INFLUENCE OF WHOLE BODY VIBRATION ON BONE DENSITY IN THE STALLED HORSE

By

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ABSTRACT

High frequency mechanical accelerations resulting from whole body vibration (WBV) have a strong osteogenic effect. It is hypothesized that WBV will maintain bone density in stalled horses equal to that of a stalled control group receiving light exercise. Radiographs of the third metacarpal were taken on d 0, 28, 56 of a stalled control receiving light exercise (n=6), and a stalled treatment group receiving WBV (n=6) for determination of bone mineral content (BMC) via radiographic bone aluminum equivalence (RBAE). No differences were observed in BMC due to treatment in the medial (P = 0 .98), lateral (P = 0.93), dorsal (P = 0.69), or palmar (P = 0.90) cortices. These results suggest that this level of WBV did not increase BMC, but maintained a baseline level similar to that of a horse in low intensity exercise, thus vibration therapy should be considered for horses subjected to stalling without exercise.

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REVIEW OF LITERATURE

The most difficult factor to assess in the conditioning and training of a horse is skeletal strength. It can be challenging to predict an issue in the skeleton until a problem actually presents itself. Once an injury or skeletal weakness is evident it is often too late to prevent a problem from occurring. As lameness is the most common cause of lost training days, prevention or early detection is key in maintaining optimal performance. In the worst cases, catastrophic bone failure can occur, and is the leading cause of death for horses at the racetrack. In fact 84.6% of horses necropsied as a result of death at a racetrack had musculoskeletal injuries (Johnson et al., 1993). Often these injuries are immediate and catastrophic, thus posing a significant welfare issue in the racehorse industry.

A thorough understanding of the skeletal system is important in designing exercise interventions and training protocols to reduce the risk of injury in the equine athlete, with further implication for animal welfare. It is important to understand the physiology of bone and how it responds to various stimuli in order to better understand bone failure and ways to prevent it.

The Skeleton

The skeleton serves the purpose of mechanical support, protection of vital soft tissues, to comprise the locomotor system for movement, and for mineral reserves. What makes bone stand apart from other tissues in the body is the fact that it is stiff yet flexible. Stiffness can be attributed to the bone mineral content (BMC) of the bone. Largely made up of inorganic calcium phosphate (hydroxyapatite) and collagen, these materials form the portion of the bone known as mineralized osseous tissue, often considered to be the bone's cement. The stiffness of bone then is in direct correlation to its bone mineral content. While bone is stiff it is also dynamic and can adapt according to prevailing demands of activity. In order to do this the bone must be able to sense demands of locomotor activity, the relationship of those demands to the current state of the bone, and to increase or decrease bone strength accordingly for safe function.

Overloading can be defined as an increase in activity from that to which the skeleton is currently adapted and disuse is a reduction in activity from which the skeleton is currently adapted. These are not assessable by just level of activity because one individual may lose bone under one level of a certain activity whereas another may gain it under the same conditions. The level at which bone would be overloaded depends on what the individual and their bones are adapted to on a regular basis. (Skerry et al., 2008)

Overloading and disuse need to be assessed for each individual but are affected by magnitude and rate of strain. What causes these changes/responses? There are several factors that can influence bon strength. Gender affects bone, as well as changes in environment, genetics, age, nutrition, and other systemic biochemical influences (Skerry et al., 2008). Of all these effects, loading has the greatest impact. A major cause of changes in bone is due to stimuli placed upon it. When activity is increased, causing high strain stimuli, there is an increase in mass or changes in architecture to increase bone strength. When there is less strain this can cause bone loss and reduction in bone strength (Skerry et al., 2008).

Bone Strain

Bones deform in response to loads or forces placed on them. The term strain is used to define how much deformation occurs in response to the application of force.

When strain is placed upon the top of a bone shortening will occur. Bones normally experience a combination of compressive, tensile, and torsional strain. Human limbs can normally withstand loads that deform the bone by 3-4x that of the strain during peak physiological activity (Skerry et al., 2008). Horses can withstand a much higher level of loading before failure than some other species. Horses are repeatedly stressing their bones, but this can be accepted if the rate of healing and modification in the bone is not overcome.

Mechanical strain can be expressed as change in length to original length and is measured in microstrains. The tibia of the horse begins to show microdamage at 6,720 microstrain with failure occurring at 15,860 microstrain (Rubin et al., 1982). For example, 1% deformation would be 10,000 microstrain units. The larger the deformations in the bone the greater the increase in bone mass because deformations stimulate increases in bone mass. Normal strains cause volumetric changes and shear strains cause angular deformations and thus bone tissue can differentiate between the different kinds of deformation (Judex et al., 2009). Only normal strains were seen to increase the degree of intracortical turnover (Rubin et al., 1996), meaning bone responds to normal, not shear strains, by increasing the remodeling of the bone. Strain magnitude determines bone mass but only dynamic strains have osteogenic potential effects. For example, when static loading applied at the same strain magnitude that would produce formation when applied dynamically produces a disuse remodeling response and causes bone resorption (Hert et al., 1971). Therefore, it can be inferred that mechanical intervention loads should be applied rapidly.

Strain rates affect the response of the bone, and beyond a critical value increased strain will result in lower strain to failure and energy absorbing capacity. The third metacarpal of healthy thoroughbreds with no history of skeletal injuries were taken and subjected to 6 different strain rates via a pair of pneumatic grips. It was found that there was an increased energy absorption capacity with increasing strain rate up to a critical point. Other studies have found this point to be at around .1 sec ^-1, but this study suggests the transition point may be lower and actually be more of a range than a specific point. Several factors contribute to this failure point, including bone mineral content and microstructure (Evans et al., 1992).

Decrease in bone mass can occur due to decreased loads on the bone, and conversely increase in bone mass can occur due to increased loads on the bone. This is due to the bones natural response to adapt to loads placed upon it, known as Wolff's Law. For example, if a horse is left with minimal movement space in a stall there is very little load on the bone due to lack of high strain loading. This is of concern because it may diminish an animal's resistance to injury if they are confined to a stall for a long period of time and then resume training. This was examined in a study conducted by Porr et al. (2008). In the study designed to evaluate the influence of stalling on bone mineral content, eleven fit horses were deconditioned (walked at 1.1m/s for 60 min daily for 4 months) and BMC measured using radiographs by radiographic photometry of the left third metacarpal. During the deconditioning period BMC decreased in the medial diaphysis ($P = 0.002$), medial metaphysis ($P = 0.006$), and lateral metaphysis ($P = 0.015$). Bone mineral content decreased in the metaphysis due to decreases in mechanical stimulation, which impact the amount of strain and rate of remodeling (Porr et al., 1998).

Strain events that exceed those normally experienced produce increased bone density. Such increased bone density was seen by Nielsen et al. with increased radiographic bone aluminum equivalent (RBAE) values in non-injured racehorses as compared to injured racehorses with lower RBAE values ($P < 0.05$). Bone density from exercised horses was greater than non-exercised horses ($P < 0.02$) in another study by Firth.

In another study 17 Arabians were used to demonstrate that bone adapts to forces placed upon it and that stalling has a negative effect on bone density. 6 horses were on full pasture turnout, 5 were kept in stalls, and 6 spent half their time on pasture and half in stalls for day 28-56 of the study. Radiographs were taken on day 0, 28, and 56. Wither, hip height, body weight, and cannon circumference were also taken every 28 days. It was found that the medial RBAE for the pasture and partial pasture group both increased significantly over 56 days ($P = .06$ and .02 respectively). The stalled group had a lower RBAE of the left third metacarpal on day 28, and was lower than the partial pasture group on day 56. The lateral RBAE for the pasture group increased overall ($P = .001$). The partial pasture group had a total lower RBAE than the pasture group, and the stalled group was yet even lower. The pasture and partial pasture groups total RBAE were greater than the stalled group. In addition to the RBAE measurements, the pasture and partial pasture groups showed increased cannon bone circumference at the end of 56 days and the stalled group did not increase. It can be concluded that stalling without exercise prevents maximal mineral deposition and that 12 hour daily turnout is enough to prevent a decrease in bone mineral content (Bell et al., 2011).

Bone Remodeling

Remodeling occurs in order to repair microdamage. The bone must be able to remodel itself to accommodate for different loads put upon it that exceed the normal for that bone.

The bone is able to change via modeling and remodeling. Modeling can be defined as alterations in size and shape of bone and is most active during long bone growth or mechanical loading. This alters the amount of bone present and determines its form. Bone remodeling is different in that there is no net change in the amount of bone and is occurring constantly in bone and occurs only at the surface via resorption and deposition of mineral. Remodeling includes five stages of quiescence, activation, resorption, reversal, formation, and back to quiescence. During the activation stage new osteoclasts are recruited to penetrate the cellular and connective tissue barrier. Next resorption can occur to remove bone that was stressed or injured. Finally the lacunae can be filled with new bone. Remodeling is a method of alteration that is necessary to combat and prevent microdamage from accumulating to dangerous levels. The resorption period is one of concern because during this time density is decreased and porosity increases. When this happens bone strength is decreased and there is a greater chance of fatigue failure. Fatigue was observed in the Nielsen et al. 1997 study of Quarter Horses in racing training where the majority of bone related injuries occurred when the RBAE was lowest. Thus it can be concluded that injuries observed may have been due to not being able to replace lost bone mineral as fast as horses that did not incur injury. Therefore, young horses with a greater initial bone mass and greater ability to increase the amount of bone may be less likely to have a skeletal injury (Nielsen et al., 1997).

The objective of a study done by Nielsen et al. (1997) was to "characterize the pattern of bone demineralization and remineralization" in 53 racing quarter horses. The horses were put into an 8-week training and then an 18-week racing period. Radiographs were taken of the left third metacarpal 86 days prior to training while the horses were on pasture, and on days 0, 62, 104, and 244 during the training. Radiographs were analyzed using radiographic bone aluminum equivalence (RBAE) to determine bone mineral content. Any horses that received non-training or soft tissue injuries were not evaluated. The remaining 43 horses were divided into groups of horses experiencing a bone related injury and horses that completed the project without injury. An increase in mineral content was seen normal for growth throughout the study. The bone density decreased by day 64 of training and remained low until day 244. This decrease in bone density was probably the result of change in bone remodeling during which the damaged tissue was removed to increase the strength of the skeleton. The medial RBAE was overall higher in the non-injured horses group than in the group that had a bone related injury ($P < 0.05$). Also, non-injured horses had a greater initial RBAE value for the lateral and medial cortices at day 0 than the group experiencing bone related injuries (Nielsen et al., 1997). Overall this study shows that bone density is directly related to bone injury. Horses with a higher density are less likely to become injured. Therefore, it is important to consider this factor in training programs.

Another study that demonstrated increased loading increases BMD used 15 thoroughbred yearlings tracked as they began training. Dorsopalmar radiographs of the third metacarpal bone were taken monthly to measure BMC via RBAE. BMC dropped from day 28-84 while the horses were confined to stalls and light exercise. Once training intensity was increased at day 84 BMC levels increased which suggests increased bone formation. Plasma calcium levels also increased once intense training started. This serves to show the important relationship between loading and bone growth (Pagan et al., 2008).

An additional study done by Firth (1999) further showed that galloping exercise produces an adaptive remodeling response in the third metacarpal and radial carpal bones of horses. Twelve 18-month old thoroughbred mares were split into a control or exercise group. The exercise group galloped on a high-speed treadmill 3 times weekly and trotted for 10 min 3 times weekly on a horse walker and walked 40 min 6 times a week. The control group only walked for 40 min a day 6 times a week. Tissues and bones were harvested immediately following the last exercise session. A sagittal slab was cut from the Cr directly opposite the radial facet slab and the thickness was measured using a slide caliper. Each bone was radiographed for increased bone density. Results showed that there was greater radiodensity in the exercised bones. BMD was also increased in the same areas as the increased radiodensity (Firth et al., 1999). Therefore, there is further evidence that loading, especially via exercise, affects bone growth and thickness. Exercised bones will be thicker than those not experiencing loading.

But how much exercise is necessary? It is still unknown exactly what speed of exercise is needed to produce a positive effect on bone. Spooner et al. (2008) predicted endurance training would not alter bone mineral content. Six Arabian geldings were exercised on a high-speed treadmill for 90 days at increasing distances. After 90 days they were put on dry lots and ran a 60 km endurance test every three weeks and also exercised twice weekly on a free flow exerciser. Control horses (5 fillies) remained on pasture. Overall no differences were seen between treatments or days as analyzed by

RBAE. This shows that slow long distance training will not increase bone strength (Spooner et al., 2008).

Vibration Therapy Effects

Overall, it is known that bone is not a static structure, but rather a dynamic tissue that is always changing due to strain and loading via modeling and remodeling. Positive remodeling occurs when strain over the normal threshold of what the individual is used to experiencing occurs. So why is there such interest in low magnitude, high frequency vibrations? Although low magnitude, it is suggested that the high frequency strain rate is still considered outside of the normal threshold that mammals are used to experiencing, and thus still can have the same overloading result as high impact strains. There has been evidence in humans and other mammals that bone remodeling does not necessarily require high impact strain, but that low impact high frequency strain can also stimulate bone growth. This is especially relevant in horses that cannot be exercised, such as during recovery from an injury, as a mechanism for maintaining or improving bone density.

High magnitude mechanical signals are not necessary to produce a positive change. Even low-magnitude mechanical signals can have an anabolic affect (Judex et al., 2009). Mechanical signals can positively affect physiological processes; it's just a matter of which parts of the loading are anabolic. How much the signal affects the bone morphology is dependent on the character of the signal. For example, bone morphology can change due to long-term exercise, as was seen in professional tennis players who had increased thickness of the cortical wall of the humerus in their dominant playing arm. Vibration therapy has been seen to produce bone and decrease resorption. When mice were subjected to daily periods of whole body vibration at 0.3g and 45hz bone formation rates were increased by 32%. Mechanical signals can serve as a safe treatment for growth factors in bone that is non-invasive and non-pharmacological (Judex et al., 2009).

The effects of vibration therapy on bone diminish with distance from the interface due to the mechanical signal damped by the soft tissues. There is a large continuum of strain levels. Once you reach a level lower than what constitutes disuse in the bone, these lower strains will cause a decrease in bone density. It is unclear how vibrations relate to the physiology of loading or disuse but possible mechanisms could be that low strain high frequency vibration could be another set of parameters that can be considered an overload from normal activity (Skerry et al., 2008).

Rubin et al. (2006) looked at the effect of vertical vibration on the hind limbs of sheep. The sheep were put on the vibrating platform for 20 min/day for 1 year and there was a control group that did not undergo any treatment. At the end of the study histomorphometric properties of their bones were compared and the treated group had increased quantity of bone in the distal femur (Rubin et al., 2006).

Further evidence that low mechanical signals at a high frequency have an anabolic effect was seen when 12 ovariectomized rats were subjected to 10 min/day of WBV at either 45 Hz (n = 6) or 90 Hz (n = 6). A control group (n = 6) did not receive any treatment. The goal of this study was to determine a WBV treatment that can prevent and counteract bone loss. After 28 days it was found that trabecular bone formation rates of the rats treated at 90 Hz was 159% (P = .02) greater that the control group and 206% (P = 0.01) greater than the 45 Hz treatment group. Also, endocortical bone formation was 31% higher (P = 0.10) in rats vibrated at 90 Hz and 40% greater (P = 0.05) than the control group. Judex et al. attributed this to greater mineral apposition rate, and greater percentage of bone surfaces mineralizing. It was thus determined that the efficacy of the signal was directly related to the frequency of the signal. The increase in bone growth was only significantly seen in the group vibrated at 90 Hz, not in the group at the lower amplitude (Judex et al., 2007).

In a human study that showed positive effects of WBV 70 women age 58-74 were split into a WBV group (n = 25), resistance training group (n = 22), and a control group (n = 23). The WBV group did static and dynamic knee extension exercise on a vibration platform at 35-40 Hz 3 times a week for 6 months. The resistance training group did knee extensions by dynamic leg press and leg extension exercises 3 times a week for 6 months, and the control group did not receive any training. Hip bone density was measured at baseline and after 6 months and was found that vibration therapy increased isometric and dynamic muscles strength ($P < .01$) and bone density of the hip increased ($P < .05$). The resistance training and control groups saw no change in hip bone density (Verschueren et al., 2004).

Very few studies have been performed to look at whether vibrations can stop bone loss or what level of loading at a low magnitude will increase bone density. This varies by species and individual but further research would be beneficial to investigate the anabolic effects of high frequency vibration on bone. WBV is a noninvasive, non-pharmacological therapy that could be used as a beneficial way to prevent bone loss or even stimulate an increase in bone density. This could be an effective way to prevent bone injuries in horses that must be confined to stalls, or to expedite the process in healing an injury, but further research is necessary to define the parameters of the potential anabolic effects in the horse.

INTRODUCTION

Bone is very important in that it provides support, protection, and locomotion and is unique because it is a dynamic stiff tissue that is constantly changing. This makes bone strength and condition very difficult to assess. Missed training time is often due to bone related injuries because problems often go undiagnosed until there are substantial injuries, such as a break or fracture.

It has been proposed and seen in other species that high frequency mechanical accelerations resulting from whole body vibration (WBV) have a strong osteogenic effect, increasing both quality and quantity of bone. Rubin et al. (2006) saw increased quality of bone in the hind limbs of sheep after being put on a vibrating platform for 20 min/day for 1 year. A group of women that stood on a vibration plate 3 times/week for 6 months also saw an increase in hip bone density (Verschueren et al., 2004).

Use of WBV has been growing in the equine industry and is anecdotally reported to positively impact the performance and health of the athletic horse, yet no research has been published to date to support such use. This project was developed to evaluate the impact of WBV on bone density in the stalled horse. It is hypothesized that WBV will maintain bone density in stalled horses, equal to that of a stalled control group receiving light exercise.

MATERIALS & METHODS

Twelve mature horses of mixed breed and age (17 ± 4 yr) under similar management were used to test the effects of whole body vibration on the bone density of stalled horses. All horses had radiographs of the left third metacarpal taken on day 0 for determination of bone mineral density. Both a dorsal-palmar and medial-lateral view were taken with the cassette placed medially and the beam centered on the midpoint of the third metacarpal parallel to the ground. The x-ray was set to 70 kV with an exposure of 0.16 seconds and focal length of 90 cm. An aluminum stepwedge penetrometer was attached to each radiograph.

All twelve horses were turned out on pasture fields for 28 d for free choice exercise to serve as a backgrounding period. On day 28 a second set of radiographs was taken and each horse was put in a stall 9.3 m^2 for an addition 28 d period. At that time, horses were randomly assigned to either the control (CON, n = 6) or treatment (VIB, n = 6) group. During the stalled period all horses were provided prairie grass hay and commercial pelleted concentrate (Purina Strategy) two times daily to maintain body condition. *Ad libitem* access to water was provided at all times throughout the study.

The CON horses worked on a mechanical panel exerciser for 60 min 6 d per week from day 28-56. The exercise protocol involved 17 min walk, 10 min trot, 3 min canter in both directions. At no time did the speed of the exerciser exceed 8 m/s, thus keeping them at low impact exercise. VIB horses stood tied on a WBV platform (Equivibe) set at 50 hertz for 45 min 5 times per wk from day 28-56. End radiographs were taken on d 56 and horses were returned to the MTSU Horse Science herd.

Bone mineral content of the third metacarpal was determined from the radiographs using radiographic bone aluminum equivalency (RBAE) for all bone cortices and total BMC, with measurements taken immediately distal to the nutrient foramen. Radiographs were taken with an aluminum stepwedge pentrometer attached to each radiographic cassette. The pentrometer 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. Radiographs were analyzed using Bio-Rad Quantity One software to form a regression model using the know thickness of the aluminum stepwedge via the method of O'Connor-Robison and Nielsen (2013). Final measurements are recorded as mm Al plus or minus standard error of the mean (SEM).

Changes in BMC were analyzed for day and treatment effects using a mixed model ANOVA with repeated measures in SAS 9.2. A P-value less than 0.05 was considered significant and trends were considered when P was less than 0.10.

RESULTS

No differences were observed in BMC due to treatment in the medial (*P* = 0 .98), lateral (*P* = 0.93), dorsal (*P* = 0.69), or palmar (*P* = 0.90) cortices (Table 1). No day effect or treatment by day differences were observed across any cortices. Data were normalized, by subtracting day 28 or 56 from day 0 to evaluate changes from baseline, and again no differences were observed due to treatment (Fig. 1-4).

Table 1- Radiographic Bone Aluminum Equivalent values (mm AL± SEM) of MCIII in horses subjected to stalling and either whole body vibration (VIB) or light exercise control (CON)

		Day 0	Day 28	Day 56	P-value
Medial	CON	35.03 ± 1.15	36.56 ± 1.15	36.02 ± 1.15	0.23
	VIB	34.95 ± 1.15	36.50 ± 1.15	35.5 3 ± 1.15	0.54
	P-value	0.96	0.97	0.77	
Lateral	CON	34.45 ± 1.66	35.43 ± 1.66	34.45 ± 1.66	0.81
	VIB	34.03 ± 1.66	33.97 ± 1.66	32.72 ± 1.66	0.72
	P-value	0.86	0.54	0.47	
Dorsal	CON	32.16 ± 1.42	34.79 ± 1.42	32.98 ± 1.42	0.40
	VIB	33.90 ± 1.42	33.89 ± 1.42	33.09 ± 1.42	1.00
	P-value	0.40	0.66	0.96	
Palmar	CON	30.92 ± 1.80	32.28 ± 1.80	30.79 ± 1.80	0.82
	VIB	32.71 ± 1.80	32.26 ± 1.80	31.48 ± 1.80	0.74
	P-value	0.49	0.99	0.79	

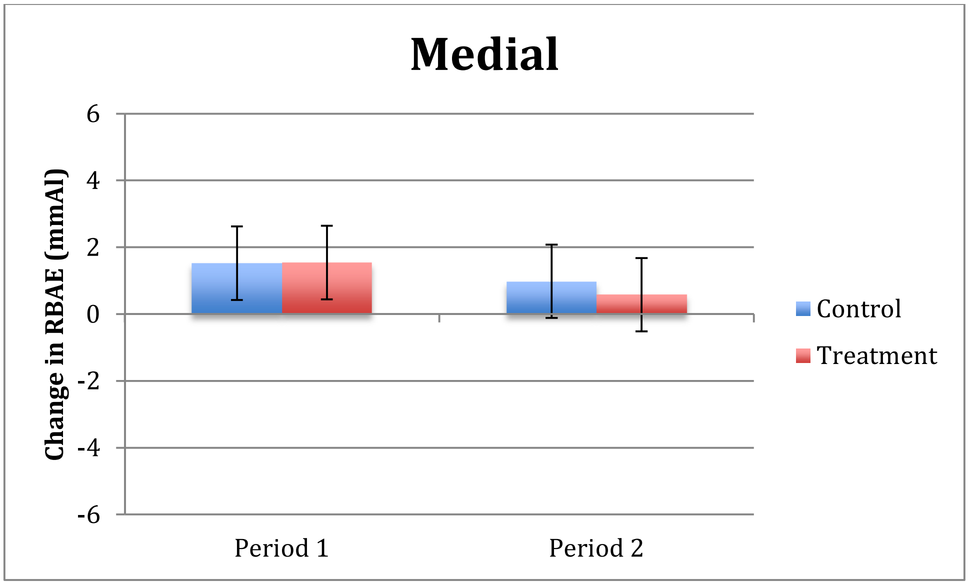

Figure 1. Changes from baseline of medial cortex RBAE (mm AL ± SEM) of horses subjected to stalling and either whole body vibration (Treatment) or light exercise (Control)

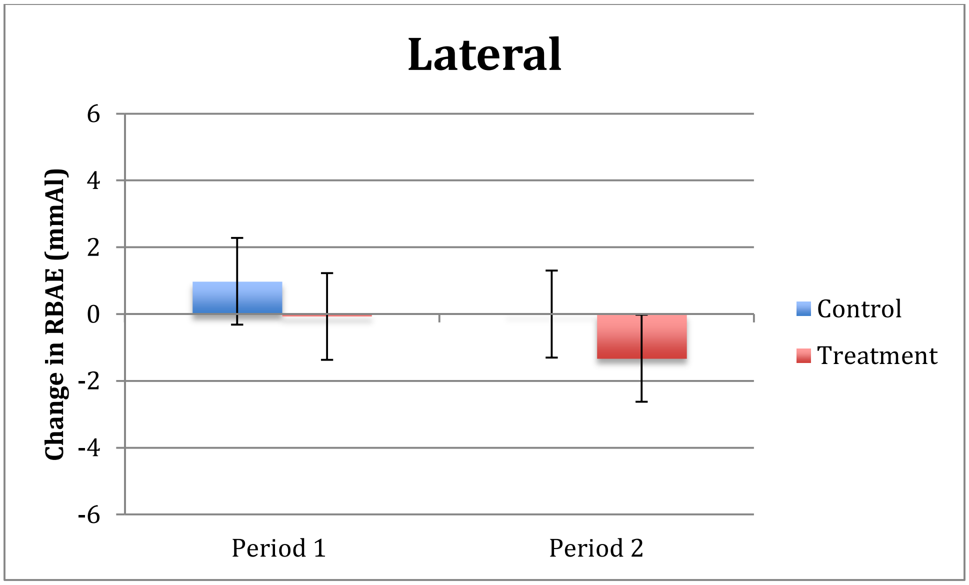

Figure 2. Changes from baseline of lateral cortex RBAE (mm AL ± SEM) of horses subjected to stalling and either whole body vibration (Treatment) or light exercise (Control)

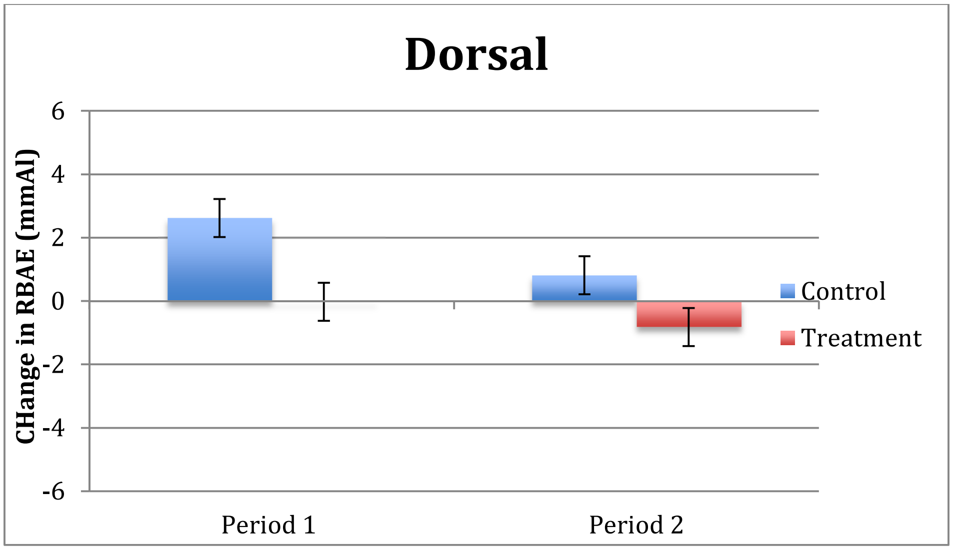

Figure 3. Changes from baseline of dorsal cortex RBAE (mm AL ± SEM) of horses subjected to stalling and either whole body vibration (Treatment) or light exercise (Control)

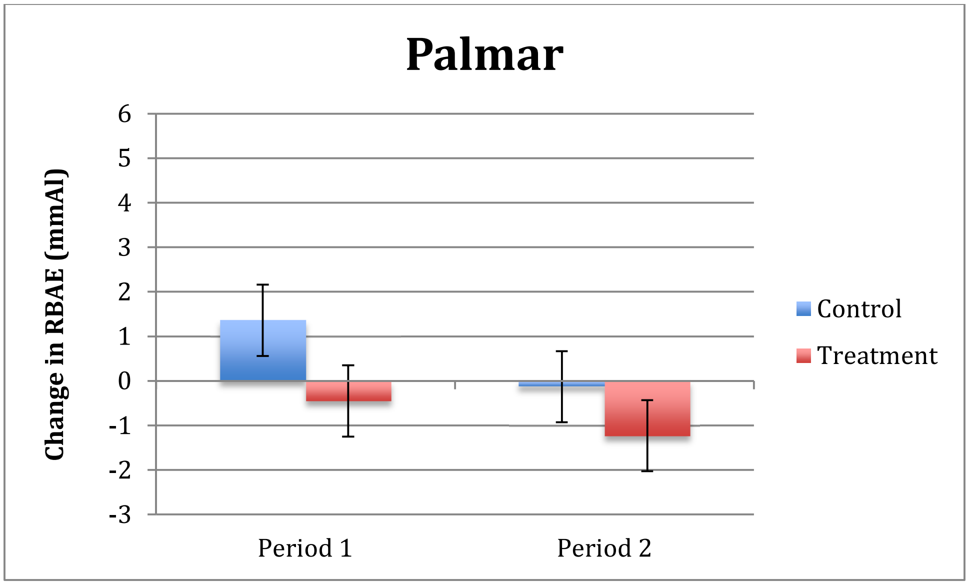

Figure 4. Changes from baseline of palmar cortex RBAE (mm AL ± SEM) of horses subjected to stalling and either whole body vibration (Treatment) or light exercise (Control)

CONCLUSION

Treating and assessing bone related injuries is of high importance since skeletal strength is one of the hardest factors to assess in the training of an athlete. It is difficult to identify or predict a skeletal issue until a problem actually presents itself, thus making it difficult to prevent bone related injuries. Preventing these injuries, such as breaks and fractures, is key in keeping a horse on track in its training. There are several factors that influence bone strength, such as loading and mechanical signals. It has been seen that loading, such as from exercise, increases bone density (Firth et al., 1999). The reverse is also true, in that lack of loading, such as in a stalled horse, decreases bone density (Bell et al., 2001). Normally it takes a change in the type of loading that the bone is adapted to in order to create a change in density, whether that be an increase in force or frequency. A decrease in bone mass is very detrimental in that it increases the chance of skeletal injury. Therefore, it would be beneficial to the industry and health of the athlete if there were a non-invasive, non- pharmacological treatment to aid in increasing the quality of bone. Whole body vibration has this potential but investigation is needed in the equine athlete.

The aim of this study was to evaluate the impact of WBV on bone density in the stalled horse. It was hypothesized that WBV would maintain bone density in stalled horses, equal to that of a stalled control group receiving light exercise. There were no differences in BMC seen in the medial, lateral, dorsal, or palmar cortices due to treatment. Even though there was no increase in BMC due to treatment, the horses did maintain the same level of BMC as when on pasture, or as in light exercise over the 28-d period of our study. This may indicate the use of vibration therapy when it is necessary to stall a horse without exercise, such as during recovery from an injury, so that BMC may be maintained, rather than decreased, and the horse may have a decreased risk of injury when put back into training.

Our results contrast the results seen by Judex et al. (2007) where mice had a 32% increase in bone formation rates when subjected to whole body vibration at 45 hz. In a later study by Judex et al. (2009) it was determined that there is a direct relation between signal frequency and efficacy on affecting bone growth. Perhaps this suggests that our vibration frequency was not vibrated at a high enough magnitude in order to produce a positive change in BMC since the mice were not vibrated at a frequency much lower than the horses, yet mice have a markedly smaller bone mass.

In a study done by Rubin et al. (2006) the hind limbs of sheep showed increased bone after a trial period of one year, standing on a vibrating platform for 20 min/day. This could mean that had our trial had a longer duration, positive effects on bone density may have occurred.

Further, we recognize the fact that there was no negative control group receiving neither exercise nor treatment. It would have been detrimental to the health and overall wellbeing of the horse to keep them in a stall for 28 days continuously. However, such a group may have show a reduction in bone density not observed in either the VIB or CON groups, adding additional evidence to support the use of vibration therapy to maintain BMC.

Further investigation is needed in whole body vibration as a treatment for the athletic horse. In previous studies, across species, there is a large discrepancy in treatment application. There is much variation in length of treatment, amount of time, and strength of vibration. Further research is needed to discover what that threshold level is that induces increased bone density. Perhaps research looking at specific bone markers, such as osteocalcin would also be useful in quantifying the effects of vibration on bone growth.

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APPENDICES

APPENDIX A: IUCAC APPROVAL

5/28/2014

Investigator(s) Name: Holly Spooner,Ph.D., John Haffner, DVM Investigator(s) Email: holly.spooner@mtsu.edu; john.haffner@mtsu.edu

Protocol Title: "Does vibration therapy alleviate bone density loss caused by stalling and/or influence kinematics of movement?" Protocol Number: 14-011

Dear Investigator,

The MTSU Institutional Animal Use and Care Committee has reviewed your research proposal identified above and has approved your research in accordance with PHS policy. Approval is granted for three (3) years. Your study expires 5/28/2017. Please note you will need to file a Progress Report annually regarding the status of your study and submit an end-of-project report.

According to MTSU Policy, an investigator is defined as anyone who has contact with animals for research purposes. Anyone meeting this definition needs to be listed on the protocol and needs to complete the IACUC training through citiprogram. If you add investigators to an approved project, please forward an updated list of investigators to the Office of Compliance before they begin to work on the project.

Any change to the protocol must be submitted to the IACUC before implementing this change. Any unanticipated harms to subjects or adverse events must be reported to the Office of Compliance at (615) 494-8918.

Also, all research materials must be retained by the PI or faculty advisor (if the PI is a student) for at least three (3) years after study completion. Should you have any questions or need additional information, please do not hesitate to contact me.

Sincerely,

Kellie Hilker Compliance Officer 615-494-8918 kellie.hilker@mtsu.edu