

Validation of the use of radiographic bone aluminum equivalence to measure bone mineral content in the equine coffin bone

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Abstract

Musculoskeletal injuries are the most common cause of lost training days in the athletic horse. Catastrophic bone failure is the most devastating of these injuries, and is a leading reason for racetrack euthanasia. As a result, interventions focusing on increasing bone quality in the equine athlete are the subject of much research. Techniques for non-invasive and economical measurement of bone health are lacking. One such technique involves the use of digital radiography, where optical density from the radiograph is compared to that of a known density of aluminum. This technique, known as radiographic bone aluminum equivalence (RBAE), has been used to measure bone mineral density (BMD) in the equine third metatarsal (cannon bone). However, this technique so far has been limited to only the cannon bone and, therefore, is in need of validation, in terms of reliability and repeatability, for other locations.

This study began the validation of the use of Radiographic Bone Aluminum Equivalence (RBAE) on the equine coffin bone, as a non-invasive estimate of BMD. The first objective was simply to determine repeatability. To evaluate bone density in the equine coffin bone (distal phalanges), digital radiographs were taken of equine coffin bones from at least 20 cadaver limbs. The dorsal and palmar locations on the bone were chosen to determine RBAE according to previously published techniques. Each location was analyzed independently two times to determine the coefficient of variation of the technique, as well as the relationship between the two locations. As a follow-up to this project, the coffin bones then will be harvested from the limb and subjected to ashing to ascertain the actual bone mineral content. This value from ashing will be correlated to the RBAE values in this study to determine the accuracy of the RBAE technique and help

identify the most accurate measurement location. This project provides an initial step into expanding our technique for non-invasive measurement of bone quality in the equine athlete.

Introduction

Many people have heard of the famous, and tragic, story of the racehorse Barbaro. He was an unforgettably adored horse, who performed at the top of his game, winning not only numerous races, but also the hearts of his fans. His quest for the 2006 Triple Crown ended with a painstaking halt when his thunderous admirers learned of the devastating news that their beloved racehorse suffered a catastrophic bone failure during the Preakness, the Triple Crown's second jewel. Despite prompt medical care at one of the nation's best facilities, Barbaro was euthanatized due to complications from his injury (supporting limb laminitis).

As exemplified in the story above, the musculoskeletal system of any horse must receive high priority in terms of attention and research. Inside this realm, bone density, or comparable amount of mineral content to square centimeters within a specific bone, is of vital importance, as it allows for an assessment of overall bone quality. Therefore, one of the main foci of any species' wellbeing should rest on developing, measuring, and sustaining appropriate musculoskeletal density, with great regard to the bones receiving the highest impact.

There are outside factors that can be considered as positive or negative influences on bone density, such as short amounts of vigorous and dynamic exercise, as they apply to all studied species. Simply put, Wolff's law indicates that bone will respond to forces placed upon it. Dr. Julius Wolff and others have shown that short amounts of high mechanical stress on bone increase BMD and resulting strength. At the same time, disuse results in reabsorption and weakening of bone, as the skeleton will only be as strong as its perceived need. Through his extensive investigations, Wolff realized that controlled

stress, then, changes both size and shape of bone, as bone reinforces itself in areas where stress is applied, a concept referred to as remodeling (Ruff et al., 2006).

So, in shifting this law to the equine model as it pertains to bone density, outside factors such as nutrition, housing, and types of exercise have been studied in order to discover what most influences a horse's bone mineral content, particularly in the lower limb area and even down into the hoof, where the highest amount of impact is made. While the earliest research often focused on nutritional interventions, a review by Nielsen and Spooner (2008) explains that changes in nutrition holds little value in terms of influencing bone strength, whereas short, high impact exercise, or a lack thereof, results in measurable changes in BMD.

A similar line of research shows that the observation of an adolescent equine's bone mineral content in relation to its age of initial training proves to be of high value in the performance horse industry. Numerous countries begin training potential athletic horses from an age as early as two, some of whom have been studied for the purpose of locating any detriment of the mineral content in the cannon bone. One study, in particular, exercised two-year-old Arabians at Michigan State University for 90 days. One group was put on an endurance-training format via treadmill, whereas the control group was not forced to exercise. They proceeded to measure bone density on each group and found that, as opposed to previous belief, gradual endurance training, in terms of BMD, produces no measureable change in BMD. This, again, agrees with Wolff's law, in that high-pressure force was not applied and, therefore, the bone did not appear to remodel as a result (Spooner et al., 2008). A third example determined that turning horses out on pasture sustains BMD, as opposed to permanent stalling, which reduces

BMC. At Michigan State University's Department of Animal Science, two groups of Arabians were either stalled or pastured for 140 days. The results concluded that there were lower RBAE values as well as lower markers of bone metabolism in the stalled group (Hoekstra et al., 1999).

Moving to exploring different techniques of measuring BMD, more common methods include, but are not limited to, computed tomography, assessing markers of bone metabolism and resorption, Dual-Energy X-Ray Absorptiometry, and RBAE. On the use of computed tomography, or CT scans, there are ample equine studies that have exercised this mechanism. One experiment, which studied the effect of age on the equine's radius and tibia, describes the technique and exactness of this procedure, explaining that, although the results from the CT agreed with the results of another method, these CT scans are not generally used in conjunction with live equines (Fürst et al., 2015). Their conclusions may stem from the fact that CT is expensive and inconvenient, seeing that sedation must be used in order to still the animal for capturing the best images, as well as being limited in availability to some of the nation's largest veterinary hospitals and universities.

Another mechanism is to evaluate markers of bone resorption or formation, which have also been used in research, and were reviewed in part by Nielsen and Spooner (2008). These markers include osteocalcin, a protein byproduct of the bone resorption process, as well as other markers of bone resorption, like pyridinoline, deoxypyridinoline, carboxy-terminal pyridinoline, and related markers of cartilage breakdown including keratin sulfate. However, complications arose while comparing these markers of bone studies, in that every marker study had conflicting results (2008).

The authors concluded that osteocalcin correlates most strongly with estimates of bone quality, and that all bone markers are most useful when comparing change over time in an equine as opposed to comparing baseline readings between or among equines.

Dual-Energy X-Ray Absorptiometry (DXA) is another possible non-invasive technique to measure BMD. However, as expressed by Stone and Turner (2012), this method comes with inconveniences as well. While exploring the ways in which DXA has been used, in humans and animals, this paper concludes that this method is highly expensive to purchase and maintain. Additionally, the units themselves are not durable.

Current research also exists that uses RBAE as a relative determinant of equine bone density. The initial study that developed the technique was done on foals' cannon bones in order to track the mineral content during the 120-470 days of their life (Meakim et al., 1981). Briefly, this technique compares the optical density of the bone to that of the optical density of a known density of aluminum that is placed on the same radiographic cassette, thus standardizing the image. The experiment by Meakim and colleagues (1981) showed high correlation between the values obtained by RBAE and the ashed BMC of the bone.

Since this initial report, RBAE has been utilized in several other equine studies because of its ease of use. In one such example, RBAE was used to examine changes in BMC in juvenile quarter horses subjected to short-duration exercise, as opposed to stalled or pastured horses with no exercise. Horses with forced exercise tended to show higher estimates of bone mineral content (Hiney et al., 2004). The authors praised the RBAE technique's ease of use and repeatability. So, although RBAE continues to be widely accepted for determining BMC, which is then believed to be reflective of whole body

bone quality, it still needs proof of consistency and repeatability in other bones. Specifically, no study has used the RBAE technique on equine coffin bone.

The coffin bone possesses a distinct and vital function to the equine's form and function. This bone is the closest to the ground, and the one that absorbs the most shock in any equine sport. Therefore, safeguarding this facet on the animal is highly important. While injuries involving the equine coffin bone are not unheard of, they typically do not result in catastrophic bone failure. Instead, problems may be more chronic in nature. Still, improving density of this structure may result in a reduction in lost training days across all performance disciplines. Furthermore, the coffin bone lies nearest the equine navicular bone. Equine navicular syndrome is one of the most diagnosed, yet least understood, ailments of the performance horse. In its easiest definition, navicular syndrome is simply heel pain. However, such pain is multi-faceted and may have many causes, including reduction in bone mineral content of the navicular bone (Turner, 1989). As the navicular is difficult to assess radiographically, the coffin bone may serve as a representative model, particularly as it relates to bone density as the bones can be expected to undergo similar stresses.

Along these lines, Middle Tennessee State University's equine laboratory has been investigating vibration plate therapy as a potential means of increasing, or at least sustaining, bone density. It is highly arguable that the most impacted bones vibration plate therapy, would be the coffin bone and navicular bone, as they rest closest to the ground, or plate, and therefore absorb the most shock. Having a technique like RBAE to evaluate bone density in a location on this distal limb could allow us to evaluate BMD in that bone, compared to an additional location more proximal on the limb.

Based on its previous success and ease of use, RBAE seems to be an efficient and inexpensive option for estimating BMD in the equine coffin bone, should it prove to be reliable. Therefore, it is the purpose of this particular experiment to ensure that an RBAE will be repeatable to estimate BMD in two locations on the bone. Thus, it shall theoretically progress the further research of this vital bone in performance horses around the world and, consequently, improve the equine industry as a whole.

Statement of Thesis Objective and Hypothesis

The objective of this project was to begin validating the use of Radiographic Bone Aluminum Equivalence (RBAE) on the equine coffin bone as a non-invasive estimate of BMD. To do so, we evaluated repeatability and correlation between two measured locations on the bone. It was hypothesized that the use of RBAE to estimate bone mineral content in equine coffin bone will be highly repeatable in at least one location (mean CV less than 5%) with significant correlation between two measurement locations ($r^2 > 0.9$).

Materials and Methods

Twenty frozen intact horse forelimbs were obtained from the Tennessee Veterinary Medical Diagnostic lab. Radiographs were taken with an aluminum stepwedge penetrometer attached to each radiographic cassette. A medial-lateral view was taken with the cassette placed medially and the beam centered on the midpoint of the coronet band (hoof- hairline junction). The x-ray was set to 70 kV with an exposure of 0.16 seconds and focal length of 90 cm. The penetrometer was used to standardize the readings from each radiograph. RBAE utilizes the known density of the aluminum stepwedge to determine the density of the bone in aluminum equivalents, based on the optical density of the aluminum stepwedge. Radiographs were analyzed using Bio-Rad Quantity One software to form a regression model using the optical density and the known thickness of the aluminum stepwedge, via the method of O'Connor-Robison and Nielsen (2013). Final measurements are recorded as mm Al. Two locations were identified for RBAE analysis (Figure 1). The first location (Dorsal) was the midway point between the top of the coffin bone near the coronary band and the bottom of the bone near the toe, while the second location (Palmar) was the midway point between the front of the bone near the toe and the back of the bone, near the navicular bone. RBAE was determined for each location twice, independently. Coefficient of variation and correlation were determined using the PROC CORR function of SAS 9.0.

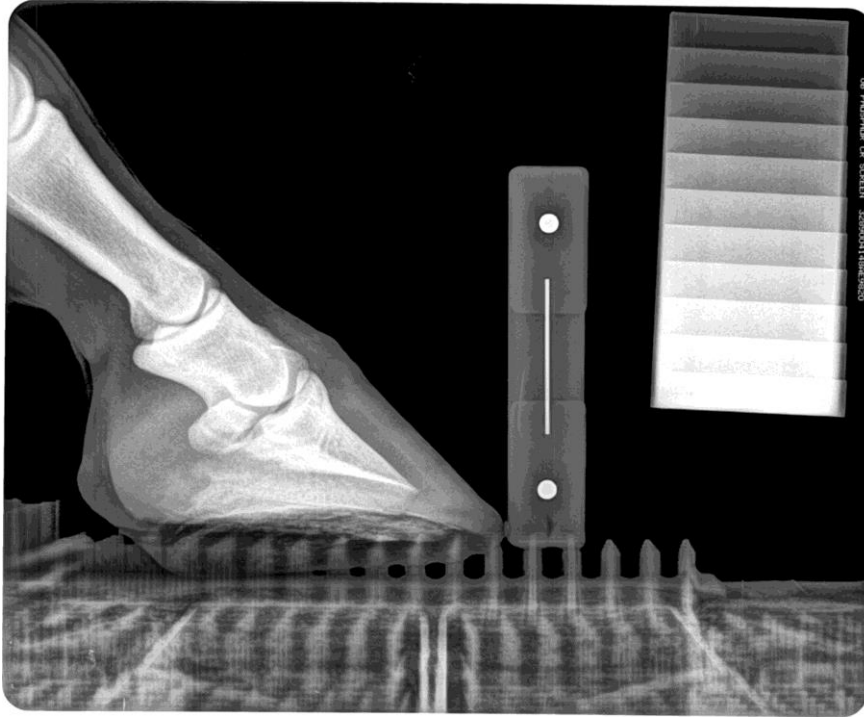


Fig 1. Radiographic image of the equine coffin bone, indicating locations of assessment (Proximal, Dorsal) via Radiographic Bone Aluminum Equivalence. Shown to the far right of the image is the aluminum stepwedge attached to each image for assessment and standardization.

Results

The mean coefficient of variation for the dorsal point is 3.15%, range (0.15-6.7%). The mean coefficient of variation for the palmar point is 3.55%, range (0.05-13.79%). Correlation between points is significant ($p < 0.05$), with $R^2 = 0.45$.

It is also valid to note that the observer found the procedure quick to learn and to complete for each radiograph. Analysis of two points on each radiograph averaged less than 5 minutes total duration, leading credence to the technique as being easy to complete.

Discussion

The results of this experiment supported our hypothesis in that radiographic bone aluminum equivalence is repeatable for both the dorsal and palmar locations. The mean CV for both locations was less than 3.6%. The two points also showed significant correlation, which was both ideal and expected. Bone density was likely highly correlated throughout a bone, especially if the areas received equivalent strain. Perhaps it would have been interesting also to have compared the values to values obtained from the cannon bone, to identify correlation between the bones. However, utilizing cadaver limbs in this experiment, this was not possible as the limbs were severed just above the fetlock, quite low onto the cannon bone.

These results were also compared to the original RBAE experiment done by Meakim. For repeatability of the scans, his CV ranged from .80 to 5.63 percent, and this experiment's range was from 0.05-13.79 percent (1981).

Furthermore, the radiographic bone aluminum equivalence was inexpensive and easily accessible, as it merely required digital radiography equipment standard in veterinary medicine with the addition of an inexpensive aluminum stepwedge, was straightforward to use, and calculated the results efficiently. When compared to the expensive DXA, the inconsistent results from the markers of bone studies, or the required use of sedation for CT scans, RBAE appears to be the best technique to non-invasively gather BMD of equines' skeletal systems.

Now, going forward from this initial step of the experiment, this method has the potential to be verified even further through comparing the results of the RBAE to the known bone mineral content of the bone, via ashing the bone. The Meakim experiment

(1981) compared the cannon bones' RBAE results to ashing results, and explained the ashing procedure. The ashing process begins by carefully extracting the bone from the leg, avoiding any damage or impairment. The bones are dried, weighed, and heated until turning to ash, which was then weighed (1981). These steps could be a potential progression of this study for the coffin bone.

Once this method is finalized, RBAE can help aid larger studies of bone, since it is straightforward to use, efficient, and inexpensive. For example the current vibration plate therapy project, underway at Middle Tennessee State University's Horse Science Center, would greatly benefit from this technique, to measure BMD in distal bones of the equine limb. The validation of the RBAE on the coffin bone is of great benefit to the MTSU study, since this bone is the closest to the ground as well as to the vibration plate; and, therefore, it receives the most impact from the whole body vibration plate as well as during the exercise. This project then has added another tool to our toolbox of non-invasively assessing bone in the equine athlete and thus may contribute to the greater goal of preventing injury in the horse.

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