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# Evaluation of Bone Mineral Content in Equine Cadavers and Pregnant Mares

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## Story in Brief

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In Experiment I, bone biopsies were taken from the twelfth rib and third metacarpal in 20 equine cadavers for analysis of calcium, phosphorus, ash as a percentage of wet weight (AWW%) and dry, fat-free ash percentage (DFF%). The AWW%, DFF% and percent phosphorus were significantly higher in the metacarpal than in the rib. However, calcium levels were similar between the two sites. In Experiment II, 10 Quarter Horse mares were equally allotted to either a control (n=5) or a treatment group (n=5) in order to study the effects of restricted movement on physical fitness. Treatment mares were restricted to 4' x 8' tie stalls while control mares were kept on native prairiegrass pasture. Biopsies from the twelfth rib were taken on d 0 (left side) and then again on d 90 (right side). Percentages of ash, calcium and phosphorus did not differ between control and treatment mares. Bone biopsy techniques may prove to be a useful tool in the future for the quantitative analysis of mineral status in the horse.

(Key Words: Equine, Bone, Mineral.)

## Introduction

Due to the dynamic nature of bone, this tissue is capable of exchanging certain ions, such as calcium and phosphorus, with the blood in order to maintain circulating levels. Because of this fact, metabolic and nutritionally induced bone disorders could be of concern, especially in the young growing horse. Bone composition and the mechanisms controlling mineral deposition and resorption have long been studied in other species (Field et al., 1974; Taylor et al., 1960). However, few reports have been published which evaluate bone mineral content (BMC) or quantify specific minerals in equine bone at various sites. Researchers have employed the use of photon absorptiometry in order to measure BMC in both equine and bovine species (Jeffcott et al., 1986; Zetterholm and Dalen, 1978). More recently, biopsy techniques have been used for determination of BMC in other species (Bobilya et al., 1991; Breur et al., 1988). Misheff et al. (1992) described a procedure in which unicortical and transcortical biopsy specimens were taken from the rib of standing horses for histologic and histomorphometric evaluation. This technique proved to have excellent success and could have potential benefits in diagnosing metabolic and nutritional bone disease. Therefore, the purpose of this study was to determine the bone composition of the twelfth rib and compare the BMC between the rib and third metacarpal of the horse.

## Materials and Methods

**Experiment I.** Twenty equine cadavers of Quarter Horse breeding were used to evaluate bone mineral status in the twelfth rib and third metacarpal (cannon bone). Bone biopsy procedures were performed approximately 12 h postmortem in three stallions, seven geldings and ten mares ranging in age from 1 to 20 yr old. Unicortical samples were taken on the left side at the midpoint of the diaphysis of the third metacarpal (cannon bone) and the twelfth rib. Biopsy samples were obtained using a 12mm internal diameter Galt stainless steel trephine which was attached to a power drill. Specimens were immediately weighed, placed in freezer bags, and stored at -20° C until chemical analysis could be performed.

Upon thawing, samples were dried to a constant weight at 100° C. Fat was removed from each

biopsy sample by washing with petroleum ether. Extracted samples were then returned to the oven and redried. The weight of the dry, fat-free bone was taken and the sample was ashed at 500° C for 12 h and allowed to cool. Weight of the cooled, ashed sample was determined after which ash weight as a percentage of wet weight (AWW%) and dry, fat-free ash percentage (FFA%; ash weight/extracted weight) were calculated. Bone samples were ground to the consistency of flour using a mortar and pestle to achieve homogeneity. An aliquot of ash weighing 100 mg was dissolved in 1ml of concentrated hydrochloric acid (HCl) and diluted with distilled deionized water to 100 ml prior to analysis of calcium (Ca) and phosphorus (P). Calcium was determined using flame atomic absorption spectroscopy<sup>6</sup> and phosphorus was measured using a colorimetric test<sup>7</sup>. Least squares means were calculated and data were analyzed using the general linear model procedure of SAS (1985).

**Experiment II.** Ten pregnant Quarter Horse mares, which were being used to study the influence of restricted movement on physical fitness, were evaluated for bone mineral status. Mares were blocked by age and expected foaling date and then randomly allotted to either the control or the treatment group (5 mares/group). Treatment mares were housed in 4' x 8' tie stalls and allowed no exercise. Control mares were kept on native prairiegrass pasture at a stocking rate of 6 acres/mare. All mares were fed a 15% CP pelleted ration on a body weight basis that was formulated to meet NRC requirements for gestating mares (Table 1). Mares on pasture were fed free choice prairiegrass hay while mares in the stalls were fed hay at 1% of their body weight. Bone biopsies were taken from the twelfth rib on d 0 and d 90 from the left and right sides, respectively. Mares were restrained in standing stocks and sedated with xylazine (300 mg intravenously). An area over the twelfth rib, approximately 20 x 20 cm<sup>2</sup>, was clipped and scrubbed for surgery. Lidocaine was administered (30 ml subcutaneously) both anterior and dorsal to the incision site to form an inverted "L" block. A skin incision was made over the rib in the center of the area which had been aseptically prepared. The subcutis and cutaneous trunci muscles were retracted to expose the rib after which the periosteum was scraped away. A 12mm Galt trephine was centered on the rib and a unicortical biopsy was taken by boring through the lateral cortex and into the medullary cavity. Following extraction, the samples were immediately weighed, placed in freezer bags, and stored at -20° C until chemical analysis could be performed. Biopsy samples were prepared and analyzed for Ca, P and ash as described in Experiment I. Following the surgical procedure, mares were treated with 30 ml of Penicillin-G twice daily and administered 2g of phenylbutazone orally once a day for 5 d. Data on percent Ca, P, and ash were analyzed using a one-way analysis of variance (ANOVA) procedure of SAS (1985).

## Results and Discussion

**Experiment I.** Mean biopsy ash weight as a percentage of wet weight (AWW%) and dry, fat-free ash percentage (DFF%, ash weight/fat free weight) for the rib and cannon bone are given in Table 2. The dry, fat-free ash ranged from 59 to 70%, which is similar to values from metacarpal bones (50 to 65%) in horses ranging in age from 1 to 30 yr (El Shorafa et al., 1979). These values are also comparable to rib bone in cattle (Little, 1972) and whole bovine, porcine and ovine bones removed from animals of different ages (Field et al., 1974).

Both AWW% and DFF% were higher in the third metacarpal than in the rib (P<.0001). Similarly, Field et al. (1974) showed in cattle, sheep and pigs that dry, fat-free ash percentages were significantly higher in the femur versus the rib. Cox and Balloun (1971) further demonstrated that the DFF% varied considerably among several individual bones in two lines of laying hens. Mean percentage ash values in these hens ranged from 52 for the tarsus bones to 64 for the humerus, with the rib and femur measuring approximately 57.

In this study, percent calcium was similar between biopsy sites while phosphorus levels were higher (P<.0001) in the cannon compared with the rib (Table 2). Likewise, calcium in bone ash was shown not to differ (P>.05) between the vertebrae, rib and femur in other species (Field et al., 1974). In laying hens, calcium levels were similar among fifteen different bones including the metacarpal, rib and femur (Taylor et al., 1960).

The difference in percent ash and phosphorus between the metacarpal and the rib appears to be due to the composition of the individual bones. The diaphysis of the long bones is comprised of compact cortical bone surrounding the medullary cavity which contains the spongy or

cancellous bone. The epiphyseal and metaphyseal regions of long bones are composed primarily of cancellous tissue made up of small particles of bone called trabeculae. The flat bones consist mainly of spongy bone surrounded by a cortex of compact bone. Furthermore, it has been suggested in lactating ewes that the spongy bones are more sensitive to resorption than the rest of the skeleton and that compact bone is extensively resorbed only during severe calcium deficiency (Benzie et al., 1955). The previous study also showed that ewes receiving a daily calcium supplement had a significantly higher percent ash in the ribs and vertebrae than those which did not receive the supplement. However, no variation in the percentage of ash was discovered in the metacarpal mid-shaft between the groups. These findings would indicate that ewes receiving the calcium deficient diet are primarily resorbing mineral from the bones containing a large proportion of cancellous tissue (i.e., flat bones). Likewise, lactating ewes fed a diet low in P, vertebral bones were the most readily affected by resorption as well as being the first to be repaired (Benzie et al., 1959). Benzie et al. (1959) confirmed that the cervical vertebrae lost 50% of the original ash, which was recovered shortly thereafter. However, the shafts of the metacarpals lost about 15% and made no recovery by the end of the experiment.

Resorption and repair of the skeleton may also vary greatly along the length of the long bones. Using radioactive calcium, it was shown in rats that uptake per gram of ash was the highest in regions of spongy bone and lowest in the compact tissue (Armstrong and Barnum, 1948). Over time, the radio-labeled Ca was redistributed equally throughout the bone so that the epiphyseal regions and vertebrae contained the same amounts as the compact bone. Likewise, femoral bones from the cat, rabbit and rat yielded lower ( $P < .01$ ) Ca and P levels in the cancellous bone versus the cortical tissue.

**Experiment II.** The restriction of exercise appeared to have no effect on bone mineral content as the percentage of ash, calcium and phosphorus in the rib was similar ( $P > .05$ ) between the two groups (Table 3). Research has shown that laying hens housed in cages often develop brittle and weak bones more often than hens maintained in floor pens (Rowland and Harms, 1970). Furthermore, layers kept on litter floors had higher tibia and humerus breaking strength and percent bone ash than birds maintained in cages (Meyer and Sunde, 1974). In contrast, broilers and laying hens reared in cages and floor pens exhibited no significant difference in percent ash of the tibia (Bond et al., 1991).

The role of exercise in preventing bone loss in humans has long been an area of speculation among researchers. There is some support, however, for the concept that disuse results in bone mass loss and that sedentary individuals have less bone mass than those that exercise. Chestnut (1993) compiled several studies demonstrating that exercising women had higher lumbar bone mineral density (BMD) than sedentary individuals.

Concerning animals, prolonged exercise in the rat had positive effects on mineral density in the femur but not the rib in which bone loss was induced by metabolic acidosis (Myburgh et al., 1989). In older female rats (25 mo) and mature mice, McDonald et al. (1986) found that bone mass and mineral content were higher in exercised versus sedentary animals. The previous study further demonstrated that exercising female rats (7-, 14- and 19-mo age groups) had a significantly higher concentration of calcium and greater  $^{45}\text{Ca}$  uptake in the femur and humerus as compared with their respective controls. It is also interesting to note that exercise in the younger rats (7 and 14 mo) increased mineralization of only the weight bearing bones (femur and humerus) while the older animals (19 mo) experienced an additional increase in bone mineral content of the rib in response to exercise.

In conclusion, the biopsy technique described in Experiment II may be helpful in diagnosing metabolic and nutritionally induced bone disorders. Results from these two experiments also indicate that bone biopsies taken from the rib could prove to be a useful diagnostic tool in evaluating the mineral status in the horse.

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Ingredient	% Diet DM	Nutrient	% Diet DM
Corn	66.95	CP	15.00
Soybean meal	15.00	DE	3.40
Cottonseed hulls	16.05	Ca	.52
TM salt	.49	P	.45
Limestone	.79	Mg	.18
Dical phosphate	.72	K	.65
		S	.16
		Na	.22
		Cl	.36

Biopsy site	AWW <sup>b</sup>	DFP <sup>c</sup>	Calcium	Phosphorus
Metacarpal	65.1 <sup>d±</sup> .64	69.5 <sup>d±</sup> .40	42.4± 1.09	14.7 <sup>d±</sup> .12
Rib	52.7 <sup>e±</sup> .64	63.0 <sup>e±</sup> .40	42.0± 1.09	13.1 <sup>e±</sup> .12

<sup>a</sup>Least squares means ± SE.  
<sup>b</sup>AWW= Ash as a percent of wet weight.  
<sup>c</sup>DFP=Dry, fat-free ash percentage.  
<sup>d,e</sup> Means within columns with different superscripts differ (P<.0001).

Item <sup>b</sup>	Control	Treatment
Ash	38.6± 2.6	40.9± 2.6
Calcium	39.7± 1.8	36.2± 1.8
Phosphorus		

	20.6± 1.2	20.0± 1.2
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<sup>a</sup>Least squares means ± SE.

<sup>b</sup>Means within a row do not differ (P>.05).

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