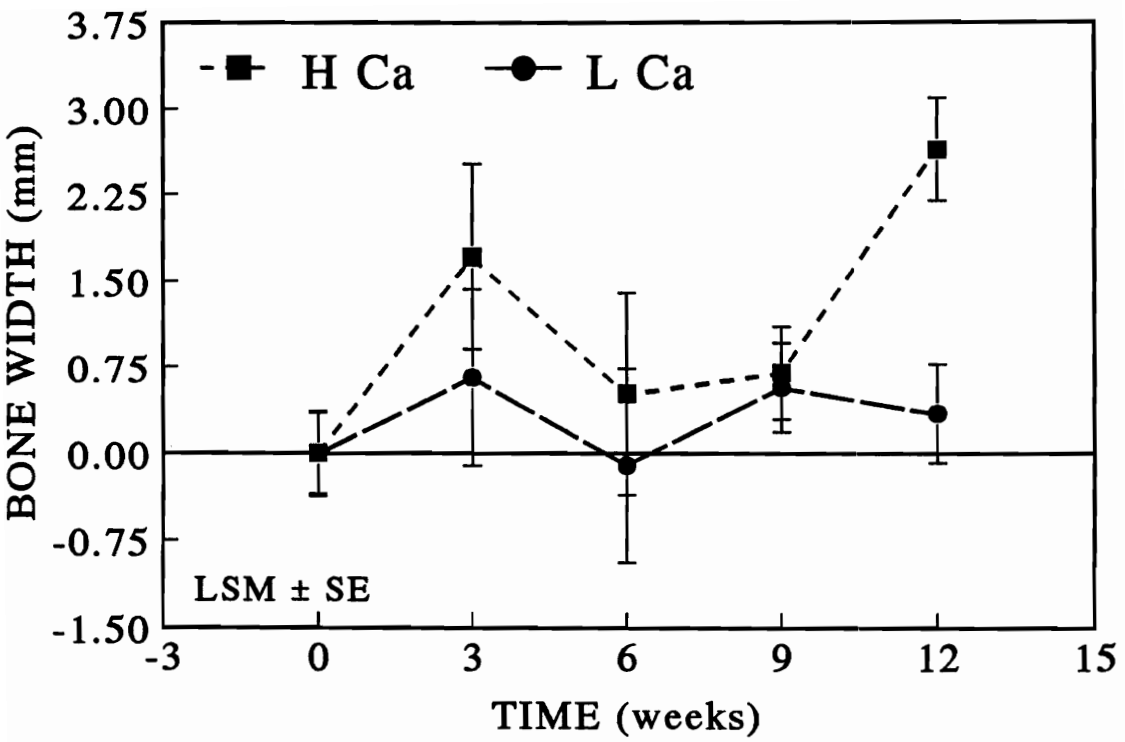

Figure 5. Dietary calcium effect (P = .091) on change in bone width of the proximal metaphysis of the equine third metacarpal bone.

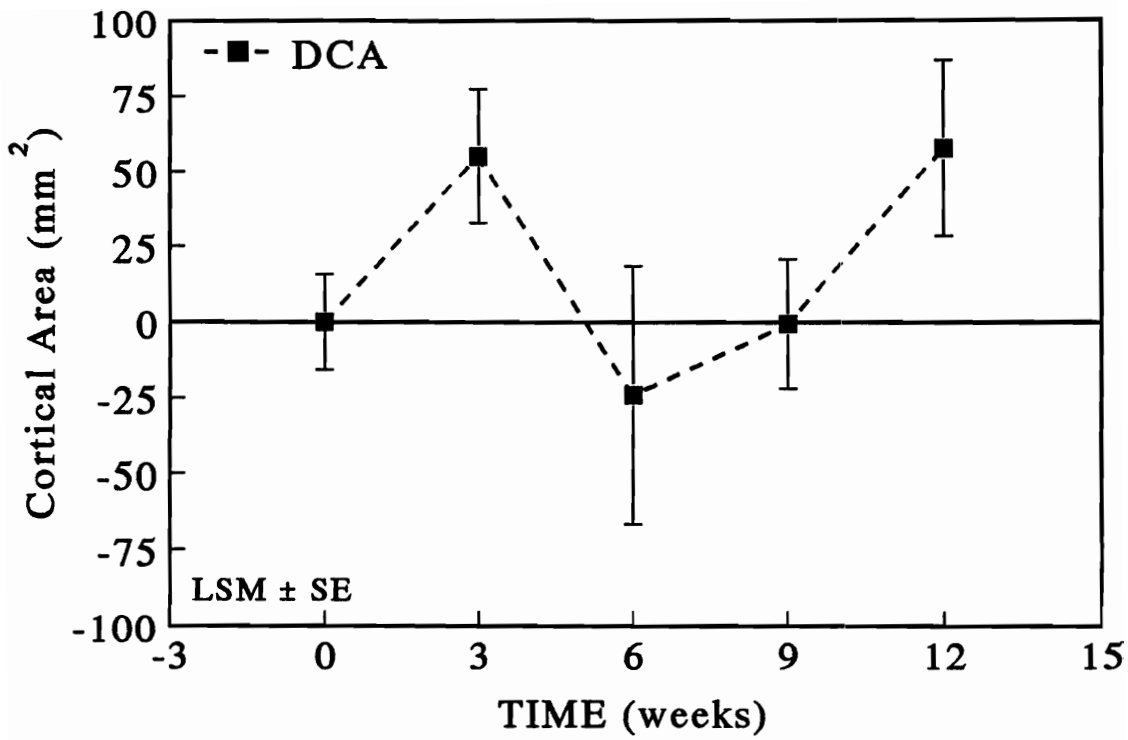

Figure 6. Training effect (P = .011) on change in estimated cortical area of the proximal diaphysis of the equine third metacarpal bone.

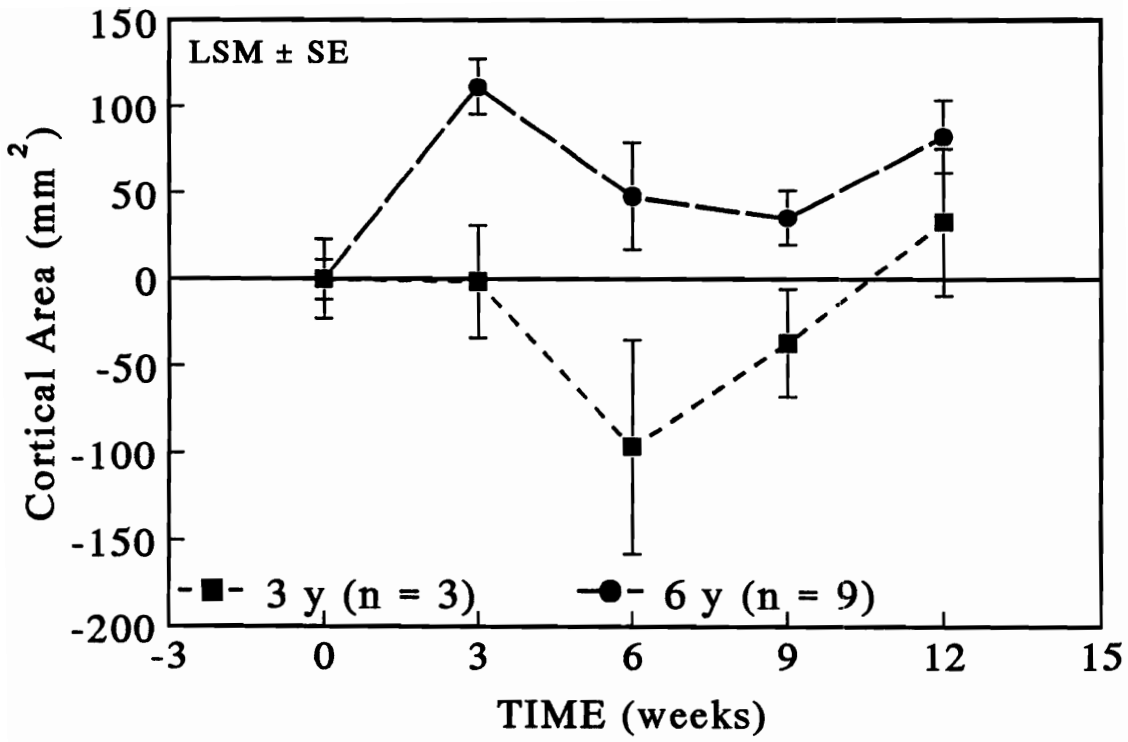

Figure 7. Age effect (P = .037) on change in estimated cortical area of the proximal diaphysis of the equine third metacarpal bone.

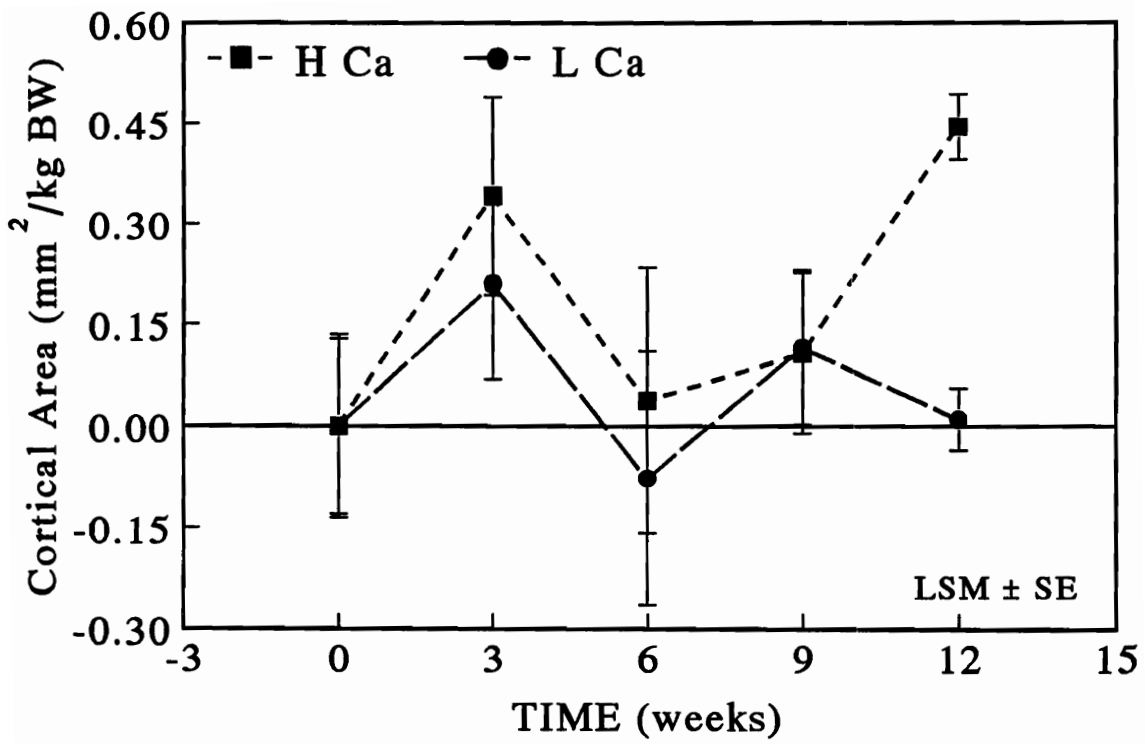

Figure 8. Dietary calcium effect (P = .069) on change in estimated cortical area per kilogram body weight of the proximal metapysis of the equine third metacarpal bone.

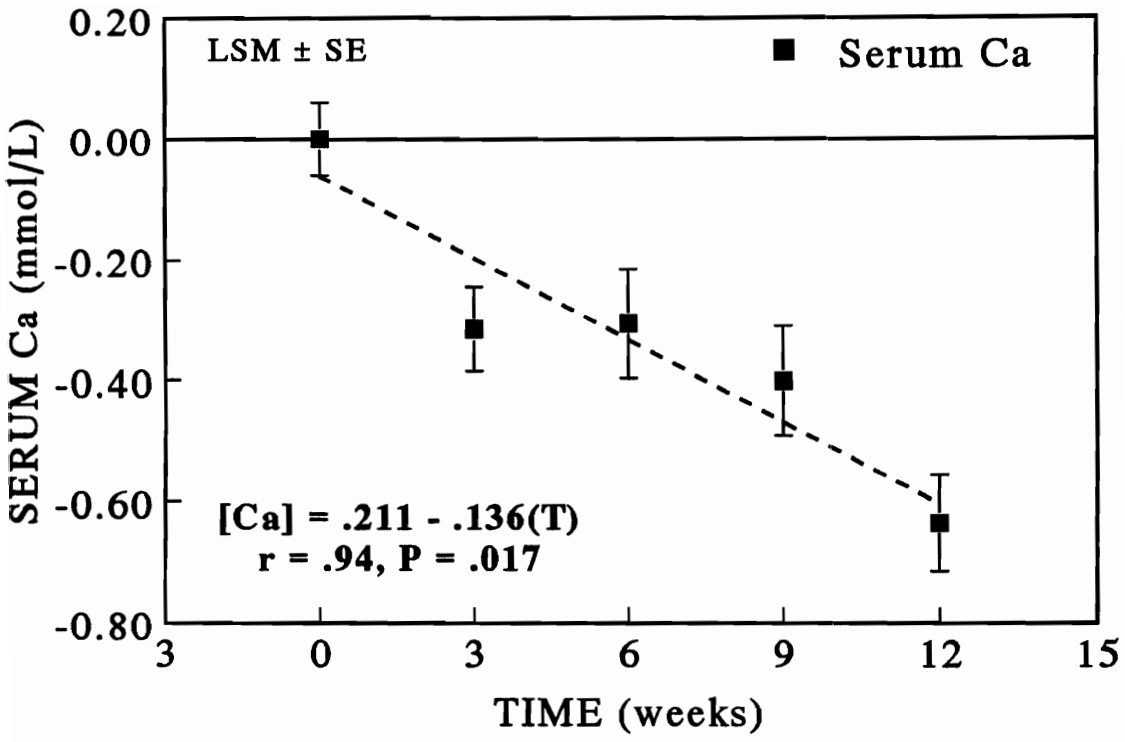

Figure 9. Training effect (P = .001) on change in resting equine serum calcium. There was a linear regression of calcium on time (P = .017).

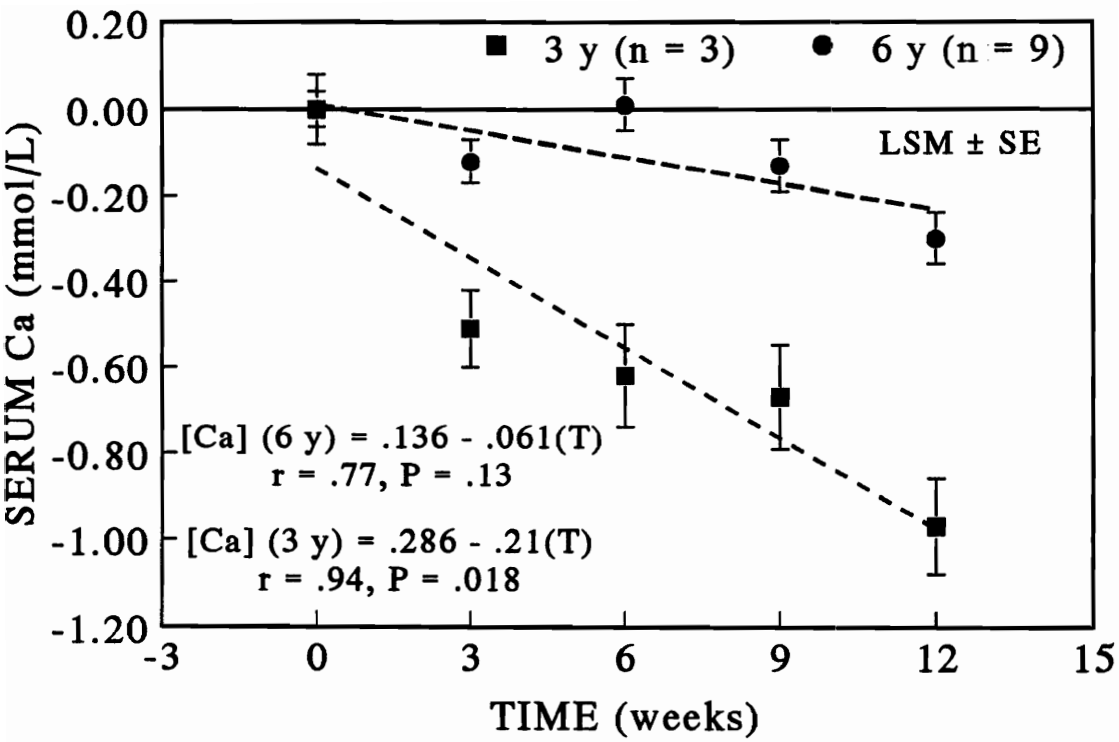

Figure 10. Age effect (P = .001) on change in resting equine serum calcium. There was a linear regression of calcium on time for three-year olds (P = .018).

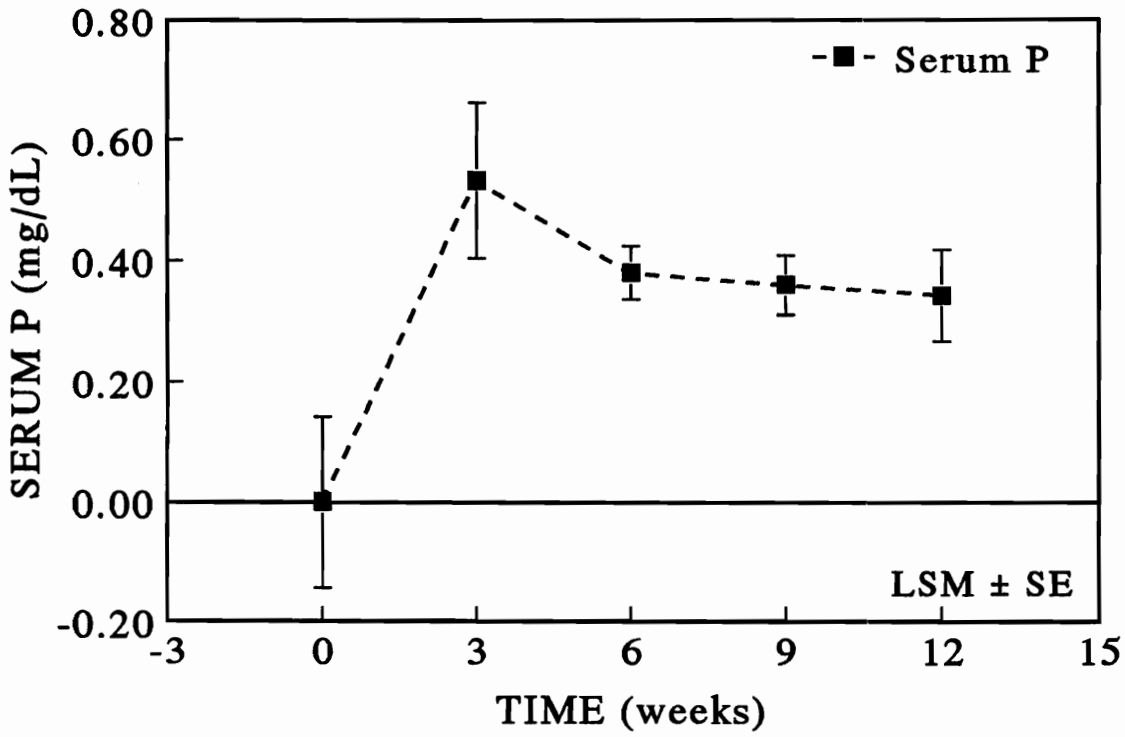

Figure 11. Training effect (P = .001) on change in resting equine serum phosphorus.

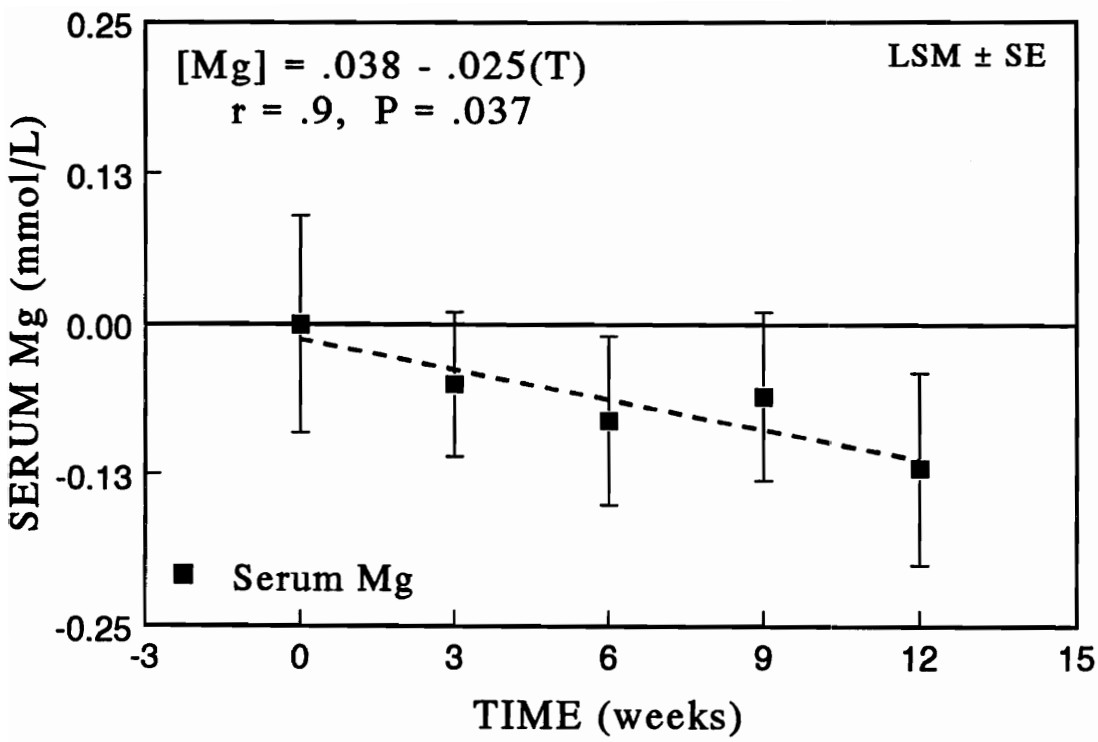

Figure 12. Training effect (P = .016) on change in resting equine serum magnesium. There was a linear regression of magnesium on time (P = .037).

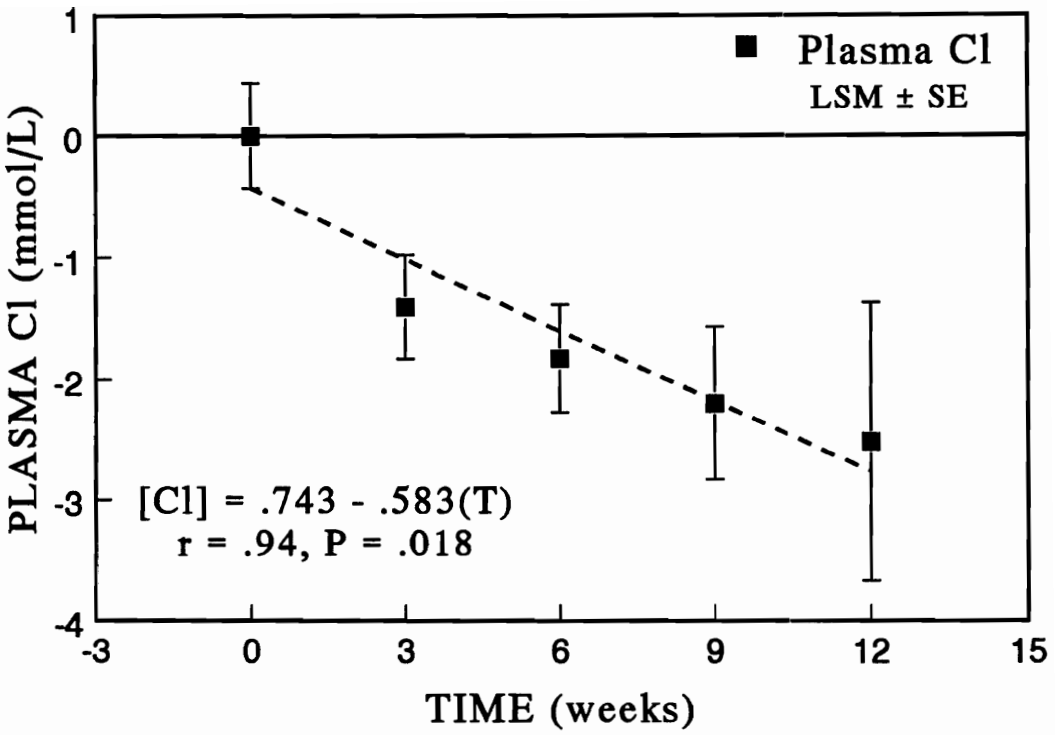

Figure 13. Training effect (P = .007) on change in resting equine plasma chloride. There was a linear regression of chloride on time (P = .018).

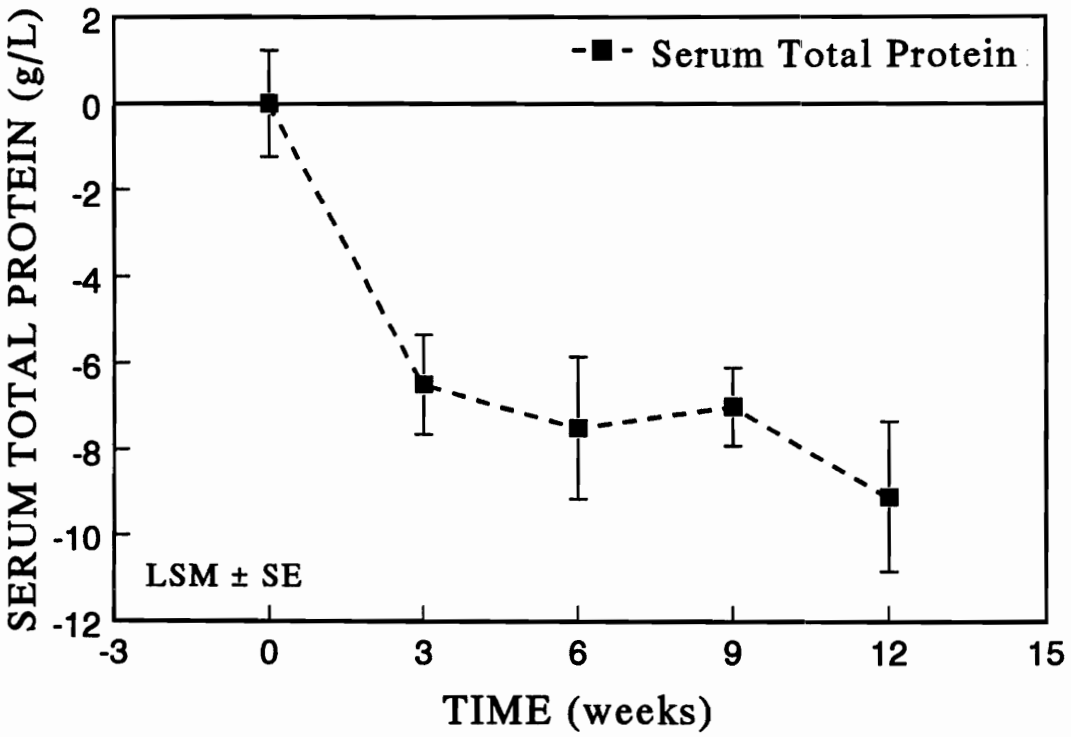

Figure 14. Training effect (P = .001) on change in resting equine serum total protein.

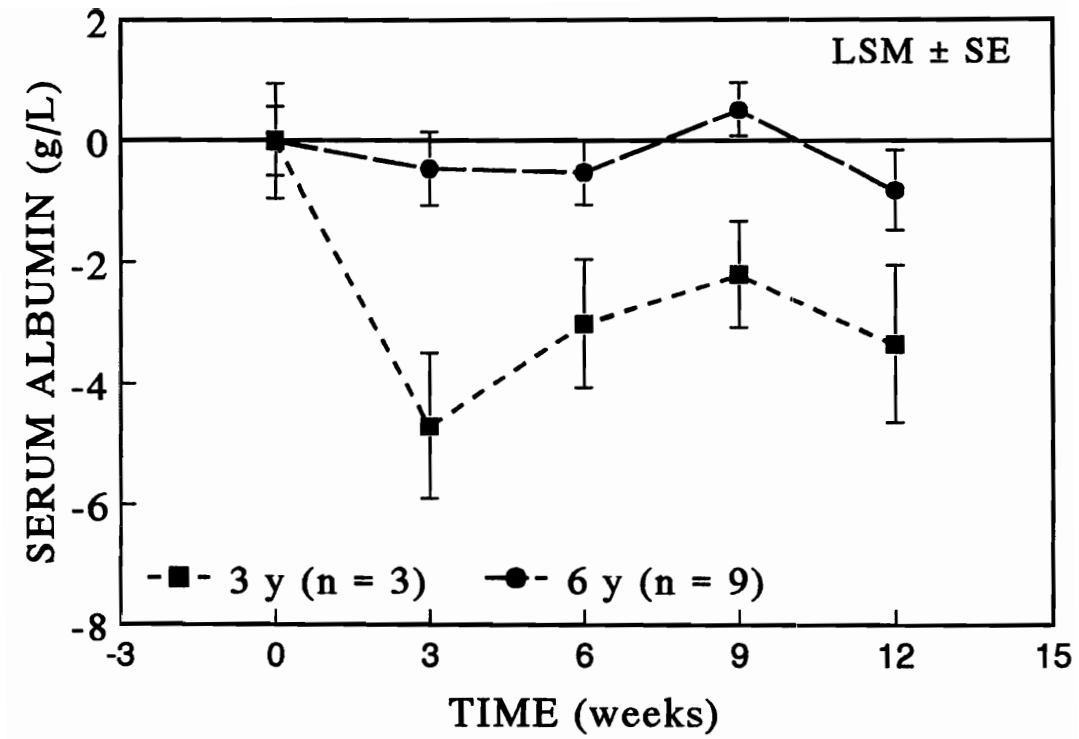

Figure 15. Age effect (P = .023) on change in resting equine serum albumin.

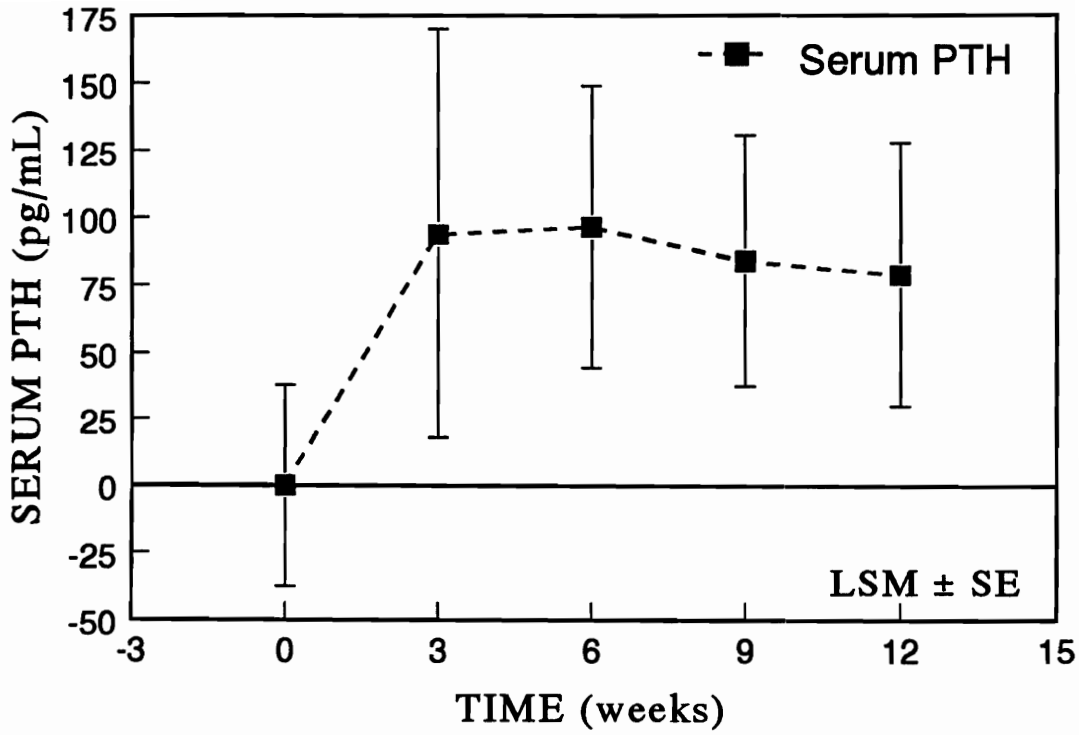

Figure 16. Training effect (P = .006) on change in resting equine serum parathyroid hormone.

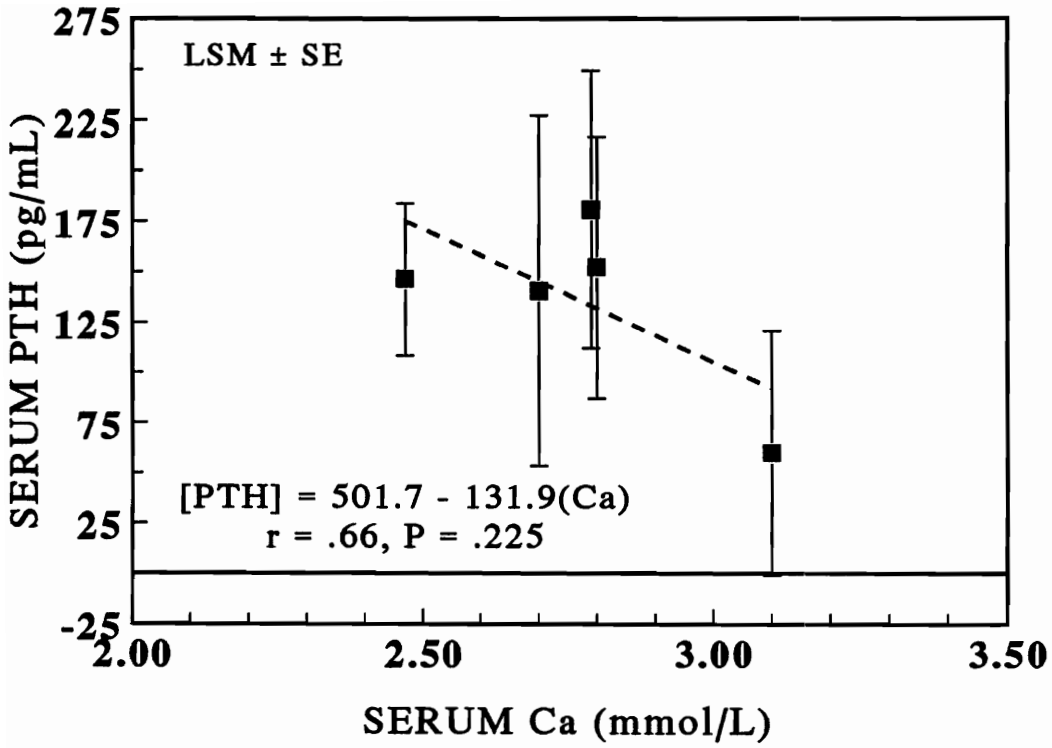

Figure 17. Regression of serum parathyroid hormone on serum calcium (P = .225) in resting equines.

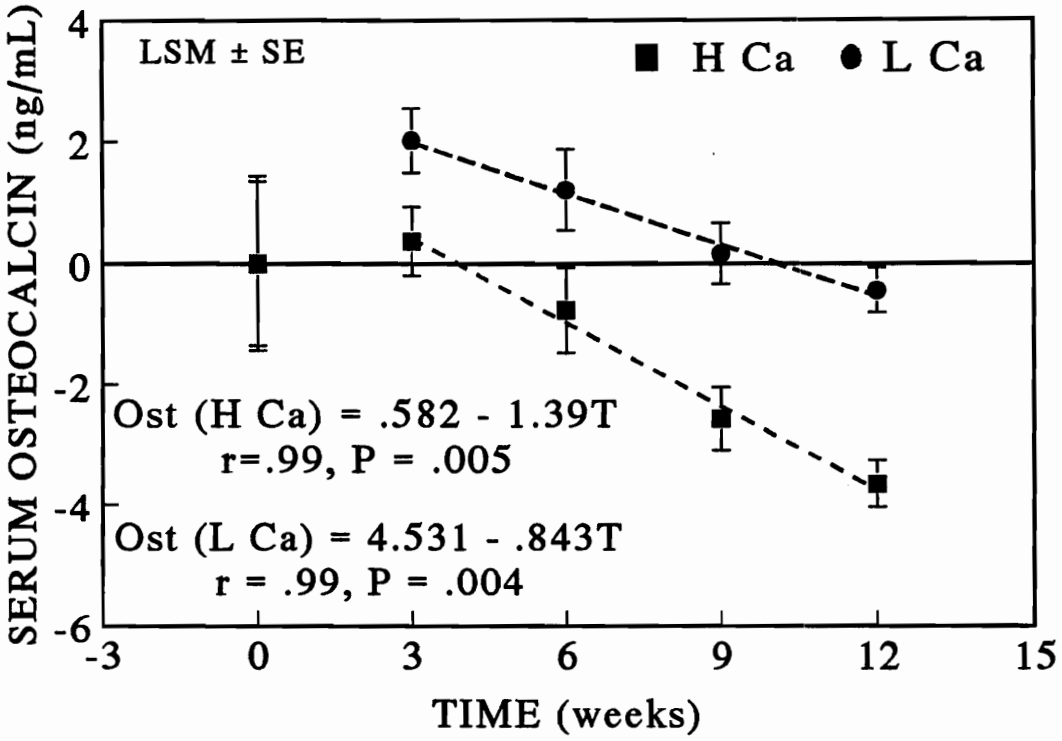

Figure 18. Dietary calcium effect (P = .003) on change in resting equine serum osteocalcin. There was a linear regression of osteocalcin on time for horses fed high calcium (P = .005) and horses fed low calcium (P = .004).

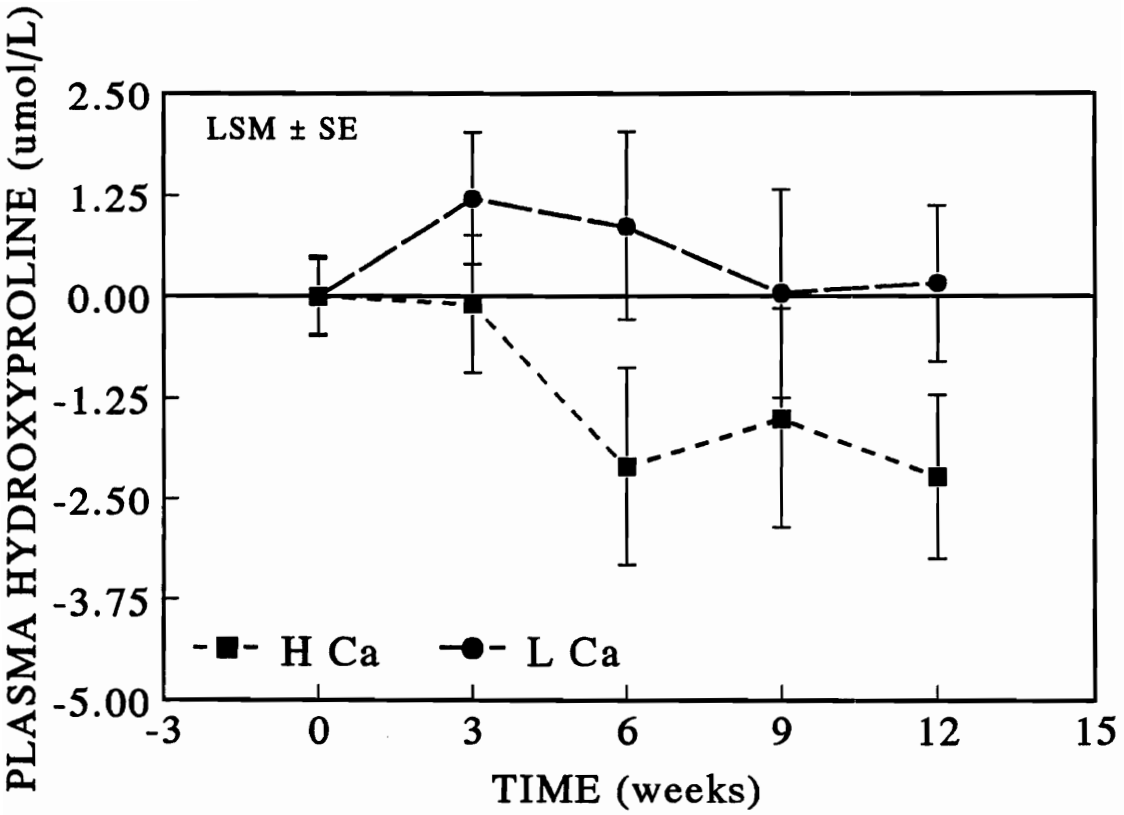

Figure 19. Dietary calcium effect (P = .081) on change in resting equine plasma hydroxyproline.

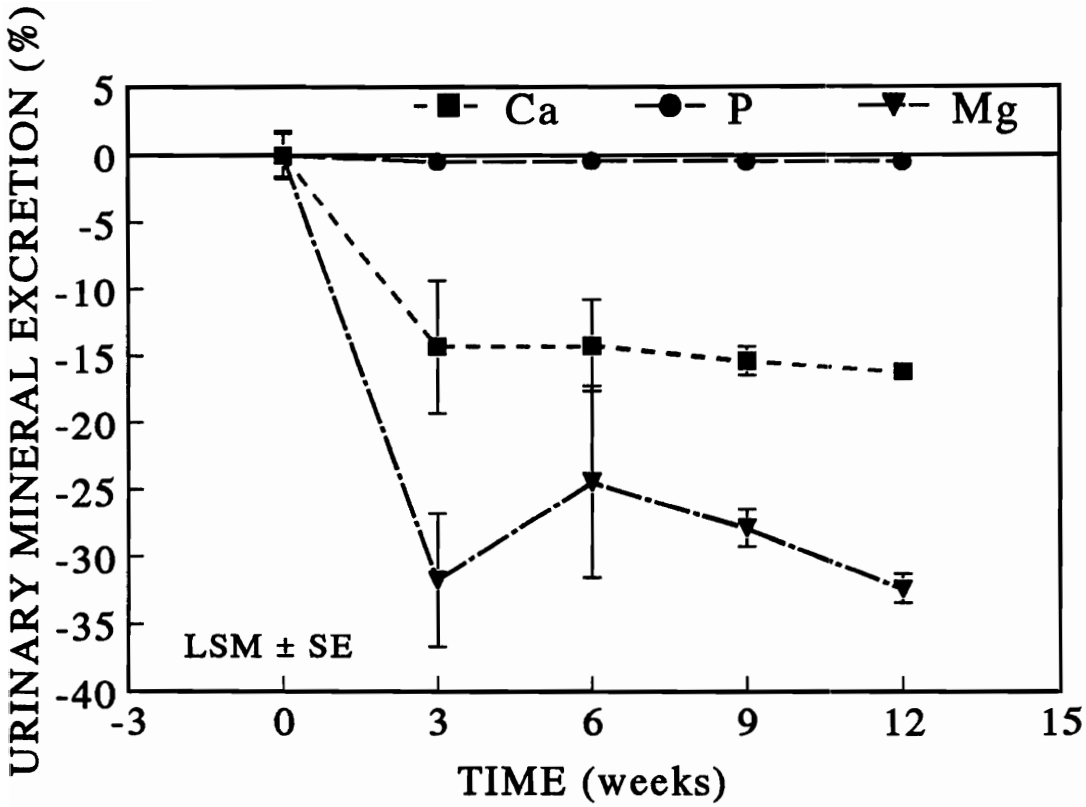

Figure 20. Diet effect on change in resting equine urinary fractional excretion of calcium (P = .001), phosphorus (P = .001), and magnesium (P = .002) in mares.

Discussion

The present findings show that repeated strenuous exercise on an equine treadmill increased BMC only in horses fed approximately twice the level of dietary Ca recommended by the NRC (1989). Conditioning resulted in a prolonged decrease in serum Ca that may have reflected bone response. Serum PTH increased between wk 3 and 12, probably in response to the decrease in serum Ca. Plasma hydroxyproline decreased between wk 6 and 12 only in horses fed the higher dietary Ca. These responses were consistent with bone remodeling during conditioning, especially in the higher Ca group. Serum osteocalcin increased initially, then decreased linearly, suggesting a decrease in bone formation that was inconsistent with the increase BMC.

Bone Remodeling

Observed increases in BMC, BW, and CA (Figures 2 - 8) were most likely due to the increased mechanical stimulation to the bone caused by the exercise. Repeated mechanical strains above a threshold are the initial stimuli for bone remodeling (Rubin, 1984). This threshold is different for different levels of activity and represents the minimum strain that must be reached before the bone responds by remodeling. When bone is strained, it becomes electrically charged with "stress generated potentials" as a result of piezoelectric forces. The voltage waveforms produced with physiologic loading have been shown to affect the function of bone cells (McLeod and Rubin, 1990). With the initiation of remodeling, osteoclasts remove bone that may

have been damaged at sites experiencing greater strains by digesting the bone surface and forming erosion cavities. Osteoblastic activity follows and is responsible for producing and mineralizing bone matrix and for forming new bone to fill in the cavities left by the osteoclasts. This results in an increased bone mass or altered distribution of mass to provide more bone at high strain locations (Norwood, 1978). Increases in BMC, BW, and CA in response to training have been seen previously in young, exercised horses (Raub et al., 1989; Porr, 1993; Sherman et al., 1995), dogs (Stoliker et al., 1976), and swine (Savio et al., 1981).

Metaphyseal BMC did not change with training. The physical structure of bone in that area is different, and it responds differently to stress. It was previously demonstrated that the McIII responds similarly to mechanical stress at locations between 20% and 70% of its length, possibly due to the fact that this coincides with the region that is free of cancellous bone (Piotrowski et al., 1983).

Bone variables responded to training only in horses fed .69% Ca (Figures 2, 3, 5, and 8). The level of dietary Ca for the low Ca diets was selected based on the NRC for Horses (1989). A subcommittee reviewed published research in the area of horse nutrition and found many gaps, unresolved conflicting reports, and a need to apply information gathered under one set of circumstances to many other situations (NRC, 1989). Values reported were selected values, some of which were extrapolated to cover the total population. Some information available on other species was used to estimate the horses' needs. It was assumed that any increased need for Ca associated

with exercise would be readily met by an obligatory increase in Ca intake as DM consumption increased to meet energy demands. However, results from this research indicate that the recommended .35% Ca (NRC, 1989) was not enough to allow bone formation in response to training, while .69% Ca was sufficient.

Calcium Status

Dietary Ca would affect bone directly through its' incorporation into hydroxyapatite or indirectly as a cation in DCAD. Dietary Cl would have an influence on bone as an anion in DCAD, but has little or no direct effect. Thus, the effect of dietary Ca (Figures 2, 3, 5, and 8) was probably not as a cation in DCAD but instead a direct effect on bone.

The linear decrease of serum Ca was more marked in the younger than older horses (Figure 10). Bones in the three-year olds, being less mature, would be expected to undergo both modeling and remodeling, possibly requiring greater amounts of Ca. Prolonged decreases in serum Ca with exercise and training have been reported for horses (Nielsen, 1992; Porr, 1993; Nielsen , 1995). One group of Thoroughbred horses in an interrupted race training program showed a decreased serum Ca between d 86 and 142, but this decreased occurred after a 30 d turn-out period which followed 56 d of training (Porr, 1993). A second group of Thoroughbreds, this one in uninterrupted training, showed a decrease in serum Ca between d 0 and 84, followed by an increase until d 112. Quarter Horse geldings in race training, fed to meet NRC recommendations for horses in training, showed an increase in serum Ca between d 14

and 42, followed by a decrease until d 84 of training (Nielsen et al., 1995). Serum Ca concentrations were higher than baseline at d 112, but not as high as they had been at d 42. These examples of prolonged decreases in serum Ca may be related to increased demands for bone remodeling.

The initial increase in serum PTH follows the expected inverse relationship with the decreasing serum Ca (Figure 16). However, serum PTH did not increase further as serum Ca continued to decrease, suggesting that an upper threshold had been met. Quarter Horse geldings in race training, fed to meet NRC recommendations for horses in training, showed a decrease in serum PTH between d 14 and 42 before increasing slightly until d 70 (Nielsen et al., 1995). Serum PTH concentrations had recovered to baseline by d 112.Horses completing the cross-country phase of a three-day event showed a decreased total Ca 10 min after exercise (Geiser et al., 1995). Ionized Ca was decreased immediately after exercise and had begun to recover by 10 min post-exercise. A decrease in total and ionized calcium was seen immediately after exercise in humans, with all subjects showing a recovery by 60 min post-exercise (Nishiyama et al., 1988). Plasma ionized Ca decreased, while total Ca and PTH increased, during five hr exercise on a bicycle ergometer in 12 men (Ljunghall et al., 1986). Increased PTH and slightly decreased total Ca were seen after prolonged physical exercise in 17 men (Ljunghall et al., 1988). Values had returned to baseline within five d post-exercise. These examples of the effects of acute bouts of exercise show blood Ca and PTH concentrations to recover to baseline very quickly. Changes in serum PTH

concentrations with exercise and training are more likely due to a training effect on serum Ca than a direct effect of exercise on serum PTH.

The increase in serum P (Figure 11) may have been associated with increased intestinal absorption of dietary P, decreased renal excretion, or bone resorption of the axial skeleton in response to the increase in serum PTH. This may be supported by the fact that both serum P and PTH increased between wk 0 and 3, then remained fairly consistent to the end of the experiment. Increases in serum P have been shown in QH in training (Nielsen et al., 1995). Increases in serum P in humans has been shown during exercise (Ljunghall et al., 1986) and during five d of recovery from intense exercise (Ljunghall et al., 1988).

The decrease in plasma Cl was not different for the two levels of dietary Cl. Decreases in plasma Cl have been associated with increased plasma volume or with alkalosis (Carlson, 1989). Neither of these accounts for the decrease observed in this study.

The decrease in serum albumin could be associated with training related increases in plasma volume. This decrease was greater for three-year olds (Figure 17) and could have contributed to the greater decrease in serum Ca seen in three-year olds (Figure 10). However, serum albumin increased between wk 3 and 12 while serum Ca continued to decrease, indicating that dilution was not the only cause of the decreased serum Ca or plasma Cl.

The lack of any significant effect of dietary Cl indicated that DCAD had little or

no effect on bone remodeling in this study. This is contrary to previous studies. A low DCAD increased the urinary excretion of Ca and Cl in horses (Topliff et al., 1989; Wall et al. 1992; Baker et al., 1993). The authors suggested that, depending on the level of dietary Ca, a low DCAD could lead to a negative Ca balance in exercising horses resulting in a deleterious effect on bone remodeling. A low DCAD also increased bone resorption in goats and cattle (Fredeen et al., 1988; Beighle et al., 1990), increased the incidence of tibial dyschondroplasia in young chickens (Halley et al., 1987; Ruíz-López et al., 1993), and was associated with lesions of dyschondroplasia in young horses (Savage et al., 1993).

Facilitation of bone remodeling by the higher Ca diets is also supported by the decreased plasma hydroxyproline (Figure 19). The decrease in plasma hydroxyproline may reflect a decreased bone resorption, since hydroxyproline is released into the blood through the degradation of collagen (Dull and Henneman, 1963).

The decrease in urinary FE of Ca (Figure 20) with training was consistent with increased Ca retention, which was suggested by the increased BMC and decreased plasma hydroxyproline

Contrary to the above observations, changes in serum osteocalcin, which should reflect bone formation (Fraher, 1993), decreased progressively from wk 3 (Figure 18). In a previous study (McCarthy and Jeffcott, 1992), plasma osteocalcin was lower in horses after 14 wk of conditioning than in controls. The conditioned horses showed an increased BMC despite lower plasma osteocalcin concentrations. Contrary to these

results (Figure 18; McCarthy and Jeffcott, 1992), serum osteocalcin increased for two wk, then decreased for four wk, before increasing to the end of the study after conditioning in Quarter Horses (Nielsen et al. 1995). These findings on Quarter Horses are consistent with an initial decrease and subsequent increase in bone formation with conditioning (Nielsen, 1992; Porr, 1993).

On the other hand, the contrary findings on serum osteocalcin and bone remodeling with conditioning (Figure 18; McCarthy and Jeffcott, 1992) may be related to changes in PTH (Figure 16). The secretion of osteocalcin in vitro is suppressed by PTH. In studies using human (Beresford et al., 1984) and rat tissue (Lian et al., 1985), cultured osteoblasts showed a suppression of osteocalcin secretion when physiologic amounts of PTH were added.

Another possible explanation for the unexpected response of serum osteocalcin to conditioning (Figure 18) would be a marginal Vitamin K deficiency. Osteocalcin is a vitamin K-dependent bone protein. Although normally associated with blood clotting, a slight deficiency in vitamin K could influence bone metabolism before any changes in blood coagulation would be detected. It has long been assumed that bacterial synthesis of menaquinone in the gastrointestinal tract provided a significant source of vitamin K to animals. However, the bacterial population is highest in the hindgut, requiring vitamin K to be absorbed under conditions of lower bile salt concentrations and less absorptive surface area. Colonic absorption of menaquinone-4, a minor bacterially produced menaquinone, and menaquinone-9, a typical bacterially produced

menaquinone, were examined in rats (Ichihashi et al., 1992). The in situ loop method was used, and results showed that approximately 23% of the menaquinone-4 was absorbed and appeared in the mesenteric venous blood. However, almost all of the menaquinone-9 administered into the loop was recovered, i.e., very little absorption occurred. This suggests that only some of bacterially produced menaquinones are absorbed from the colon, indicating that dietary vitamin K may be a primary source. The vitamin K content of the feeds used in this study have not been reported, but green, leafy materials, fresh or dry, are rich sources. Fecal vitamin K is also fairly high, and may have provided a source of the vitamin.

Implications

Bone remodeling in response to conditioning was indicated by changes in BMC, BW, and CA, serum Ca and PTH, plasma hydroxyproline, and urinary FE of Ca. It was unaffected by dietary Cl, hence DCAD. It was observed in horses fed .69% Ca on a DM basis but not in those fed .35% Ca, the level currently recommended for intense exercise (NRC, 1989). Thus, a dietary Ca of .69% would be preferred to .35% for horses subjected to repeated intense exercise, that is, conditioning. Any increase in dietary Ca should be integrated in relation to other nutrients, such as P and vitamin D.

Effects of dietary calcium and deconditioning on

calcium status and bone remodeling

Introduction

Skeletal unloading, which is experienced when a limb is placed in a cast or an animal is restricted from normal movement, is associated with a loss of bone mass due to continued resorption and decreased bone formation (Sessions et al., 1989; Machwate et al., 1994). This loss may also occur when an animal has been in training and is placed in a stall in order to recover from an injury. The decrease in bone mass could then affect the animals performance when training is resumed, predisposing them to a bone related injury. Knowledge of the rate and extent bone loss could be used to develop retraining programs that include a skeletal strengthening component.

Dietary Ca has been shown to influence bone remodeling in response to training (Experiment 1). Dietary Ca appeared to act directly on the bone as Ca, instead of through the dietary cation-anion difference (DCAD) as a cation.

Objective

The objectives of this study were to evaluate the effects of two levels of dietary Ca and deconditioning on bone remodeling and Ca status in conditioned Arabian horses kept in stalls for 12 wk.

Materials and Methods

Animals

Eleven Arabian horses, six geldings and five mares, were assigned to one of two treatment groups. The horses ranged from four to eight years of age. All horses had been conditioned on a high speed treadmill (Mustang 2200, Kagra International, Fahrwangen, Switzerland) for 12 wk and then performed a program of exercise designed to maintain condition for 7 mo (Table 9).

Experimental Procedure

Housing. Horses were housed in 2 x 4 m box stalls. They were walked on an automatic walker at 1.1 m/s for 60 min/d in two 30 min sessions 7 d/wk.

Diet. Horses were fed between 0700 and 0900, and 1600 and 1800 h. Animals were initially fed 2 % of their body weight, then adjustments were made in response to maintain body weight. Horses were assigned to and fed one of two diets (Table 10). Hay was chopped and combined with the rest of the dietary ingredients, creating a complete mixed diet. A trace mineral mix was designed to meet 1.6 to 2.0 times the 1989 NRC recommendations for horses. It was based on analysis of the hay and typical values (NRC, 1989) of concentrates (Table 11). Diets were designated LC (.35% Ca) and HC (.7% Ca from a 63% dicalcium phosphate-37% limestone mix). The P level was adjusted to maintain a Ca:P of 1.4:1 (NRC, 1989). Nutrient analysis of the diets is shown in Table 12. The DCAD are shown in Table 13. Included are totals for:

$[(Ca^{2+} + Mg^{2+} + Na + K) - (P + Cl + S)$

Dietary intakes, averaged over the experiment, are shown in Table 14.

Feed samples. Feed samples were collected three times during the experiment and analyzed for DM, crude fiber, CP using AOAC methods (1991), and Ca, P, Mg, Na, and K, using Induced Coupled Plasma Spectrometry (ICAP 9000, Thermo Jarrell-Ash, Franklin, MA). Samples for Cl analysis were sent to the DHIA laboratory, Itaca, NY.

Measurements

Animals were weighed weekly on an electronic scale (Dyco Incorporated, Model 1200, Scottsdale, AZ). Blood and urine samples, and dorsopalmar (DP) radiographs of the left metacarpus, were taken at 21 d intervals.

Blood. Serum and plasma samples were collected from all horses by jugular venipuncture (Vacutainer, Becton Dickinson, Rutherford, NJ). An arterial sample was also taken in lithium heparin blood-gas syringes from a carotid loop located under the skin. The loop was translocated surgically at least 1 year prior to the experiment. All samples were collected between 0730 and 0900 h and spun in a refrigerated centrifuge (1660 x g for 12 min at 4°C). Serum or plasma were stored at -20°C within 2 h.

Unless otherwise stated, blood analysis was by colorimetric assay using diagnostic kits (Sigma Diagnostics, St. Louis, MO). One blood tube was allowed to clot at room temperature before being centrifuged to collect serum. Serum was used for creatinine (Procedure 557), Ca (Procedure 587), inorganic P (Procedure 360-UV), Mg (Procedure 596), total protein (Procedure 541), and albumin (Procedure 631) determination. One

blood tube was kept on ice until centrifuged to collect plasma. Plasma was used for hydroxyproline analysis by the method of Fujii et al. (1981). One blood tube was kept on ice until centrifuged to collect serum. Serum was used for osteocalcin analysis (Osteocalcin Radioimmunoassay Kit, INCSTAR Corporation, Stillwater, MN). Plasma was used for Na, K, and Cl analysis (Clinical Chemistry Slide, Kodak Ektachem Clinical Products, Eastman Kodak Company, Rochester, NY).

Arterial blood was analyzed for ionized Ca using a potentiometric method (Ciba Corning 288 Blood Gas System, Ciba Corning Diagnostic Corp., Medfield, MA) within 2 h.

Urine. Mares were cleaned (Operand Iodine Scrub, Redi Products, Prichard, WV) and catheterized and the bladder drained. The mares were placed back in stalls for one hr before being catheterized a second time. The contents of the bladder were collected and an aliquot taken for analysis. A blood sample was collected at the time of each catheterization. Urine was frozen and stored at -20°C within 2 h.

Unless otherwise stated, urine analysis was by colorimetric assay using diagnostic kits (Sigma Diagnostics, St. Louis, MO). Urine was analyzed for Ca, P, Mg, and creatinine excretion.

Radiographs. Dorsopalmar radiographs (Kodak film, Kodak cassette with lanex screen) of the left front metacarpus were taken (80 kv, 20 ma, .04 s, 75 cm focal distance) with a portable x-ray machine (MinXRay 803, MinXRay Inc., Northbrook, IL) approximately every 21 d. An aluminum step wedge was exposed simultaneously

with the McIII and used as a reference standard, providing radiographic bone aluminum equivalents (RBAE) for estimation of bone mineral content (BMC) using an image analyzer (Southern Micro Instruments, Inc., Atlanta, GA) as described by Meakim et al. (1981). Three sites on the medial and lateral sides of the McIII, at the nutrient foramen and one cm above and one cm below the nutrient foramen, were used to measure RBAE, bone width (BW), and medullary width (MW). Medial and lateral BMC were estimated using the equations described by Ott et al. (1987):

$$\text{medial BMC} = .87(\text{RBAE}) - 2.35 \quad (r^2 = .92)$$

$$\text{lateral BMC} = .93(\text{RBAE}) - 2.86 \quad (r^2 = .93)$$

The three values were averaged to provide BMC for the proximal diaphyseal medial and lateral cortices. Values for BW and MW were each averaged and used for the calculation of cortical area (CA) based on circular bone geometry (Ott et al., 1987):

$$CA = (BW)^2 - (MW)^2 \quad (r^2 = .88)$$

Medial and lateral BMC, BW, MW, and CA were also measured at one site in the proximal metaphyseal region of the McIII.

Statistical Analysis

Data were analyzed using the GLM procedures of SAS (1991) and repeated measures analysis of variance, evaluating the effects of dietary Ca, sex, age, and interactions. Data from wk 0 was subtracted from subsequent data points, and these differences were also evaluated using the GLM procedures of SAS (1991) and repeated measures analysis of variance. Variables that were not significant were

dropped from the model. Data for diets and nutrient intakes were reported as means ± standard errors, while data for serum, plasma, urine, and radiographs were reported as least squares means ± standard errors. Significance was inferred at $P < .05$, and a trend at $P < .10$.

Urinary FE data for one mare fed the HC diet was dropped due to having in excess of six times the standard deviation of the other mares (as calculated by a z-test). This left only one mare on the HC diet.

Table 9. Exercise protocols used during 10 mo of conditioning and maintenance of level of condition using a high speed equine treadmill

Speed, m/min	Gait	Slope %	Conditioning Time, min	Maintenance Time, min
72	walk	0	2.5	2
72	walk	6	2.5	2
192	trot	6	4	2
420	gallop	6	3	2
192	trot	6	3	2
480	gallop	6	3	2
192	trot	6	3	2
540	gallop	6	3	2
192	trot	6	3	2
72	walk	0	2	2

Table 10. Feed composition as a percentage of dietary DM

Ingredient	HC	LC
Orchardgrass Hay	86.9	88.2
Molasses	10.0	10.0
Trace mineral mix[a]	.5	.5
NaCl	1.0	1.0
Calcium mix[b]	1.3	---
Vitamin premix[c]	.002	.002

[a] See Table 9
[b] Dicalcium phosphate - 63%, Limestone - 37%
[c] Hoffman-LaRoche, provided per kilogram of mix: vitamin A, 1,380,000 IU; vitamin D_3, 258,000 IU; vitamin E, 26,450 IU; riboflavin, 700 mg; niacin, 3,010 mg; folic acid, 66 mg; thiamin mononitrate, 1,520 mg; d-biotin, 40 mg; ß-carotene, 3,520 mg.

Table 11. Trace mineral mix

Mineral Salt	g salt/100 lb premix[a]
$FeSO_4$,7 H_2O	2082.57
$ZnSO_4$	1248.75
$CuSO_4$,5 H_2O	536.87
$MnSO_4$,1 H_2O	1788.06
Na_2SeO_3	3.99
KI	2.38

[a] soybean meal used as a carrier

Table 12. Nutrient analysis of diets[a, b, c]

Nutrient, %	HH	HL	LH	LL
DM	87.5 ± .1	87.6 ± .5	87.4 ± .3	87.6 ± .7
CP	8.9 ± .8	9.0 ± .7	9.3 ± .7	9.1 ± .4
ADF	38.4 ± 2.5	36.9 ± 2.7	38.7 ± 1.9	37.9 ± 1.3
Ca	.58 ± .06	.65 ± .09	.35 ± .06	.37 ± .03
P	.37 ± .04	.42 ± .04	.23 ± .02	.25 ± .01
Mg	.24 ± .01	.25 ± .02	.23 ± .01	.22 ± .01
S	.21 ± .01	.24 ± .03	.21 ± .02	.2 ± .02
Na	.32 ± .07	.44 ± .1	.37 ± .05	.35 ± .03
Cl	1.7 ± .06	1.3 ± .04	1.8 ± .09	1.1 ± .1
K	2.0 ± .04	2.2 ± .01	2.0 ± .1	1.9 ± .01

[a] dry matter basis
[b] means ± SE
[c] average of three samples for each diet

Table 13. Calculated dietary cation-anion difference expressed as mEq/kg DM

Diet	$(Ca^{2+}+Mg^{2+}+Na+K) - (P+Cl+S)$
High Calcium	596.6
Low Calcium	489.6

Table 14. Dietary intake data

Horse	Diet	Body weight (kg)[a]	Intake/d (kg)[a]	Intake as percent of body weight
4	HH	409	7.3	1.8
7	HH	530	9.1	1.7
9	HH	459	8.2	1.8
3	HL	442	8.6	2.0
10	HL	449	9.5	2.1
11	HL	406	7.7	1.9
1	LH	422	7.7	1.8
5	LH	448	9.1	2.0
8	LH	492	10	2.0
2	LL	484	9.1	1.9
12	LL	472	10	2.1

[a] data are averaged over the 12 wk of the experiment

Results

All horses participating in the study completed the protocol in good health.

Body Weight

Body weight was between 422 and 486 kg. There was a mean 2.5% decrease (P = .004) during 12 wk of deconditioning (Figure 21).

Radiographic Photometry

Bone Mineral Content. Proximal diaphyseal cortical bone mineral content (DBMC) was between 19.1 and 21.2 g/2 cm section on the medial aspect and between 17.2 and 19.5 g/2 cm section on the lateral aspect. Proximal metaphyseal cortical bone mineral content (MBMC) was between 17.2 and 19.5 g/2 cm section on the medial aspect and between 10 and 18.4 g/2 cm section on the lateral aspect. These were similar to values reported previously (Nielsen, 1992; Porr, 1993).

Medial DBMC decreased (P = .002) with deconditioning. There was a linear relationship for DBMC with time (Figure 22):

$$DBMC = .606 - .266(T)$$

$$r = .97 \quad P = .066$$

There was no effect of deconditioning (P > .10) on lateral DBMC. Medial MBMC (P = .006) and lateral MBMC (P = .015) decreased with deconditioning. There was a linear response of medial MBMC with time (Figure 23):

MBMC = .891 - .35(T)

r = .93 P = .022

Medullary and Bone Width. Proximal diaphyseal medullary width (DMW) was between 13.7 and 15.9 mm and bone width (DBW) was between 30.1 and 33.5 mm. Proximal metaphyseal medullary width (MMW) was between 16.3 and 20.5 mm and bone width (MBW) was between 33.8 and 40.5 mm.

There were no effects of deconditioning or diet on DMW or DBW.

Horses fed the LC diet tended to have a greater MMW as compared to horses fed the HC diet (P = .068), but there was no difference when changes from wk 0 were compared. Seven-year olds had a greater MMW as compared to four-year olds (P = .005), but there was no difference when changes from wk 0 were analyzed.

Proximal metaphyseal bone width (MBW) tended to decrease from wk 0 to 9, then increased to wk 12 (P = .059) with deconditioning (Figure 24).

Cortical Area. Estimated cortical area of the proximal diaphysis (DCA) was between 663.1 and 922.2 mm^2, and of the proximal metaphysis (MCA) was between 910.1 and 1271.3 mm^2. There was no effect of deconditioning or diet on estimated proximal diaphyseal (DCA) or metaphyseal cortical area (MCA).

Serum and Plasma Analysis

Serum Calcium. Serum Ca was between 2.6 and 3.2 mmol/L (10.2 and 12.8 mg/dL). There was an increase in serum Ca with deconditioning (P = .004). Changes from wk 0 showed a trend for an increase only in horses fed the HC diet (P = .067)

(Figure 25).

Plasma Ionized Calcium. Plasma ionized Ca was between 1.38 and 1.51 mmol/L. Plasma ionized Ca increased with deconditioning (P = .048) (Figure 26).

Serum Phosphorus. Serum Phosphorus was between 1.2 and 1.6 mmol/L (3.8 and 4.9 mg/dL). There was no effect of deconditioning or diet.

Serum Magnesium. Serum Mg was between 1.1 and .7 mmol/L (1.5 and 2.1 mEq/L). There was no effect of deconditioning or diet.

Serum Osteocalcin. Serum osteocalcin was between 9.6 and 29.3 ng/mL. There was a decrease in serum osteocalcin with deconditioning (P = .001). There was a tendency for horses fed the LC diet to have greater concentrations of serum osteocalcin (P = .073) between wk 3 and 12 (Figure 27). The greatest decrease in serum osteocalcin in the HC group was seen between wk 3 and 9.

Plasma Hydroxyproline. Plasma hydroxyproline was between 2.8 and 8.7 μmol/L. There was an increase in plasma hydroxyproline between wk 0 and 3 (P = .001), followed by a decrease to wk 0 values with deconditioning (Figure 28). Four-year olds had higher concentrations as compared to seven-year olds (P = .046), but changes from wk 0 showed no difference.

Serum Total Protein. Serum total protein was between 5.8 and 7 g/dL. There was a decrease in serum total protein with deconditioning (P = .001). Horses fed the HC diet tended to have a higher serum total protein at wk 6 and 9 (P = .08) (Figure 29).

Serum Albumin. Serum albumin was between 3.2 and 3.6 g/dL. Horses fed the HC diet had a greater serum albumin at wk 6 (P = .006) as compared to horses fed the LC diet (Figure 30). There was no effect of deconditioning.

Urinary Fractional Excretions (Creatinine Ratios)

There was no effect of diet or deconditioning on urinary FE of Ca or P. Changes from wk 0 showed no effect of deconditioning or diet on urinary FE of Mg.

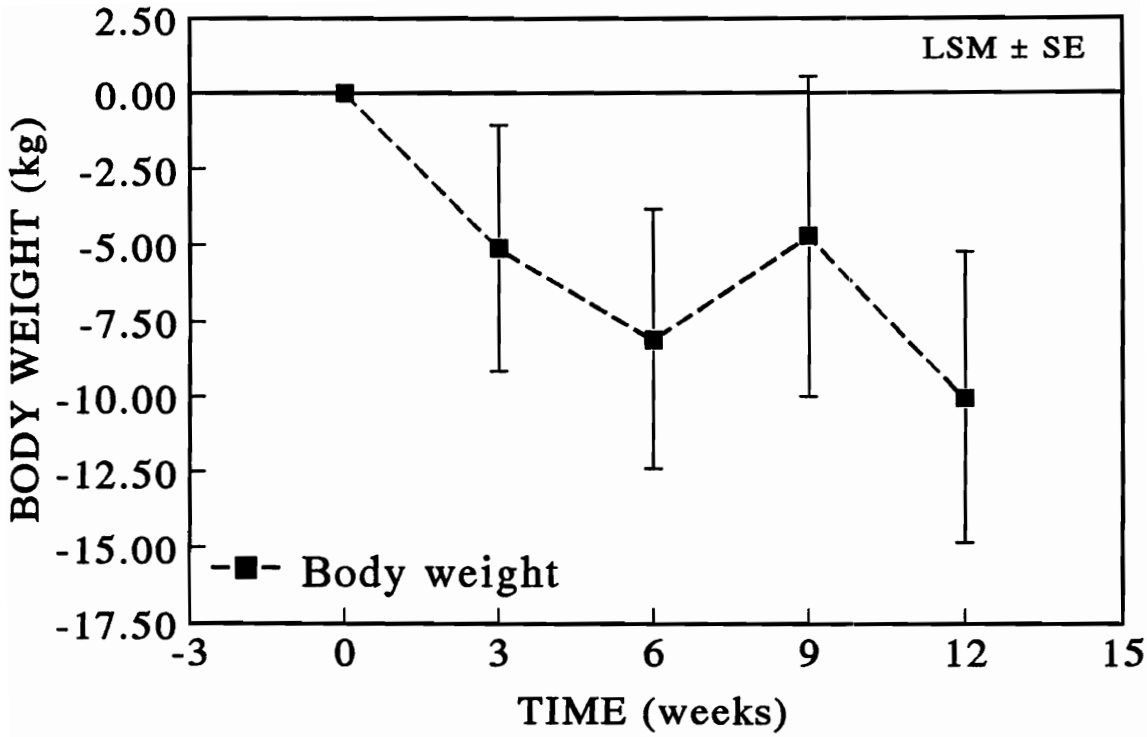

Figure 21. Deconditioning effect (P = .004) on change in equine body weight.

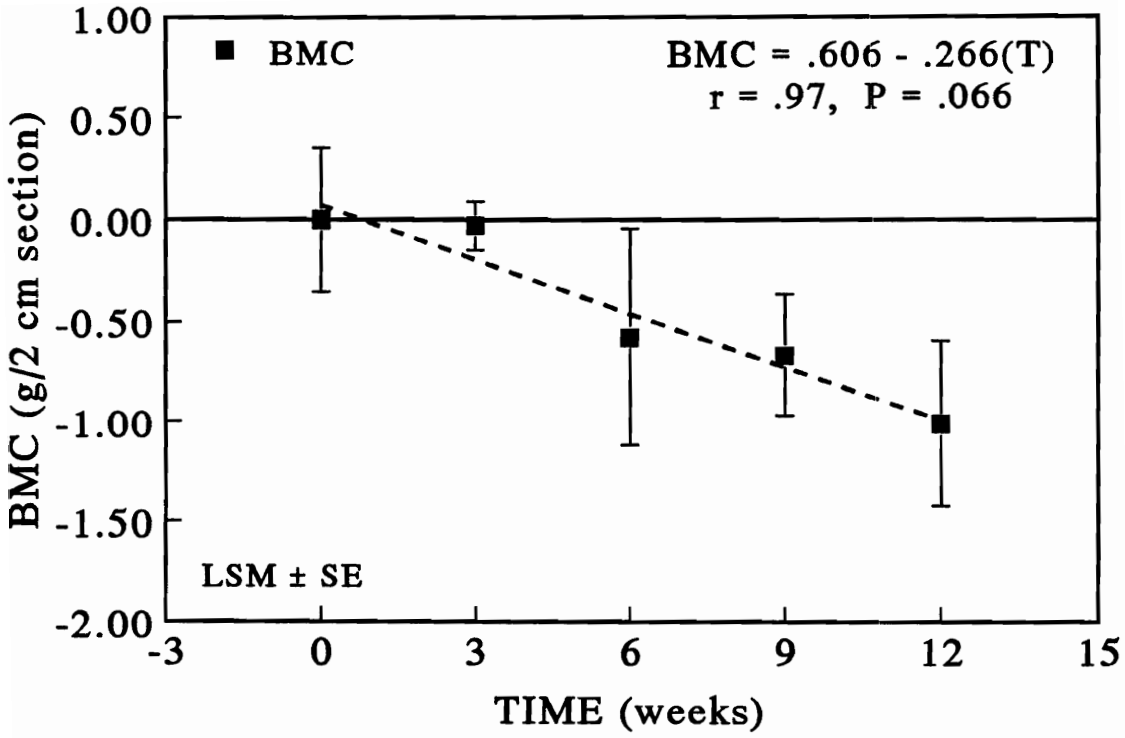

Figure 22. Deconditioning effect (P = .002) on change in estimated bone mineral content of the medial cortex of the proximal diaphysis of the equine third metacarpal bone. There was a linear regression of bone mineral content on time (P = .066).

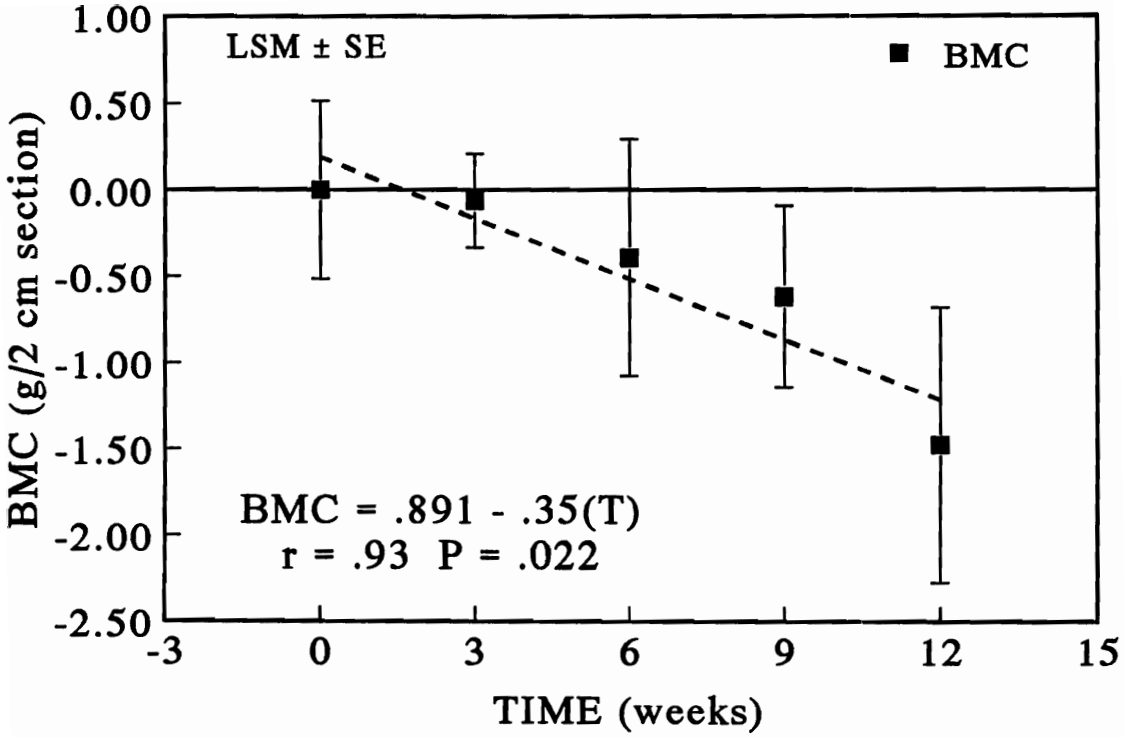

Figure 23. Deconditioning effect (P = .006) on change in estimated bone mineral contect of the medial cortex of the proximal metaphysis of the equine third metacarpal bone. There was a linear regression of bone mineral content on time (P = .022).

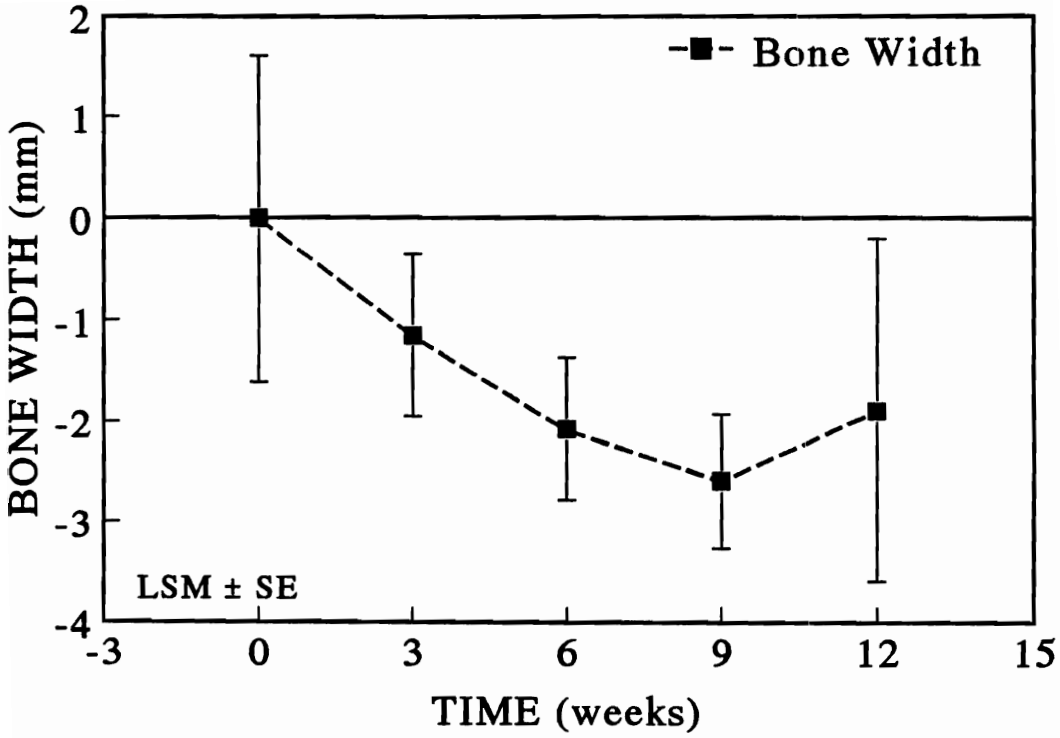

Figure 24. Deconditioning effect (P = .059) on change in bone width of the proximal metaphysis of the equine third metacarpal bone.

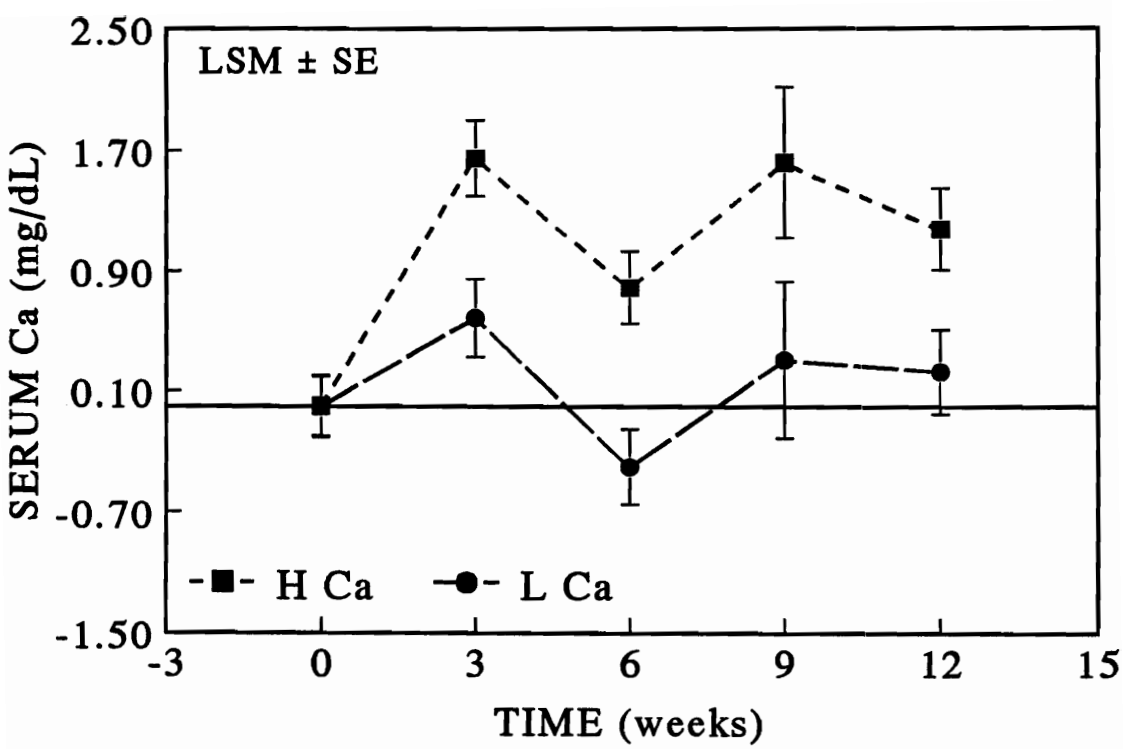

Figure 25. Dietary calcium effect (P = .067) on change in resting equine serum calcium.

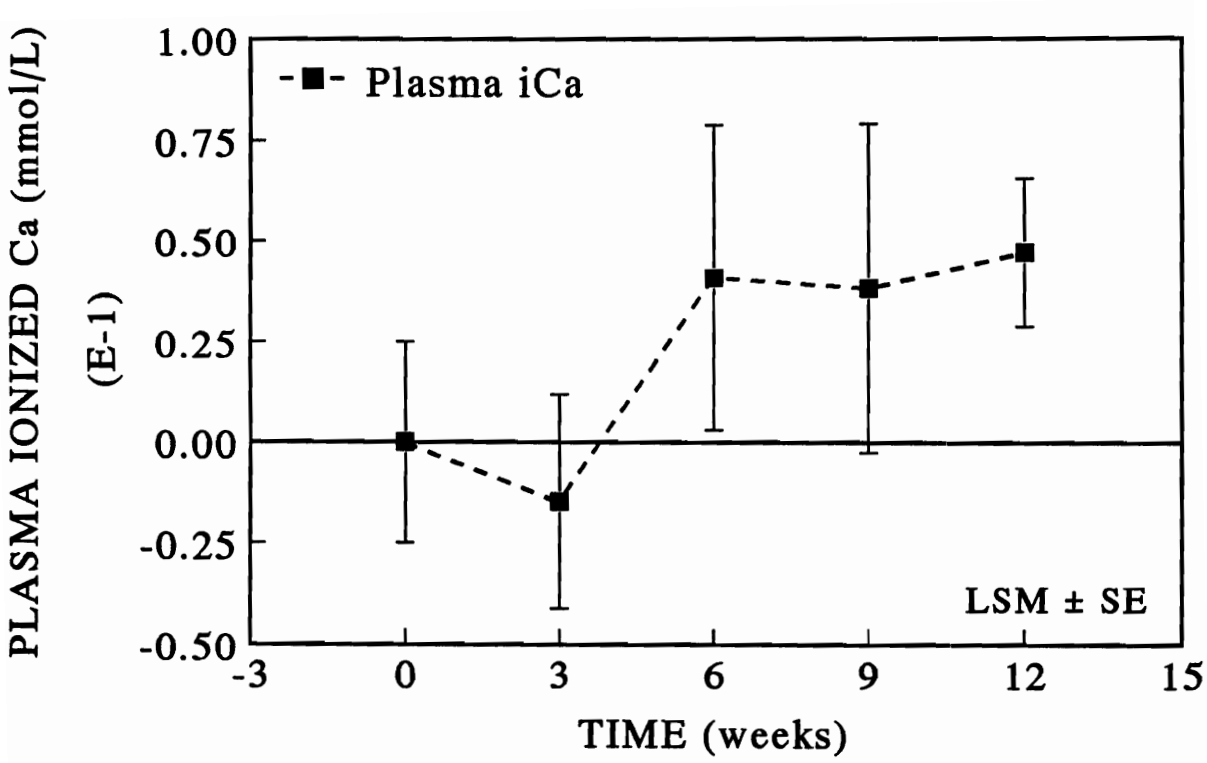

Figure 26. Deconditioning effect (P = .048) on change in resting equine plasma ionized calcium.

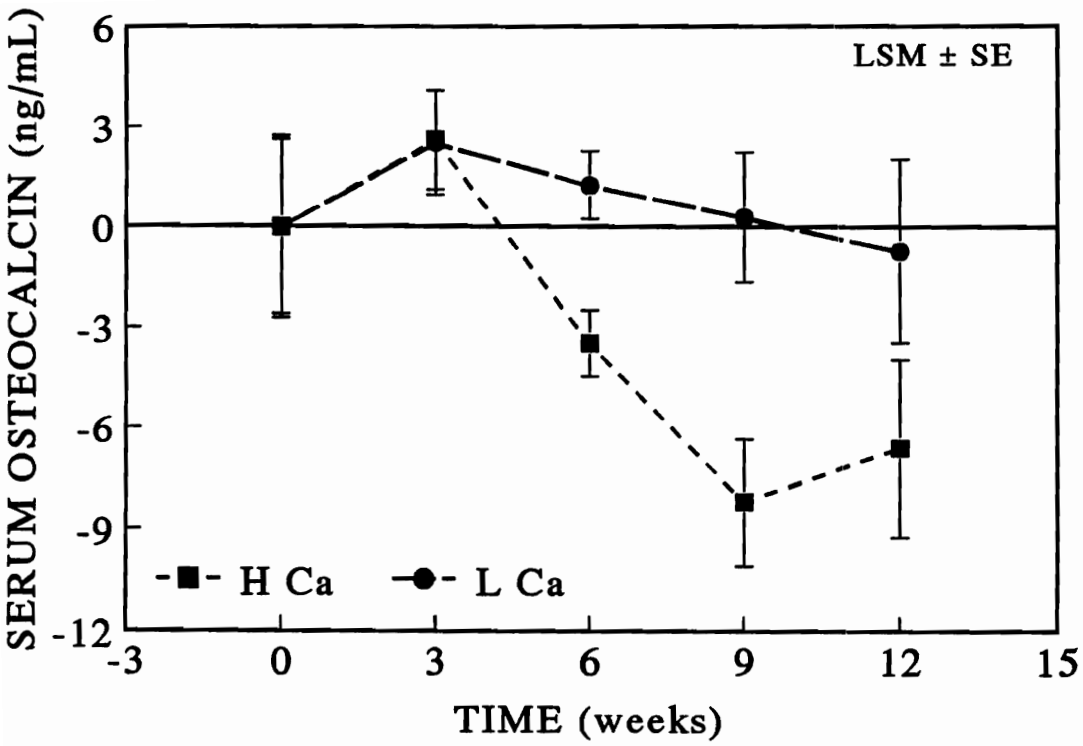

Figure 27. Dietary calcium effect (P = .001) on change in resting equine serum osteocalcin.

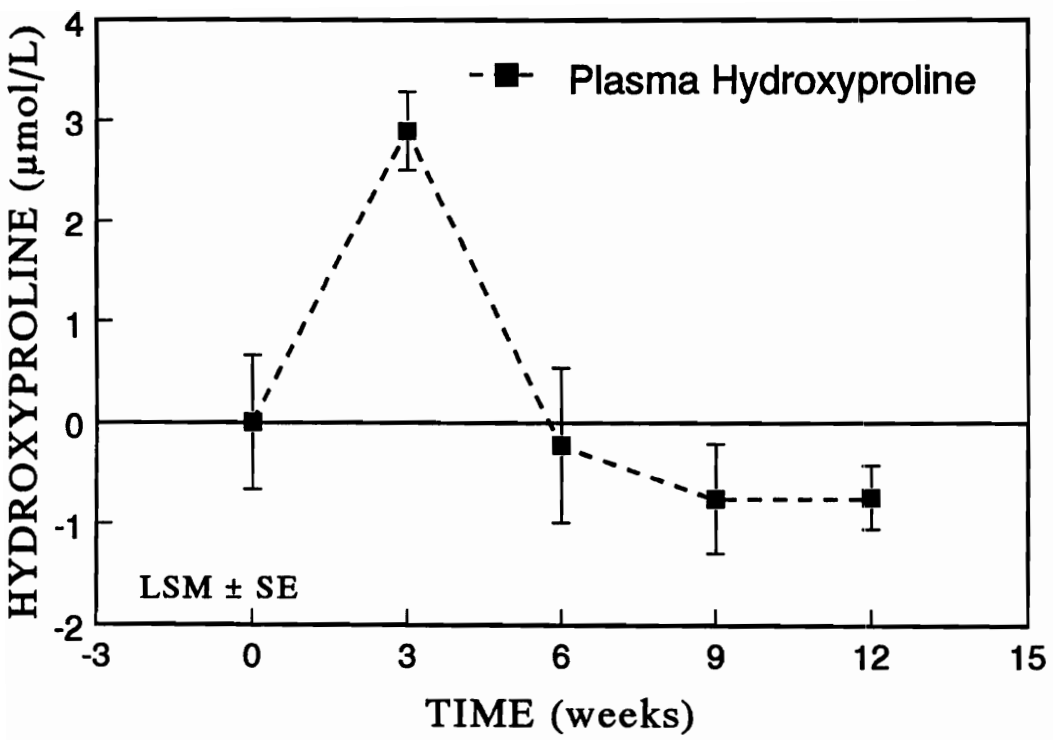

Figure 28. Deconditioning effect (P = .001) on change in resting equine plasma hydroxyproline.

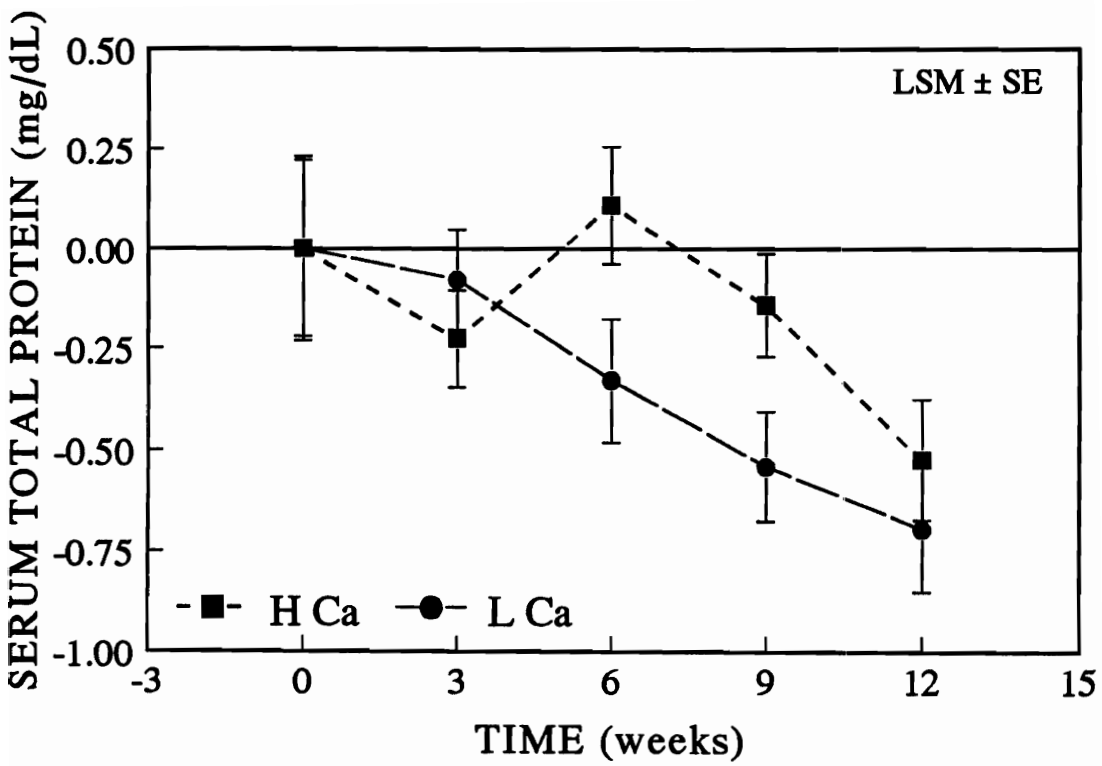

Figure 29. Dietary calcium effect (P = .08) on change in resting equine serum total protein.

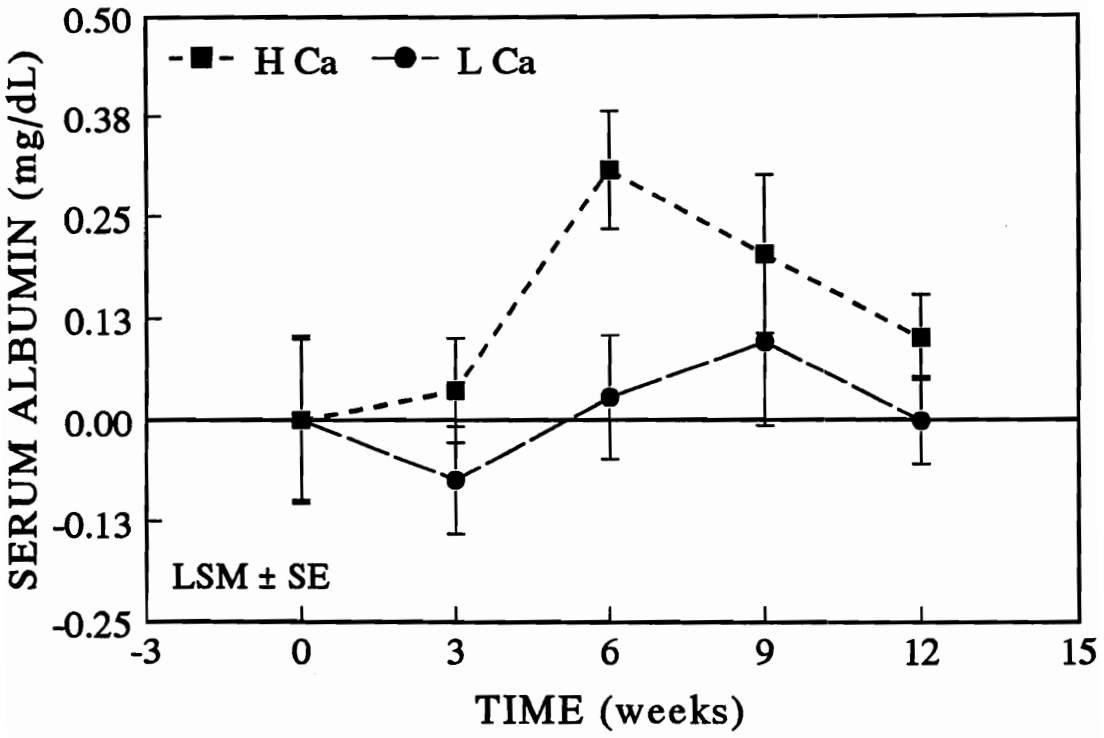

Figure 30. Dietary calcium effect (P = .006) on change in resting equine serum albumin.

Discussion

Bone loss during deconditioning of fit horses was revealed by decreases in BMC and BW. Bone resorption was indicated by a transient increase in plasma hydroxyproline and an increase in serum Ca in the group fed .65% Ca. Also, a decrease in bone formation was indicated by a decrease in serum osteocalcin in the .65% Ca group only. Thus, dietary Ca of .65%, compared to a control of .35%, provided no protection against bone loss during deconditioning.

Bone Remodeling

The progressive decrease in BMC (Figures 22 and 23) was most likely associated with a decrease in mechanical stimulation, i.e. fewer, less forceful movements. Skeletal unloading has been associated with a loss of bone mass due to increased bone resorption and decreased formation (Sessions et al., 1989; Machwate et al., 1994). Static histomorphometry has shown that exercised horses had a smaller area of resorption spaces (associated with osteoclastic activity) and a greater bone apposition than unexercised horses, indicating an increased bone formation in the exercised horses (McCarthy and Jeffcott, 1992). With this in mind, a decrease in the intensity of exercise would most likely decrease the rate of formation associated with the exercise. Immobilization of the left forelimb of horses for eight weeks resulted in a decreased BMC in both front limbs (Buckingham and Jeffcott, 1991).

Calcium Status

Deconditioning was associated with an increase in serum Ca in horses fed the high Ca diet (Figure 25). The increase in serum Ca was greater than the increase in serum albumin, indicating that a decrease in fluid volume was not responsible for all of the change seen. Increased dietary Ca has been shown to slow bone resorption in pre-menopausal women (Raisz and Fujita, 1992), but did not affect the rate of bone resorption in this study.

An increased rate of bone resorption may have been reflected in the transient increase in plasma hydroxyproline (Figure 28). As the bone remodeled to adjust to the new level of stimulus, the hydroxyproline concentrations returned to baseline.

A decreased rate of bone formation was indicated by a decrease in serum osteocalcin in horses fed .65% Ca . The decrease in serum osteocalcin may have been due to a decreased rate of bone formation. In this study, osteocalcin concentrations were higher in the horses after prolonged training (8.5 pg/mL in experiment one vs. 17.2 pg/mL in experiment 2 two) as compared to when the same horses were initially being conditioned. This is similar to previous comparisons between trained and untrained horses (Geor et al., 1993), and on athletic and non-athletic humans (Nishiyama et al., 1988). Serum osteocalcin concentrations reported here were similar to some previously reported values for horses (Lepage et al., 1991; Nielsen, 1995), but were higher than others (Geor et al., 1993). The higher concentrations reported here may be due to the longer period of time in which the horses had been in training.

Foals transferred into stalls were shown to have a transient decrease in serum osteocalcin, which was associated with a decrease in bone formation (Mäenpää et al., 1988).

Implications

Rest for a few weeks is often prescribed for sick or lame horses. Bone loss during rest, or deconditioning, may make the horse vulnerable to bone injury when conditioning is resumed. A diet containing .65% Ca on a DM basis provided no protection against bone loss during 12 wk when compared to the level of .35% recommended for intense exercise.

General Discussion

These two studies demonstrated expected changes in BMC, an increase during conditioning and a decrease during deconditioning. A higher dietary Ca (.69%) than has been recommended for intense exercise (.35%) by the NRC (1989) favored the response to conditioning by not deconditioning. Changes in dietary Cl, hence DCAD, had no influence on bone or Ca status during conditioning.

These findings have two practical implications:

1. Horses should be fed more than the current recommended level of Ca during conditioning, and .69% may be sufficient for this purpose. This level could be used as a positive control in future experiments.

2. Bone loss in fit horses during rest periods creates risks that should be kept in mind when conditioning is resumed. This bone loss was not mitigated by .65% Ca as compared to .35% Ca.

These studies had several advantages over previous research in this field. The use of a treadmill allowed for controlled intensity and duration of exercise as compared to the training and exercise on a track. The use of a complete feed, i.e., the hay was ground and mixed with other components of the diet, allowed for more accurate measure of intake (less feed loss, e.g., hay in the bedding). All horses used in these studies were of one breed, which prevented breed related differences influencing the results. Also, 10 of the horses used in the deconditioning study were also in the

training study. This decreased the chance that differences in level of condition at the start of the deconditioning study would have influenced the results. Finally, the time period used for training, 12 wk, was long enough to stimulate a response in bone remodeling in mature horses. The time period used for deconditioning, also 12 wk, was long enough to stimulate a measurable response in bone remodeling and simulated a time interval appropriate for many typical convalescent periods.

These studies also had certain limitations. The labor and time constraints required to care for and exercise horses on a treadmill limited the number of animals to 12. The use of 12 horses fed one of four diets further reduced the numbers for statistical analysis, but analysis of variance compared three horses/diet, and six horses for each main effect. In the event, only the main effect of Ca proved to be consistently significant during conditioning, and diet had no effect during deconditioning.

The lack of an effect of dietary Cl, hence DCAD, was unexpected, but the range used (Table 7) was reasonable for practical diets. In contrast, previous workers have used more extreme (Baker et al., 1992; Popplewell, 1993; Cooper et al., 1995) ranges that may have little practical relevance. Other incidental sources of variation were sex and age. Fortunately, there were no interactions of sex or age with diet or training. The low number of mares, only one mare on each of three diets, restricted interpretation of urinary fractional excretion in response to diet. Some other considerations include:

1. The serum Cl decrease during conditioning was unexplained by any known cause of hypochloridemia.

2. The serum osteocalcin decreases during conditioning were opposite to those expected. The two possible explanations offered (p. 80-81) were tenuous.

3. An obviously missing measurement was calcitonin. However there was no existing method available for use with horses. There is one currently under investigation.

These studies pave the way for further refinement of the optimal dietary Ca level for competitive equine athletes. A dietary level of .69% Ca could be used as the control in future studies.

Recent advances in biochemical markers of bone metabolism (Fraher, 1993; Risteli and Risteli, 1993; Price et al., 1995) indicate directions for further work.

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APPENDIX A

RAW DATA - EXPERIMENT ONE

Appendix A

Appendix Table 1. Estimated bone mineral content (g/2 cm section) of the lateral diaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	18.94	17.61	19.48	19.24	19.97
2	M	6	HL	18.50	17.87	17.47	17.45	21.16
3	M	6	HH	17.60	18.74	16.87	18.32	17.21
4	M	6	LL	17.51	17.57	17.63	17.13	17.02
5	M	6	HH	18.64	17.98	18.26	17.96	19.21
6	G	6	HL	20.09	18.72	19.61	20.31	20.62
7	G	3	HL	19.45	19.61	20.28	20.68	21.32
8	G	6	LL	17.91	18.67	17.71	18.01	18.07
9	G	6	HH	17.93	18.35	18.17	18.08	19.70
10	G	6	LH	20.30	21.04	21.22	20.82	20.52
11	G	3	LH	18.17	17.71	18.47	18.69	17.30
12	G	3	LL	18.11	18.47	18.16	18.58	18.70

Appendix Table 2. Estimated bone mineral content (g/2 cm section) of the medial diaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	19.95	20.06	19.42	19.21	19.24
2	M	6	HL	20.44	20.73	20.04	20.46	21.19
3	M	6	HH	20.16	20.68	19.53	19.94	20.29
4	M	6	LL	20.14	20.10	20.44	20.40	19.39
5	M	6	HH	19.66	20.90	20.36	19.94	20.82
6	G	6	HL	20.91	20.53	20.97	20.97	21.39
7	G	3	HL	21.27	21.79	20.71	21.27	21.54
8	G	6	LL	20.10	20.08	19.67	20.55	19.87
9	G	6	HH	20.20	20.44	20.36	20.19	20.90
10	G	6	LH	20.41	20.52	20.12	20.34	19.63
11	G	3	LH	19.88	19.27	17.73	19.66	19.05
12	G	3	LL	19.41	20.42	21.16	21.31	21.44

Appendix Table 3. Extimated bone mineral content (g/2 cm section) of the lateral metaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	18.25	18.38	17.91	17.27	17.77
2	M	6	HL	18.32	18.90	18.05	17.83	18.98
3	M	6	HH	17.42	17.78	17.20	18.75	17.78
4	M	6	LL	17.59	16.86	17.31	17.19	16.72
5	M	6	HH	18.00	17.93	18.01	17.99	19.00
6	G	6	HL	.	19.13	18.45	18.54	18.42
7	G	3	HL	18.25	18.18	17.81	18.76	19.06
8	G	6	LL	17.26	18.37	17.56	17.92	17.94
9	G	6	HH	18.38	18.47	18.53	18.31	19.20
10	G	6	LH	18.35	18.28	20.05	18.53	18.33
11	G	3	LH	17.79	17.08	17.14	16.66	16.77
12	G	3	LL	18.91	19.03	18.19	19.36	19.70

Appendix Table 4. Estimated bone mineral content (g/2 cm section) of the medial metaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	17.92	18.66	17.08	17.07	17.52
2	M	6	HL	19.70	19.77	19.03	19.32	19.84
3	M	6	HH	18.56	19.16	17.66	16.33	18.35
4	M	6	LL	18.63	18.23	18.93	18.28	17.56
5	M	6	HH	18.09	19.08	18.90	18.82	19.51
6	G	6	HL	.	19.27	19.57	19.64	19.31
7	G	3	HL	19.98	20.41	19.37	19.90	20.20
8	G	6	LL	18.43	19.09	18.41	18.35	18.61
9	G	6	HH	18.84	19.29	19.72	19.04	19.87
10	G	6	LH	18.76	18.32	18.56	18.65	17.63
11	G	3	LH	19.10	18.45	16.70	18.56	18.14
12	G	3	LL	18.41	18.97	18.60	19.60	19.89

Appendix Table 5. Medullary width (mm) of the proximal diaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	14.06	14.52	14.29	14.02	14.36
2	M	6	HL	13.72	13.77	13.61	13.61	14.08
3	M	6	HH	12.18	12.24	11.55	11.64	11.91
4	M	6	LL	15.74	15.19	15.79	15.46	15.30
5	M	6	HH	14.20	14.42	14.64	14.69	15.75
6	G	6	HL	14.86	15.73	15.68	15.25	15.55
7	G	3	HL	13.69	13.80	14.08	13.80	14.24
8	G	6	LL	18.21	19.08	17.71	18.56	19.04
9	G	6	HH	14.35	14.46	14.07	14.62	15.34
10	G	6	LH	15.57	15.68	16.17	15.79	15.95
11	G	3	LH	15.94	16.11	16.10	16.02	15.77
12	G	3	LL	15.37	15.50	15.61	15.38	16.55

Appendix Table 6. Bone width (mm) of the proximal diaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	30.37	32.40	30.30	30.91	31.15
2	M	6	HL	31.59	34.81	32.09	32.13	32.57
3	M	6	HH	33.39	32.56	32.96	32.34	34.10
4	M	6	LL	34.65	35.08	35.36	34.48	33.88
5	M	6	HH	34.25	35.36	35.70	34.19	35.86
6	G	6	HL	33.44	37.27	35.25	36.13	35.11
7	G	3	HL	35.71	37.22	36.65	36.72	37.97
8	G	6	LL	37.20	39.42	37.97	38.06	40.40
9	G	6	HH	34.78	35.27	34.27	34.00	37.42
10	G	6	LH	33.95	35.44	35.66	35.05	35.16
11	G	3	LH	35.70	36.64	35.45	36.45	36.39
12	G	3	LL	37.81	37.46	34.47	36.65	38.63

Appendix Table 7. Medullary width (mm) of the proximal metaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	11.45	12.91	15.23	16.07	16.89
2	M	6	HL	12.3	12.79	14.27	14.27	12.29
3	M	6	HH	13.46	14.1	15.43	14.26	12.95
4	M	6	LL	13.77	11.97	13.44	11.48	14.26
5	M	6	HH	18.72	17.08	18.9	20.55	16.74
6	G	6	HL	14.27	12.78	14.26	14.1	13.78
7	G	3	HL	13.91	15.73	14.9	15.56	16.89
8	G	6	LL	17.73	16.75	19.2	17.92	17.55
9	G	6	HH	16.72	15.4	18.05	16.89	18.54
10	G	6	LH	19.38	19.54	19.05	18.39	20.2
11	G	3	LH	16.73	16.06	16.54	17.1	15.23
12	G	3	LL	16.21	16.39	19.03	18.85	19.11

Appendix Table 8. Bone width (mm) of the proximal metaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	39.82	39.73	36.76	39.08	40.09
2	M	6	HL	39.85	41.31	38.7	38.85	41.81
3	M	6	HH	38.88	40.98	40.06	40.16	41.14
4	M	6	LL	40.16	40.33	40.49	40.49	39.72
5	M	6	HH	40.41	41.44	42.09	40.44	41.93
6	G	6	HL	.	43.93	43.93	43.61	44.77
7	G	3	HL	40.56	43.07	42.22	42.39	43.71
8	G	6	LL	42.39	47.09	43.71	43.46	43.88
9	G	6	HH	41.89	43.54	40.07	41.39	45.37
10	G	6	LH	43.05	42.71	43.38	42.56	42.44
11	G	3	LH	40.92	42.39	40.03	41.16	40.54
12	G	3	LL	40.84	41.06	41.7	42.5	43.21

Appendix Table 9. Estimated cortical area (mm^2) of the proximal diaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	840.11	715.24	760.55	764.92	1454.53
2	M	6	HL	1026.41	845.17	847.91	867.92	1436.73
3	M	6	HH	910.45	953.78	913.25	1022.90	1330.48
4	M	6	LL	1000.01	1002.51	950.63	915.12	1423.21
5	M	6	HH	1043.09	1059.62	953.19	1037.49	1282.53
6	G	6	HL	1142.78	999.10	1072.55	991.70	.
7	G	3	HL	1196.97	1145.48	1157.97	1240.39	1451.63
8	G	6	LL	1189.64	1128.22	1104.89	1270.18	1482.56
9	G	6	HH	1034.97	978.31	943.19	1166.82	1475.21
10	G	6	LH	1011.96	1011.26	979.77	981.59	1477.72
11	G	3	LH	1083.63	998.85	1074.38	1077.14	1394.55
12	G	3	LL	1164.79	946.24	1107.72	1220.44	1405.14

Appendix Table 10. Estimated cortical area (mm^2) of the proximal metaphysis of the equine third metacarpal bone.

ID	Sex	Age	Ca	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	1454.53	1411.80	1119.34	1269.00	1321.94
2	M	6	HL	1436.73	1542.93	1294.06	1305.69	1597.03
3	M	6	HH	1330.48	1480.55	1366.72	1409.48	1524.80
4	M	6	LL	1423.21	1483.23	1458.81	1507.65	1374.33
5	M	6	HH	1282.53	1425.55	1414.36	1213.09	1477.90
6	G	6	HL	.	1766.52	1726.50	1703.02	1814.46
7	G	3	HL	1451.63	1607.59	1560.52	1554.80	1625.29
8	G	6	LL	1482.56	1936.91	1541.92	1567.65	1617.45
9	G	6	HH	1475.21	1658.57	1279.80	1427.86	1714.71
10	G	6	LH	1477.72	1442.33	1518.92	1473.16	1393.11
11	G	3	LH	1394.55	1538.99	1328.83	1401.74	1411.54
12	G	3	LL	1405.14	1417.29	1376.75	1450.93	1501.91

Appendix Table 11. Resting serum calcium (mg/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	12.4	11	10.9	11.7	10.7
2	M	6	HL	11	10.4	10.4	10.6	10.2
3	M	6	HH	11.7	11.3	11.3	10.4	10.6
4	M	6	LL	11.7	10.9	11.7	10.6	10.3
5	M	6	HH	12.1	11.2	11.2	9.4	10.2
6	G	6	HL	11.4	11.1	11.9	11.5	10.5
7	G	3	HL	12.4	11.6	11	11.1	7.6
8	G	6	LL	11.6	11.2	11.8	11.7	11
9	G	6	HH	10.7	11.9	12.5	11.2	9.3
10	G	6	LH	11.5	10.9	12.4	11.2	10.1
11	G	3	LH	13.1	11	11.8	11.3	9.2
12	G	6	LL	13.8	11.4	11.1	11.1	11.4

Appendix Table 12. Resting serum ionized calcium (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	1.26	1.15	1.2	1.19	1.01
2	M	6	HL	1.31	1.12	1.19	1.08	1.22
3	M	6	HH	1.18	1.11	1.23	1.1	1.42
4	M	6	LL	1.17	1.14	1.11	1.09	1.45
5	M	6	HH	1.27	1.19	1.2	1.05	1.24
6	G	6	HL	1.18	1.15	1.03	1.38	1.11
7	G	3	HL	1.29	1.15	1.17	1.15	1.17
8	G	6	LL	1.24	1.11	1.27	1.22	1.04
9	G	6	HH	1.2	1.39	1.22	1.15	1.33
10	G	6	LH	1.18	1.21	1.39	1.02	1.34
11	G	3	LH	1.18	1.19	1.14	1.06	1.11
12	G	3	LL	1.24	1.32	1.1	0.98	1.2

Appendix Table 13. Resting serum phosphorus (mg/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	2.24	4.78	3.31	3.36	3.37
2	M	6	HL	2.57	4.2	4.07	3.69	3.3
3	M	6	HH	2.82	4.21	3.71	3.51	3.9
4	M	6	LL	2.28	4.44	3.81	3.31	3.6
5	M	6	HH	2.81	3.23	4.12	3.78	4.33
6	G	6	HL	2.54	4.32	3.89	3.73	4.11
7	G	3	HL	2.72	4.78	4.27	4.36	3.09
8	G	6	LL	2.86	3.84	3.85	3.29	3.76
9	G	6	HH	2.47	4.55	3.92	3.81	3.57
10	G	6	LH	3.14	3.56	3.57	3.71	3.48
11	G	3	LH	2.69	4.34	3.57	3.63	3.79
12	G	6	LL	2.63	4.08	3.75	3.73	4

Appendix Table 14. Resting serum magnesium (mEq/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	1.48	1.68	1.83	1.88	1.93
2	M	6	HL	1.86	1.79	1.73	1.86	1.57
3	M	6	HH	2.11	1.91	1.74	1.73	1.79
4	M	6	LL	2.05	2.16	1.90	1.97	1.88
5	M	6	HH	1.81	1.74	1.68	1.57	1.66
6	G	6	HL	1.79	2.07	1.80	1.88	1.75
7	G	3	HL	1.94	2.09	1.89	1.93	1.41
8	G	6	LL	1.89	1.84	1.84	1.82	1.79
9	G	6	HH	1.93	1.91	2.03	1.80	1.57
10	G	6	LH	1.97	1.82	1.80	1.61	1.47
11	G	3	LH	2.11	1.75	1.69	1.80	1.59
12	G	6	LL	2.04	1.63	1.67	1.70	1.80

Appendix Table 15. Resting plasma sodium (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	132	133	134	133	137
2	M	6	HL	135	133	134	131	133
3	M	6	HH	135	135	133	134	139
4	M	6	LL	135	135	132	135	139
5	M	6	HH	136	137	138	135	137
6	G	6	HL	134	135	133	134	138
7	G	3	HL	133	133	134	134	135
8	G	6	LL	134	133	132	134	138
9	G	6	HH	133	134	133	134	140
10	G	6	LH	134	134	133	136	139
11	G	3	LH	137	134	135	134	138
12	G	3	LL	136	135	133	133	138

Appendix Table 16. Resting plasma chloride (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	99	97	99	98	98
2	M	6	HL	100	99	98	97	97
3	M	6	HH	100	99	98	97	101
4	M	6	LL	99	99	99	99	102
5	M	6	HH	102	102	100	99	98
6	G	6	HL	101	98	97	97	101
7	G	3	HL	99	97	97	97	96
8	G	6	LL	100	98	96	96	99
9	G	6	HH	101	98	96	98	101
10	G	6	LH	102	101	98	98	102
11	G	3	LH	99	97	98	98	94
12	G	3	LL	99	97	95	95	95

Appendix Table 17. Resting plasma potassium (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	3.84	3.64	3.58	3.9	3.55
2	M	6	HL	3.63	3.05	3.42	3.51	3.11
3	M	6	HH	4.02	3.49	3.56	3.44	3.69
4	M	6	LL	4.13	3.94	3.58	3.74	3.57
5	M	6	HH	3.89	3.35	3.44	3.53	3.39
6	G	6	HL	3.35	3.84	3.33	3.39	3.54
7	G	3	HL	4.49	3.63	3.78	3.78	3.67
8	G	6	LL	3.86	3.83	3.46	3.27	3.59
9	G	6	HH	3.52	3.6	3.49	3.73	3.84
10	G	6	LH	3.48	3.95	3.65	3.41	3.59
11	G	3	LH	4.11	3.69	3.59	3.71	3.57
12	G	3	LL	4.43	3.89	4.02	3.93	3.88

Appendix Table 18. Resting blood pH in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	7.403	7.394	7.419	7.4	7.393
2	M	6	HL	7.435	7.429	7.428	7.393	7.416
3	M	6	HH	7.413	7.418	7.413	7.42	7.414
4	M	6	LL	7.424	7.418	7.409	7.411	7.404
5	M	6	HH	7.378	7.417	7.41	7.41	7.397
6	G	6	HL	7.385	7.429	7.416	7.427	7.39
7	G	3	HL	7.401	7.394	7.402	7.393	7.391
8	G	6	LL	7.413	7.418	7.43	7.441	7.463
9	G	6	HH	7.406	7.424	7.42	7.411	7.404
10	G	6	LH	7.371	7.378	7.415	7.395	7.375
11	G	3	LH	7.39	7.403	7.396	7.376	7.399
12	G	3	LL	7.387	7.412	7.423	7.388	7.401

Appendix Table 19. Resting serum total protein (g/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	7.28	6.72	7.17	6.66	7.21
2	M	6	HL	6.4	5.97	6.16	5.79	5.89
3	M	6	HH	7.74	6.96	6.56	6.58	6.12
4	M	6	LL	7.25	6.77	6.51	6.55	6.17
5	M	6	HH	7.22	6.23	6.33	6.47	6.01
6	G	6	HL	6.57	6.73	6.14	6.48	5.84
7	G	3	HL	6.86	5.85	5.63	6.27	5.81
8	G	6	LL	6.74	6.88	6.17	6.21	5.64
9	G	6	HH	6.97	6.41	6.37	6.63	6.16
10	G	6	LH	6.59	6.72	5.83	6.08	5.62
11	G	3	LH	6.79	6.1	6.02	6.42	6.35
12	G	3	LL	7.13	7.06	6.56	6.46	6.02

Appendix Table 20. Resting serum albumin (g/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	2.9	2.84	2.9	3.01	3.18
2	M	6	HL	3.34	3.32	3.28	3.44	3.16
3	M	6	HH	3.34	3.38	3.19	3.62	3.31
4	M	6	LL	3.26	3.25	3.34	3.33	3.13
5	M	6	HH	3.5	3.22	3.55	3.61	3.48
6	G	6	HL	3.36	3.44	3.49	3.52	3.37
7	G	3	HL	3.72	3.01	3.38	3.48	3.55
8	G	6	LL	3.46	3.54	3.48	3.41	3.21
9	G	6	HH	3.7	3.42	3.38	3.69	3.51
10	G	6	LH	3.45	3.36	3.18	3.26	3.28
11	G	3	LH	3.88	3.43	3.29	3.32	3.28
12	G	3	LL	3.52	3.38	3.48	3.39	3.1

Appendix Table 21. Resting serum parathyroid hormone (ng/mL) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	65.52	224.89	152	73.82	210.5
2	M	6	HL	111.14	123.29	236.79	173.56	132.39
3	M	6	HH	19.99	135.32	246.15	233	156.68
4	M	6	LL	337.59	633.09	529.32	566.3	425.45
5	M	6	HH	61.66	50.62	59.14	92.26	76
6	G	6	HL	53.87	35.11	33.56	30.8	40.74
7	G	3	HL	23.42	331.91	121.39	79.35	166.48
8	G	6	LL	19.59	72.97	132.66	77.67	86.91
9	G	6	HH	18.1	83.06	237.68	193.57	262.03
10	G	6	LH	10.77	12.79	31.68	103.6	23.6
11	G	3	LH	3.96	44.26	21.61	27.61	45.45
12	G	3	LL	.74	46.29	44.53	57.69	66.41

Appendix Table 22. Resting plasma osteocalcin (pg/mL) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	3.56	5.64	3.89	5.86	3.97
2	M	6	HL	9.29	9.57	8.82	8.87	7.13
3	M	6	HH	10.34	12.22	11.48	11.76	9.93
4	M	6	LL	10.19	12.29	14.9	12.92	11.71
5	M	6	HH	7.99	6.57	7.33	7.08	5.68
6	G	6	HL	12.22	11.10	9.70	10.03	6.86
7	G	3	HL	18.97	20.22	18.93	14.28	13.29
8	G	6	LL	9.83	11.50	10.64	12.14	8.23
9	G	6	HH	14.16	14.35	12.04	10.64	8.84
10	G	6	LH	9.08	11.05	9.68	7.71	7.61
11	G	3	LH	16.00	18.12	14.75	12.26	12.79
12	G	3	LL	7.56	8.68	7.56	6.28	6.49

Appendix Table 23. Resting plasma hydroxyproline (μmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/21/94	1/11/95
1	M	6	LH	7.48	8.58	5.26	5.93	5.48
2	M	6	HL	9.69	9.47	6.37	7.03	6.15
3	M	6	HH	9.03	9.69	4.82	7.03	7.03
4	M	6	LL	8.80	10.58	7.92	6.37	7.25
5	M	6	HH	10.13	10.82	6.59	8.14	7.25
6	G	6	HL	9.69	8.80	7.25	7.48	7.92
7	G	3	HL	14.12	11.02	11.46	11.24	9.91
8	G	6	LL	8.587	7.48	7.48	7.25	8.36
9	G	6	HH	9.91	9.03	6.15	7.48	7.48
10	G	6	LH	9.91	7.70	7.92	8.14	8.36
11	G	3	LH	10.58	9.95	9.69	7.92	9.91
12	G	3	LL	13.45	8.14	12.35	11.02	9.47

Appendix Table 24. Body weight (kg) of exercising Arabian horses.

ID	Sex	Age	Diet	10/20/94	11/10/94	12/1/94	12/22/94	1/10/95
1	M	6	LH	425.00	425.00	425.00	413.64	418.18
2	M	6	HL	488.64	477.27	488.64	481.82	488.64
3	M	6	HH	443.18	438.64	422.73	420.45	425.00
4	M	6	LL	422.73	406.82	409.09	397.73	400.00
5	M	6	HH	500.00	488.64	493.18	475.00	479.55
6	G	6	HL	515.91	506.82	511.36	511.36	515.91
7	G	3	HL	506.82	502.27	509.09	495.45	515.91
8	G	6	LL	495.45	488.64	488.64	486.36	488.64
9	G	6	HH	468.18	459.09	459.09	454.55	461.36
10	G	6	LH	484.09	481.82	484.09	481.82	488.64
11	G	3	LH	393.18	388.64	393.18	388.64	400.00
12	G	3	LL	452.27	434.09	443.18	438.64	452.27

Appendix B

Raw Data - Experiment Two

Appendix Table 1. Estimated bone mineral content (g/2 cm section) of the lateral diaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Ca	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	20.38	19.10	16.58	16.74	18.83
2	M	7	LL	17.97	17.67	18.32	17.89	16.73
3	M	7	HL	17.61	19.70	17.23	18.44	18.77
4	M	7	HH	17.47	17.56	17.14	17.51	16.65
5	M	7	LH	18.18	16.60	17.86	18.15	19.26
7	G	4	HH	21.20	20.65	20.03	19.01	17.64
8	G	7	LH	18.06	18.03	17.20	16.94	16.67
9	G	7	HH	18.92	20.23	18.72	17.17	18.92
10	G	7	HL	21.13	18.60	20.32	19.28	19.02
11	G	4	HL	18.38	19.09	18.61	16.60	17.97
12	G	4	LL	18.36	18.24	18.14	17.73	17.65

Appendix Table 2. Estimated bone mineral content (g/2 cm section) of the medial diaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Ca	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	19.91	19.55	18.47	18.68	18.50
2	M	7	LL	20.06	20.16	20.55	20.33	19.64
3	M	7	HL	20.45	20.78	19.63	20.07	19.36
4	M	7	HH	20.36	20.42	19.68	19.47	19.37
5	M	7	LH	18.89	19.06	20.51	19.27	19.51
7	G	4	HH	22.10	22.23	21.04	21.16	20.69
8	G	7	LH	19.90	20.18	19.33	18.93	19.14
9	G	7	HH	20.50	21.05	20.12	19.62	19.43
10	G	7	HL	19.76	19.89	20.37	19.71	18.99
11	G	4	HL	19.60	19.44	18.47	18.81	19.14
12	G	4	LL	21.36	21.09	20.57	20.28	19.04

Appendix Table 3. Extimated bone mineral content (g/2 cm section) of the lateral metaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Ca	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	18.46	19.18	17.60	17.52	16.95
2	M	7	LL	17.29	17.94	18.08	18.10	17.09
3	M	7	HL	18.09	17.07	17.87	15.74	16.15
4	M	7	HH	17.29	17.29	17.05	16.92	16.15
5	M	7	LH	16.49	17.09	17.74	16.90	17.33
7	G	4	HH	19.02	18.38	17.95	17.94	17.75
8	G	7	LH	18.12	17.70	16.79	16.14	16.22
9	G	7	HH	18.68	18.53	17.16	17.06	16.33
10	G	7	HL	19.20	17.75	18.29	17.47	17.39
11	G	4	HL	17.29	17.41	17.34	16.35	17.05
12	G	4	LL	18.82	18.80	18.60	18.10	18.53

Appendix Table 4. Estimated bone mineral content (g/2 cm section) of the medial metaphyseal cortex of the equine third metacarpal bone.

ID	Sex	Age	Ca	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	18.85	18.06	17.35	17.34	16.36
2	M	7	LL	19.13	19.14	19.31	19.33	18.25
3	M	7	HL	19.16	18.45	18.39	17.61	17.21
4	M	7	HH	18.48	18.54	17.52	18.24	17.57
5	M	7	LH	17.01	17.21	18.98	17.48	17.50
7	G	4	HH	20.76	20.76	20.00	19.80	16.71
8	G	7	LH	18.67	18.68	17.21	17.04	17.39
9	G	7	HH	19.33	19.13	18.03	17.96	17.03
10	G	7	HL	17.92	18.37	18.97	17.83	16.47
11	G	4	HL	17.64	18.36	17.40	17.88	18.35
12	G	4	LL	19.74	19.39	19.03	18.45	17.56

Appendix Table 5. Medullary width (mm) of the proximal diaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	14.66	13.85	13.84	13.79	13.84
2	M	7	LL	13.03	13.40	13.46	13.18	13.17
3	M	7	HL	12.18	11.42	11.85	11.93	11.36
4	M	7	HH	15.79	15.59	15.65	15.63	15.70
5	M	7	LH	13.95	14.44	14.88	14.12	14.54
7	G	4	HH	14.38	14.05	13.70	13.63	13.90
8	G	7	LH	18.28	18.38	18.32	18.15	18.37
9	G	7	HH	14.37	14.53	14.21	14.22	14.25
10	G	7	HL	15.65	15.65	15.87	15.57	15.49
11	G	4	HL	16.13	15.63	15.85	16.06	16.33
12	G	4	LL	15.51	15.69	16.11	16.07	15.93

Appendix Table 6. Bone width (mm) of the proximal diaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	25.08	29.85	28.00	26.98	27.97
2	M	7	LL	30.61	31.75	30.51	31.10	27.87
3	M	7	HL	33.37	31.99	32.51	28.84	29.22
4	M	7	HH	33.79	34.08	32.45	33.66	32.73
5	M	7	LH	30.14	29.48	31.81	28.80	33.15
7	G	4	HH	33.51	33.40	31.48	32.87	34.92
8	G	7	LH	36.62	37.16	33.89	31.77	33.63
9	G	7	HH	32.28	30.10	28.08	29.64	31.55
10	G	7	HL	27.88	27.88	25.38	27.24	28.43
11	G	4	HL	31.99	30.83	30.28	30.77	32.15
12	G	4	LL	35.76	34.42	34.02	32.90	35.31

Appendix Table 7. Medullary width (mm) of the proximal metaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	17.92	18.23	19.05	19.62	18.48
2	M	7	LL	18.4	17.75	19.06	17.32	18.82
3	M	7	HL	17.78	17.12	17.12	15.75	16.95
4	M	7	HH	20.24	20.22	19.9	19.61	21.54
5	M	7	LH	23.78	21.82	23.45	19.13	19.33
7	G	4	HH	15.53	16.51	17.01	15.7	17.12
8	G	7	LH	23.84	23.67	24	24.03	25.08
9	G	7	HH	22.37	23.85	22.7	21.24	19.68
10	G	7	HL	21.2	21.69	21.52	22.06	20.19
11	G	4	HL	16.51	16.34	16.5	16.02	16.44
12	G	4	LL	20.24	20.74	20.57	20.92	17.79

Appendix Table 8. Bone width (mm) of the proximal metaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	34.69	36.96	34.68	33.20	35.27
2	M	7	LL	38.76	39.56	37.79	38.56	34.25
3	M	7	HL	40.93	39.15	39.46	39.70	33.40
4	M	7	HH	39.17	39.63	39.83	38.89	38.85
5	M	7	LH	36.81	34.70	37.30	33.35	38.66
7	G	4	HH	40.04	38.40	34.68	35.97	38.83
8	G	7	LH	42.93	41.96	40.32	37.11	38.14
9	G	7	HH	37.88	38.69	36.73	37.75	36.82
10	G	7	HL	35.22	35.37	35.22	35.13	35.96
11	G	4	HL	38.43	36.27	34.63	35.62	36.27
12	G	4	LL	42.29	39.53	39.84	38.56	39.66

Appendix Table 9. Estimated cortical area (mm^2) of the proximal diaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	414.09	698.91	592.64	537.84	590.87
2	M	7	LL	767.48	828.71	749.60	793.20	603.10
3	M	7	HL	965.51	892.87	916.69	689.42	724.95
4	M	7	HH	892.77	918.07	807.86	888.92	824.66
5	M	7	LH	713.52	660.26	790.77	629.87	887.39
7	G	4	HH	916.01	918.06	803.60	894.75	1026.43
8	G	7	LH	1006.74	1042.92	813.14	680.24	793.42
9	G	7	HH	835.41	694.69	586.66	676.12	792.46
10	G	7	HL	532.27	532.37	392.39	499.31	568.14
11	G	4	HL	763.40	705.88	665.56	688.76	767.17
12	G	4	LL	1038.11	938.44	897.83	824.17	993.03

Appendix Table 10. Estimated cortical area (mm^2) of the proximal metaphysis of the equine third metacarpal bone.

ID	Sex	Age	Diet	7/28/95	8/16/95	9/6/95	9/29/95	10/20/95
1	M	7	LH	882.27	1033.71	839.80	717.30	902.46
2	M	7	LL	1163.78	1249.93	1064.80	1186.89	818.87
3	M	7	HL	1359.14	1239.63	1264.00	1328.03	828.26
4	M	7	HH	1124.63	1161.69	1190.42	1127.88	1045.35
5	M	7	LH	789.49	727.98	841.39	746.27	1120.95
7	G	4	HH	1362.02	1201.98	913.36	1047.35	1214.67
8	G	7	LH	1274.64	1200.37	1049.70	799.71	825.65
9	G	7	HH	934.48	928.09	833.80	973.92	968.41
10	G	7	HL	791.01	780.58	777.34	747.47	885.49
11	G	4	HL	1204.28	1048.52	926.99	1012.14	1045.24
12	G	4	LL	1378.79	1132.47	1164.10	1049.23	1256.43

Appendix Table 11. Resting serum calcium (mg/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	11.1	12.5	11.0	12.5	12.1
2	M	7	LL	11.1	12.5	9.9	11.2	11.1
3	M	7	HL	9.6	12.3	11.4	13.2	11.5
4	M	7	HH	11.1	12.2	11.0	11.9	11.1
5	M	7	LH	11.8	12.6	11.3	10.9	12.3
7	G	4	HH	11.4	12.3	11.4	11.6	11.4
8	G	7	LH	11.5	11.9	10.9	13.2	11.3
9	G	7	HH	11.2	11.8	11.5	12.7	12.2
10	G	7	HL	10.7	12.0	11.7	12.8	12.3
11	G	4	HL	10.5	12.6	11.8	13.1	12.8
12	G	4	LL	12.4	11.8	12.5	12.6	12.4

Appendix Table 12. Resting serum ionized calcium (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	1.49	1.50	1.57	1.51	1.54
2	M	7	LL	1.43	1.41	1.42	1.39	1.39
3	M	7	HL	1.35	1.39	1.53	1.55	1.47
4	M	7	HH	1.40	1.41	1.46	1.42	1.43
5	M	7	LH	1.52	1.42	1.45	1.48	1.53
7	G	4	HH	1.42	1.36	1.43	1.41	1.43
8	G	7	LH	1.41	1.36	1.40	1.55	1.41
9	G	7	HH	1.43	1.44	1.42	1.44	1.43
10	G	7	HL	1.45	1.39	1.38	1.47	1.45
11	G	4	HL	1.34	1.39	1.41	1.51	1.47
12	G	4	LL	1.43	1.40	1.46	1.40	1.47

Appendix Table 13. Resting serum phosphorus (mg/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	4.39	4.42	3.98	4.31	4.23
2	M	7	LL	4.41	4.78	4.47	3.88	5.22
3	M	7	HL	4.59	4.77	4.52	3.58	4.66
4	M	7	HH	3.91	4.31	3.36	3.61	4.18
5	M	7	LH	4.25	4.79	4.58	4.8	4.73
7	G	4	HH	4.29	4.83	4.98	4.38	5.11
8	G	7	LH	3.67	4.35	4.55	2.58	4.21
9	G	7	HH	3.37	4.46	4.19	4.22	4.59
10	G	7	HL	3.78	3.91	3.57	3.5	3.96
11	G	4	HL	4.47	4.58	4.11	4	3.62
12	G	4	LL	5.4	4.28	4.32	3.77	4.22

Appendix Table 14. Resting serum magnesium (mEq/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	1.46	1.55	1.5	1.61	1.5
2	M	7	LL	1.66	1.43	1.37	1.43	1.55
3	M	7	HL	1.46	1.45	1.43	1.44	1.37
4	M	7	HH	1.83	2.32	1.6	1.53	1.56
5	M	7	LH	1.34	2.09	1.55	1.7	1.64
7	G	4	HH	1.73	2.19	1.63	2.46	1.78
8	G	7	LH	1.58	2.16	1.53	1.92	1.63
9	G	7	HH	1.46	1.96	1.57	1.57	1.66
10	G	7	HL	1.39	1.81	1.66	1.52	1.58
11	G	4	HL	1.51	1.68	1.7	1.68	1.77
12	G	4	LL	1.52	1.49	2.02	1.48	1.45

Appendix Table 15. Resting plasma sodium (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	134.3	135.9	138.3	133.2	137.1
2	M	7	LL	139.2	137.6	136.4	132	134.1
3	M	7	HL	139.1	137.8	139	136.8	134.3
4	M	7	HH	135.6	137.6	136.4	133.2	134.4
5	M	7	LH	134.3	139.4	136.9	139.2	133.8
7	G	4	HH	139.3	137.4	135.3	137.1	137.2
8	G	7	LH	135	137.5	138.1	140.8	138.4
9	G	7	HH	139.3	139.1	139.5	137.9	138
10	G	7	HL	134	137	138.5	138.7	137.3
11	G	4	HL	132.6	135.7	135.7	135.4	135.7
12	G	4	LL	133.7	137.4	137.6	136.7	136.9

Appendix Table 16. Resting plasma chloride (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	99	100	98	96	97
2	M	7	LL	100	100	98	98	97
3	M	7	HL	99	100	103	102	99
4	M	7	HH	99	101	99	99	98
5	M	7	LH	101	101	100	98	96
7	G	4	HH	99	100	99	98	97
8	G	7	LH	98	99	99	102	96
9	G	7	HH	99	99	98	99	98
10	G	7	HL	99	99	98	98	99
11	G	4	HL	97	98	96	96	96
12	G	4	LL	97	98	98	97	97

Appendix Table 17. Resting plasma potassium (mmol/L) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	3.68	4.39	4.39	4.06	4.41
2	M	7	LL	4	3.85	3.93	3.6	3.93
3	M	7	HL	3.67	3.91	3.86	3.97	4.01
4	M	7	HH	3.9	4.55	4.24	4.19	4
5	M	7	LH	3.8	4.35	4.35	4.69	4.38
7	G	4	HH	4.55	4.72	4.43	4.57	4.86
8	G	7	LH	4.05	3.96	4.14	4.37	3.97
9	G	7	HH	3.98	3.88	4.21	4.16	3.99
10	G	7	HL	3.99	4.21	4.16	4.11	4.29
11	G	4	HL	3.62	4.44	4.33	4.63	4.48
12	G	4	LL	4.17	4.64	4.82	4.7	4.93

Appendix Table 18. Resting blood pH in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	7.428	7.408	7.428	7.413	7.435
2	M	7	LL	7.449	7.437	7.464	7.441	7.432
3	M	7	H	7.428	7.402	7.423	7.396	7.411
4	M	8	HH	7.429	7.433	7.445	7.425	7.404
5	M	7	LH	7.456	7.456	7.449	7.432	7.450
7	G	4	HH	7.406	7.447	7.407	7.419	7.406
8	G	7	LH	7.448	7.463	7.440	7.403	7.447
9	G	6	HH	7.429	7.434	7.451	7.413	7.429
10	G	6	HL	7.408	7.417	7.451	7.428	7.423
11	G	4	HL	7.453	7.484	7.443	7.394	7.416
12	G	4	LL	7.434	7.455	7.431	7.458	7.431

Appendix Table 19. Resting serum total protein (g/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	7.42	7.40	7.42	7.41	7.43
2	M	7	LL	7.44	7.43	7.46	7.44	7.43
3	M	7	HL	7.42	7.40	7.42	7.39	7.41
4	M	7	HH	7.42	7.43	7.44	7.42	7.40
5	M	7	LH	7.45	7.45	7.44	7.43	7.45
7	G	4	HH	7.40	7.44	7.40	7.41	7.40
8	G	7	LH	7.44	7.46	7.44	7.40	7.44
9	G	7	HH	7.42	7.43	7 45	7.41	7.42
10	G	7	HL	7.40	7.41	7.45	7.42	7.42
11	G	4	HL	7.45	7.48	7.44	7.39	7.41
12	G	4	LL	7.43	7.45	7.43	7.45	7.43

Appendix Table 20. Resting serum albumin (g/dL) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	2.96	2.81	3.16	2.94	3.06
2	M	7	LL	3.22	3.05	3.36	3.08	3.24
3	M	7	HL	3.06	3.11	3.39	3.28	3.17
4	M	7	HH	3.02	2.91	3.25	3.2	3.17
5	M	7	LH	3.49	3.34	3.52	3.45	3.62
7	G	4	HH	3.43	3.19	3.48	3.28	3.29
8	G	7	LH	3.48	3.39	3.43	3.58	3.23
9	G	7	HH	3.3	3.38	3.76	3.39	3.26
10	G	7	HL	3.03	3.43	3.49	3.32	3.03
11	G	4	HL	3.42	3.76	3.91	4.01	3.61
12	G	4	LL	3.28	3.43	3.26	3.65	3.24

Appendix Table 21. Resting serum parathyroid hormone (pg/mL) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	132.00	1899.70	2126.10	1441.60	1882.70
2	M	7	LL	88.40	121.30	111.40	165.00	168.90
3	M	7	HL	196.00	171.40	259.80	91.50	181.60
4	M	7	HH	790.10	610.50	733.30	279.40	704.10
5	M	7	LH	83.60	83.80	98.20	47.90	61.00
7	G	4	HH	85.20	126.80	202.00	185.40	155.90
8	G	7	LH	44.30	63.00	66.80	27.70	107.20
9	G	7	HH	226.40	312.70	155.30	186.30	102.60
10	G	7	HL	84.10	32.30	121.60	40.90	47.10
11	G	4	HL	62.90	29.70	43.60	22.70	36.70
12	G	4	LL	37.90	33.30	33.70	38.60	39.60

Appendix Table 22. Resting plasma osteocalcin (pg/mL) in exercised Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	4.35	5.17	4.47	4.12	3.05
2	M	7	LL	12.56	16.22	17.85	17.11	13.74
3	M	7	HL	15.65	19.48	12.84	12.50	16.54
4	M	7	HH	14.31	16.93	17.48	11.10	13.83
5	M	7	LH	9.39	10.91	11.04	10.13	10.04
7	G	4	HH	31.36	39.53	30.25	24.86	26.62
8	G	7	LH	14.54	19.72	19.89	12.61	15.66
9	G	7	HH	27.58	32.59	30.61	19.26	16.76
10	G	6	HL	18.32	16.48	17.53	10.41	10.41
11	G	4	HL	30.18	28.45	18.24	10.86	11.98
12	G	4	LL	10.55	12.06	12.46	13.03	10.25

Appendix Table 23. Resting plasma hydroxyproline (μmol/L) in exercised Arabian horses.

ID	Sex	Age	Ca	7/28/95	8/18/95	9/8/95	9/29/95	10/20/95
1	M	7	LH	1.91	3.18	0.90	0.13	2.17
2	M	7	LL	4.20	6.74	3.95	2.17	3.18
3	M	7	HL	2.93	5.47	2.17	1.66	3.18
4	M	7	HH	3.18	5.22	2.93	1.91	2.17
5	M	7	LH	2.17	5.73	3.18	0.90	1.66
7	G	4	HH	9.29	13.10	5.98	8.01	7.51
8	G	7	LH	4.20	6.74	1.91	3.18	4.71
9	G	7	HH	4.71	6.74	3.18	4.20	5.73
10	G	7	HL	2.93	5.47	2.17	4.45	3.18
11	G	4	HL	4.20	7.52	5.47	3.95	3.44
12	G	4	LL	5.47	8.52	7.00	7.25	4.71

Appendix Table 24. Body weight (kg) of exercising Arabian horses.

ID	Sex	Age	Diet	7/28/95	8/17/95	9/7/95	9/28/95	10/19/95
1	M	7	LH	418	424	422	424	420
2	M	7	LL	494	488	488	476	476
3	M	7	HL	450	444	438	438	442
4	M	7	HH	422	412	410	406	396
5	M	7	LH	452	450	444	452	440
7	G	4	HH	536	528	524	534	530
8	G	7	LH	506	492	492	484	486
9	G	7	HH	458	464	464	458	452
10	G	7	HL	452	450	450	446	446
11	G	4	HL	410	406	404	410	402
12	G	4	LL	478	470	468	474	468

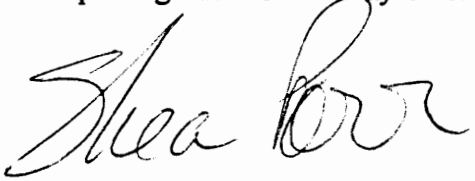
Vita

Cheryl Ann Porr, known to her friends as Shea, was born in Medina, Ohio, on February 22, 1967. She and her family moved frequently while she was young, as her father was in the military. When he retired from active service, they settled in Texas. She graduated from the High School for the Performing and Visual Arts in Houston, Texas, in May of 1985.

In August of 1985, Shea entered Texas A&M University, where she majored in Animal Science, with an emphasis in equine science. She graduated with a Bachelor of Science degree in May of 1990.

In January of 1992, she entered the University of Florida to pursue a graduate degree in Animal Science, emphasising equine nutrition and exercise physiology. Under the advice and council of Dr. E.A. Ott, she graduated with a Master of Science degree in December of 1993.

In January of 1994, Shea entered Virginia Polytechnic Institute and State University to continue her academic pursuits. Dr. D.S. Kronfeld guided her through her program. She is currently a candidate for the degree of Doctor of Philosophy and anticipates graduation in May of 1996.



Cheryl Ann (Shea) Porr