THE EFFECT OF WHOLE BODY VIBRATION ON BONE DENSITY AND OTHER PARAMETERS IN THE EXERCISING HORSE

by

Kayleigh E. Maher

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Thesis Committee:

Dr. Holly Spooner, Chair

Dr. Rhonda Hoffman

Dr. John Haffner

ABSTRACT

Maintenance of bone density has implications in preventing catastrophic bone failure in the horse, while whole body vibration (WBV) may have an osteogenic effect. It was hypothesized WBV would increase bone density in the exercising horse, with similar changes in markers of bone metabolism. Radiographs of the third metacarpal were taken of a control group (CON, n = 5), and a treatment group that received WBV (VIB, n = 6) to determine bone mineral content (BMC) via radiographic bone aluminum equivalence over a 28 d treatment period, while serum pyridinoline crosslinks (PYD) and plasma osteocalcin (OC) were analyzed throughout. No differences were observed in BMC due to treatment in the medial (P = 0.99), dorsal (P = 0.48), lateral (P = 0.38) or palmar (P = 0.45) cortices nor in PYD (P = 0.95) or OC (P = 0.70). These results suggest WBV had little impact on BMC or bone markers in the exercising horse.

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CHAPTER 1. LITERATURE REVIEW

The maintenance of bone density has been a topic of importance in research involving human health and physiology. Interventions to maintain bone density have been extensively researched in human and some animal models such as mice and rats (Oxlund et al. 2003; Stuermer et al. 2014; Wei et al. 2014). More recently, research in whole body vibration and its effect on bone density has become an important topic of research as an aid in maintaining or increasing bone density for many different reasons. Prevention of fractures in frail people, prevention and treatment for osteoporosis as well as skeletal injuries (Gusi et al, 2006; Verschueren et al, 2004) are a few areas that have been researched using whole body vibration. Whole body vibration has been used to improve bone density of postmenopausal women (Slatkovska et al. 2010; Verschueren et al. 2004;). Whole body vibration has also been shown to have an effect on endocrine functions as well as muscle development and balance which in turn can affect bone density in a positive way (Stuermer et al, 2014; Torvinen et al. 2002; Wei et al, 2014).

Injuries, lack of or inability to exercise, hormonal changes, and age are only a few factors that can have an effect on bone density. These same factors apply to animal models, especially the horse. Like humans and other animal models, horses require loading and adequate levels of strain in order to maintain bone density. Skeletal strength is one of the most difficult factors to assess in a horse especially in regards to training and conditioning. It is difficult to predict a possible issue in the skeleton until it presents itself. Too often a first assessment of skeletal strength takes place once an injury has occurred; this is when skeletal weakness becomes evident (Nielsen et al, 1997). Horses

on stall rest or minimal exercise for any reason can lose bone density rapidly, due to a decrease in loading (Bell et al., 2001; Carrier et al., 1997; Hoekstra et al., 1999; Porr et al., 1998). Thus finding ways to maintain skeletal strength prove important. The use of whole body vibration on horses is a fairly new concept but has readily gained industry acceptance for a variety of conditions, and may prove a useful intervention to maintain or increase skeletal strength.

This review will discuss bone growth, remodeling, density and the effects of whole body vibration on bone density and related variables including muscle strength, effects on locomotion and endocrine function.

Bone Growth and Remodeling

Bone is a tissue that is subjected to constant turnover. The constant change in bone is in response to mechanical loading as well as paracrine and endocrine factors (Sims and Gooi, 2008). Bone turnover can be described as a dynamic process of metabolic activities that work in balance with each other. Osteoclastic cells reabsorb the old bone and osteoblasts complete the apposition of newly formed bone (Lepage et al., 2001, Pagani et al., 2005). During bone growth and adaptations to specific changes in loading, a bone's shape and architecture change via cellular activity to remove and form bone. Remodeling is a set of actions that are co-coordinated between osteoclasts, osteoblasts and osteocytes (Sims and Gooi., 2008). Modeling includes alterations in size and shape of bone and takes place most often in young growing animals, during long bone growth or mechanical loading (Nielsen et al., 1997; Price et al., 1995).

Remodeling is how the dynamic nature of the skeleton is achieved. There are three phases of bone remodeling; initiation, transition and termination (Matsuo and Irie, 2008). The direction of communication between the initiation phase and the transition phase are opposite one another, from osteoblast to osteoclast precursors in the initiation phase, and from osteoclasts to osteoblasts in the transition phase. The initiation phase includes bone resorption and activation of osteoclasts. During the transition phase bone resorption is inhibited, osteoclasts undergo apoptosis and osteoblast cells are recruited. The part of the bone reabsorbed in the initiation phase is prepared for bone formation. Following the transition phase is the termination phase. This phase includes new bone formation, mineralization, and finally entry into quiescence (Matsuo and Irie, 2008). Because osteoblastic bone formation is a much slower process than bone resorption, the termination phase is much longer then the initiation phase.

In its simplest interpretation, damage stimulates bone to constantly change and grow. Within the matrix of the bone, microdamage occurs in response to repetitive loading cycles beyond a threshold level. According to Wolff's law, bone and its internal structures change in order to accommodate new stresses that are placed upon it (Prisby et al, 2008). How a bone remodels in response to its mechanical environment ensures that bone mass and architecture are able to withstand the often changing loads that are required throughout the life of the animal (Price et al., 1995). The continuation of microdamage however, can ultimately lead to a total fracture of the specific structure (Nielsen et al., 1997; Carrier et al., 1997). Understanding the effects of microdamage and bone remodeling are important to exercising horses.

Understanding methods for training a horse to provide the appropriate amount of loading and strain on the horse's bones can prevent potential fractures and other musculoskeletal injuries later on in life as well as to prevent wastage in horses entering training (Carrier et al., 1997; Nielsen et al., 1997; Porr et al., 1998). Nielsen et al (1997) characterized bone demineralization and remineralization in the third metacarpal, using radiographic measures of bone density in young Quarter Horses entering into training. The goal was to determine the relationship between changes in bone and injuries that occurred during the training process. The most optically dense portion of each medial cortex within the third metacarpal was examined in horses that completed the study without injury compared to horses that experienced an injury. Overall, the medial radiographic bone aluminum equivalent (RBAE) was higher in the non-injured group than in the group that had experienced some form of bone related injury during the study (Nielsen et al., 1997). The results of this study indicate the start of training initiated transient changes in remodeling and increased the amount of remodeling space or area on the bone where remodeling is taking place, which could cause a temporary increase in resorption of mineral. In theory the increase in resorption could decrease the ability to resist stress, which leaves the horse more susceptible at a critical time in training.

Although skeletal strength is hard to assess it would appear that skeletal mass is the best measurable determinant (Kimmel, 1993). A decrease in RBAE values reflected a decrease in stiffness and lowered resistance of the third metacarpal (Nielsen et al., 1997). The decrease in resistance makes the bone less able to handle strain. A young horse starting training may experience loads that exceed the rate of healing and modification,

this can be difficult for the young skeleton to handle at times. Microdamage can only be repaired via remodeling. As the workload increases the bone needs to be able to remodel itself in order to handle the new amount of strain that is being placed on it.

Bone Density in Equines

The inability of bone to adapt to its mechanical environment can lead to injury. Because the majority of bone modeling occurs in animals when they are younger, bone growth and deposition in horses that are still growing can be improved by their environment. In horses skeletal unloading has been associated with bone loss (Bell et al., 2001; Nielsen et al., 1997; Porr et al., 1998). Bone loss is due to the continuation of resorption and a lack of bone formation. Lameness in horses is one of the major causes of loss of training time (Oliver et al., 1997; Rossdale et al., 1985), which can lead to stall rest and inevitably bone loss. Bone mineral content of the third metacarpal decreased by 0.45% weekly throughout a 12-week deconditioning period (Porr et al., 1998). This loss of BMC can lead to an increased risk of skeletal injury, especially if a horse is put back into hard work right away.

In the racing industry, catastrophic injuries are the leading cause of track deaths (Johnson et al., 1994). Musculoskeletal injuries are the major cause of Thoroughbred fatalities in California (Carrier et al., 1997). A large number of the injuries observed in California racehorses are skeletal fractures. Carrier et al. (1997) examined the incidence of humeral fractures in relation to lay-ups in California Thoroughbred racehorses. The return to training and racing after a lay-up of 60 days or more predisposed horses to an

increased risk for humeral but not a pelvic fracture after returning back to work (Carrier et al., (1997).

Loading and strain are important factors on bone mineral content in the equine model. The effects of trotting and supplemental weight, i.e. increased loading, on the development of the metacarpal bone was explored using radiographs and RBAE over a 108 d period (Nielsen et al., 2002). The results showed an initial decrease in RBAE from the baseline to the pre-conditioning measurements. Because the horses were exercised counter clockwise, the greatest increase was observed in the left foreleg, which was the inside leg during exercise. The RBAE increased in both weight groups but remained low in the control group. The most notable difference was the decrease in RBAE in all treatment groups in the beginning of the study when horses were confined in stalls before the conditioning began. This decrease in RBAE again shows the importance of loading and weight bearing on bones.

Because bone is more adaptable in young growing horses, ensuring they get an early start in proper bone development is important to help prevent skeletal related injuries later in life (Hoekstra et al., 1999). Daily access to pasture has proven to increase bone mineral content in weanlings, compared to complete stalling (Bell et al., 2001). Both full time and 12 h per day pasture groups had greater total RBAE than the stalled group (Bell et al., 2001). Understanding how lay-ups and stalling for extended periods of time can affect the mineral content of a horse's bones is important in preventing possible injuries once the horse is put back into work.

Radiographic Measurements of Bone

Radiographic photodensitometry has been a standardized method to measure and track changes in bone mineral content in the distal limbs of horses. Radiographs are used to determine radiographic aluminum equivalent (RBAE) or the optical density as compared to the known density of aluminum (mm Al) for each cortex, using an aluminum stepwedge attached to the radiographic cassette. Radiographic aluminum equivalent also takes into account changes in bone density as well as overall changes in bone size (Meakim et al., 1981). In horse research, particular attention is paid to the third metacarpus, because the development of the RBAE method compared third metacarpal RBAE (mm Al) of equine cadaver legs to 2 cm section of ashed bone (BMC grams) from the same third metacarpal (Meakim el al., 1981). Optical density is plotted against mm Al and the equation of the subsequent line is determined. Previously radiographic photodensitometry methods used traditional film radiographs but in this digital age, a switch from traditional to digital radiographs has required a validation of digital technology compared to traditional radiographs (O'Connor-Robison and Nielsen, 2013). Because digital radiographs have an increased clarity, researchers and veterinarians are able to detect treatment differences as well as monitor changes that would be associated with nutrition and training.

Biochemical Measurements of Bone

Biochemical markers to assess bone turnover have developed over the past 20 years (Delmas et al., 2000). Today these markers are more sensitive to detecting abnormalities in bone turnover. There are bone markers to detect bone formation as well

as bone resorption. Alkaline phosphatase (ALP) plays an important role in osteoid formation and mineralization (Delmas et al., 2000). Total ALP serum originates from various tissues including liver, bone, spleen and kidney. ALP is important in determining liver function and bone disorders, with specific assays for bone-specific alkaline phosphatase having been developed. Osteocalcin (OC) is another important biochemical marker involved in bone formation. OC is synthesized by osteoblasts and contains gamma-carboxyglutamic acid which are responsible for calcium binding properties of the protein (Delmas et al., 2000). Serum OC is a specific marker used to assess osteoblast function. Serum or plasma can be used to quantify osteocalcin. Biochemical markers for bone resorption are degradation products of bone collagen. Both pyridinoline (PYD) and deoxypyridinoline (DPD) are important bone resorption markers. Both PYD and DPD are released when degradation of mature collagens takes place. The measurements of these two components show a high specificity for skeletal issues (Delmas et al., 2000).

Evaluation of bone formation as a measure of osteocalcin has proven useful in horses as well as humans (Brown et al., 1984; Lepage et al., 1990). Assessing biochemical markers in relation to bone mineral content in horses has become an easier process due to more sensitive assays. Osteocalcin can be a useful biochemical indicator of bone turnover. When osteocalcin is newly synthesized a fraction of it goes into circulation, which allows it to be measured in horses by an enzyme immunoassay. Age of horses can effect concentrations of osteocalcin. An inverse correlation has been seen between the age of the animal and the concentration of serum osteocalcin. There is a decrease in the first 30 months of life showing a slowdown in the rate of bone formation

in adult horses compared to foals. Lepage et al. (1990) found a significant inverse correlation between age and serum osteocalcin concentrations in horses, with serum osteocalcin concentrations being lowest in the oldest group. The decline in serum osteocalcin suggests a decrease in bone turnover as an animal ages, which leads to the necessity of loading on bones from a young age and the need for bone density maintenance.

Serum pyridinoline (PYD) assays provide a quantitative measure of pyridinoline crosslink excretion and can be measured either in horse urine or in the serum (Seibel et al., 1992). Immunoassays have made improvements in sensitivity, which result in the ability to measure free PYD levels in serum. The ability to measure free PYD allows for a novel method for researching bone and cartilage degradation. Bone and cartilage contain structural collagens such as type I and II and are cross linked with their alpha chains as well as between adjacent molecules. This helps to provide strength and rigidity to the collagen fibril that results. When bone or cartilage collagen is broken down or degraded, PYD is released into circulation and excreted in the urine.

Whole Body Vibration

It is known that bone is a very dynamic structure and is constantly changing via modeling and remodeling. When strain is over a certain threshold positive remodeling takes place. Strain is determined by how much something deforms due to the application of a force (Skerry, 2008). Many studies have shown the importance of strengthening the skeleton through exercise during the years of skeletal growth during adolescence and early adult hood. Early strengthening may be a promising way of reducing the likelihood

of skeletal fractures as aging occurs (Bell et al., 2001; Hoekstra et al., 1999; Nielsen et al., 1997; Xie et al., 2006). Exercise has been proven to increase bone formation, decrease bone resorption, raise peak bone mass and enhance bone strength not only in humans but animal models as well, specifically in equines. Although skeletal loading is important for skeletal strength it should be approached with caution. Specific aspects of mechanical loading can be harmful and can potentially cause skeletal issues. There is a great deal of interest in low-intensity, high frequency vibration and how it can influence strain and therefore bone formation. Many studies in humans as well as other mammals show evidence that bone remodeling does not necessarily require high impact strain to increase bone formation but that low-intensity high frequency strain can also stimulate bone growth (Gusi et al., 2006; Oxlund et al., 2002; Xie et al., 2006).

Whole body vibration (WBV) is currently used for many different reasons not only on humans but equines as well. One of the main uses for WBV is on postmenopausal women to help prevent osteoporosis and maintain bone density (Verschueren et al., 2004). The use of WBV has been proven effective in increasing bone density in mammals such as rats (Oxlund et al., 2002; Stuermer et al., 2014; Wei et al., 2014), but little research has documented its effects in equines.

The effect of low-level mechanical vibrations on bone resorption and formation in the growing skeleton of eight-week old female mice was researched by Xie et al. (2006). Compared to age-matched controls, mice that received 15 min per day of WBV treatment had lower osetoclastic activity in both the epiphyseal and the metaphyseal regions of the tibia by 30%. Osteoclastic activity in the metaphysis of the control mice remained

unchanged throughout the duration of the study but was decreased in WBV mice. The results of this study show that extremely low levels of mechanical signals have the ability to reduce the levels of trabecular bone resorption. The bone that was formed was the same quality as previously existing bone, which is important in any study examining bone growth and formation.

As people age, osteoporosis and other bone diseases become an increasing concern. In elderly people the incidence of fractures increases dramatically. Patients that experience a hip fracture have an increased rate of mortality by roughly 12-20%, compared to those of similar age and gender who do not suffer from a fracture (Autier et al., 2000). Verschueren et al. (2004) conducted a randomized controlled 6- month study examining the musculoskeletal effects of high-frequency WBV training in postmenopausal women, with a focus on the bone density of the hip. The results of this study showed that total hipbone mineral density (BMD) increased over time in the WBV group, but there were no changes in BMD in the resistance training group or the age-matched control group (Verschueren et al., 2004).

Along with their gain in BMD, women in the vibration group also showed improved recovery of balance after performing ballistic abduction of the arms and experienced an increase in muscle strength and a decline in fat mass (Verschueren et al., 2004). The changes that were seen in muscle mass were similar to changes observed in the resistance-training group. The gain in BMD during the 6-month study was shown to be statistically unrelated to the increase in isometric or dynamic strength. This suggests that the osteogenic effect that was observed was not mediated by reflexive muscle

contractions. This assumption is supported by the fact that the gain the resistance-training group saw in lower limb extension strength was not paralleled by an associated increase in bone density. The results of this study show a promising effect of WBV on not only bone density but its effect on muscle as well. This can be helpful for those who may not be able to complete load-bearing exercises, such as the elderly as well as in the equine industry with regards to skeletal injuries and rehabilitation.

WBV can assist in preventing problems such as osteoporosis without causing further strain or damage to the person or animal receiving it. Rubin et al. (2002) investigated the effects of mechanical intervention on the quantity and quality of trabecular bone in the femur. This specific study used the hind limbs of sheep and stimulated them with vertical oscillation on the cortex of the tibia for 20 min per day, 5 days per wk at 30 Hz. Bone quantity increased due to not only increasing the number of trabeculae but also an increase in the thickness of trabeculae that already existed (Rubin et al., 2002). The new trabeculae enhanced the stiffness and strength of the trabecular bone, which increases the quality of that bone. Low-level mechanical stimuli presented at a frequency that is similar to contractility of muscle, are strongly anabolic. The changes observed by Rubin et al. (2002) are promising indications of the efficacy of WBV with respect to treatment and prevention of diseases such as osteoporosis.

WBV may also have a hormonal effect, which in turn can also affect BMD. Previous studies have shown that gut-derived serotonin acts as an important regulatory factor in inhibiting an increase in the number of osteoblasts and well as an increase in bone formation (Inose et al., 2011; Modder et al., 2010). Higher levels of circulating

serotonin in humans increased bone turnover and reduced bone formation in the human model (Modder et al., 2010). Wei et al. (2014) used ovariectomized rats to investigate the effect of WBV on serum serotonin levels. The lack of estrogen in the ovariectomized rats resulted in an increase in circulating serum serotonin. The treatment with WBV reduced the level of circulating serum serotonin. This suggests WBV can be utilized as a non-invasive and pharmacological alternative to preventing bone loss and can be helpful for postmenopausal women who are experiencing a decrease in estrogen, which can lead to a change in serotonin levels.

The use of WBV in athletics has become very popular and is now a part of many training regimes for athletes (Torvinen et al., 2002). The same is true in the equine industry with the to-date untested notion that WBV has a positive impact on bone and muscle characteristics as well as stride length, blood flow and endocrine influences. The effects of WBV on not only bone but muscular performance and balance have been reported in several studies (Kawanabe et al., 2007; Prisby et al., 2008; Rehn et al., 2007; Torvinen et al., 2002; Verschueren et al., 2004). The effect of a short exposure to vibration on muscular performance and body balance of 16 healthy subjects showed that a single 4-min bout of vibration loading had a transient increase in isometric extension strength of the lower extremities, jump height and body balance (Torvinen et al., 2002). These results were observed 2 min after the vibration treatment but did decrease greatly by the 60 min mark. It is evident that a short bout of vibration is beneficial for physical performance, yet no similar studies to-date have been performed in the athletic horse model.

To date only one study has addressed WBV in the equine, when the effects of WBV were compared to a stalled group receiving light exercise (Hulak, 2015). There were no differences seen in BMC between WBV treated and lightly exercised horses, indicating that stall-rested horses receiving WBV treatment maintained bone density similar to horses in light exercise (Hulak, 2015).

In all, WBV is an extensive area of research that has been studied in various animal models as well as humans. It is known to have benefit in increasing bone mineral density specifically in osteoporosis. On the other hand, very few studies have addressed whether vibration treatments can stop or reduce bone loss. It is also unclear the level of vibration magnitude required for bone density to begin to increase. More research needs to be done in equines to determine the effects WBV has on other parameters such as anabolic effects and endocrine functions. WBV is a non-invasive, non-pharmacological alternative to many treatments regarding bone loss and maintenance. Going forward WBV could be an effective treatment and preventative method in bone injuries in horses. For horses that require stalling for rest or even those in work, WBV may be used to maintain bone density already acquired. Also examining the effects on muscle in regards to soreness and biomechanics (such as stride length) in horses would be very beneficial.

CHAPTER 2. THE EFFECT OF WHOLE BODY VIBRATION ON BONE

DENSITY AND OTHER PARAMETERS IN THE EXERCISING HORSE

Introduction

In the horse lameness is the major contributor to loss of training time. Morbidity and mortality are caused largely by skeletal injuries (Price et al., 1995). Bone injuries are hard to predict and are often only found after they already happen. The use of WBV has been recently introduced to the equine industry to try and combat many different issues. It has been used in other species to impact bone density and thus its use in the horse should be investigated.

WBV has been studied in many species including humans, mice, and sheep. Rubin et al. (2002) found low level mechanical stimuli improved the quantity and quality of the trabecular bone in hind legs of female sheep. WBV has also been used extensively in postmenopausal women to maintain bone density. Hulak, 2015 studied the effects of WBV on stalled horses compared to stalled horses receiving light exercise. This study found that while WBV did not increase BMC in the treatment group, it did maintain BMC in the stalled horse to the same level as those subjected to stalling with light exercises.

The use of WBV in the equine industry is relatively new. It is being used anecdotally to help prevent injury as well as a method of recovery from lameness and skeletal injuries. WBV is also being used to promote blood circulation, performance, hoof development and many other areas in the equine industry. It is thought that WBV should have the same effects seen in other species but no research has been published

supporting this claim to date. This project was designed to investigate the effects of WBV

on the exercising horse's stride length, heart rate and gaskin and forearm circumference

(as a measure of muscle hypertrophy). This study also examined the effects of WBV on

bone mineral content and osteocalcin and serum PYD, markers of bone formation and

resorption, respectively, compared an exercising control group that did not experience

WBV. The hypothesis of this study is that BMC measured via RBAE's will increase in

the treatment group receiving WBV, with corresponding changes in PYD and OC. It is

also hypothesized that stride length and gaskin/forearm circumference will increase due

to treatment. Heart rate will reflect acceptance of the treatment by the horse compared to

the control group not receiving WBV.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee of

Middle Tennessee State University (Protocol #14-011, Appendix A).

Horses

Eleven mixed breed horses of various ages (17 ± 4 yr) were obtained from the

teaching and research herd of Middle Tennessee State University. The six mares and 5

geldings were under similar management prior to study initiation. All horses were out on

pasture for a backgrounding period from day -28 to 0. During the experimental period,

the horses were stalled in 9.3 m^2 stalls. Prior to the start of the study horses were

randomly assigned into two groups either a control group (CON, n = 5) or an

experimental group (VIB, n = 6). During the stalling period all horses were fed prairie

grass hay and commercial pelleted concentrate (Purina Strategy) twice daily to maintain

body condition. *Ad libitum* access to water was provided throughout the duration of the study. All horses were weighed on days 0 and 28.

General Experimental Design

All horses were exercised daily in a free-stall, motorized, circular equine exercise machine (EquiGym, Lexington, KY), 6 d/wk for 1h daily. The exercise protocol consisted of 10 min at a walk, 10 min at a trot, 3 min at a canter and 7 min at a walk in each direction. At no time throughout the study did the speed of the exerciser exceed 8m/s. In doing so this insured all horses were kept at a level considered to be low impact exercise. The treatment group was placed on a whole body vibration plate (EquiVibe, Malcom, NE) 5 days a week for 45 min. The vibration plate was set to 50 hertz. The treatment group remained tied while on the vibration plate and were provided access to prairie grass hay for the duration of the treatment.

Radiographs

Radiographs were taken on days -28, 0, and 28 of the study. Digital radiographs of both the medial-lateral and dorsal-palmer views of the left third metacarpal were taken. After completion of (d -28) radiographs all horses were turned out on pasture for a 28 d wash out period with free choice exercise. For all radiographs, an aluminum stepwedge penetrometer was attached to the radiographic cassettes. The x-ray was set to 70 kVp for the lateral views and 72 kPv for the dorsal-palmer views, with a distance of roughly 66 cm and an exposure of 1.8 mAs. The radiographs were used to determine the bone mineral content (BMC) of the third metacarpal using radiographic bone aluminum equivalency (RBAE) for all bone cortices and total BMC. Total RBAE was examined

roughly 1 cm distal to the nutrient foramen. The radiographs were analyzed using Bio

Rad Quantity One software. Using the values provided from the software a regression

model was formed using the known thickness of the aluminum stepwedge, in accordance

with the method reported by O'Connor-Robison and Nielsen (2013).

Blood Samples

Blood samples were collected on days 0, 1, 2, 7, 8, 9, 14, 15, 16, 21, 22, 23, 28,

via jugular venipuncture into 10 ml glass serum and lithium heparin tubes. Lithium

heparin tubes were immediately inverted. All samples were centrifuged and plasma and

serum were aliquoted for later analyses. All samples were stored in a -20 freezer.

Osteocalcin and serum pyridinoline crosslinks (PYD) were analyzed via colorimetric

assays previously validated in the horse (MicroVue Osteocalcin EIA Kit and MicroVue

Serum PYD EIA Kit) for days 0, 1, 2, 14, 28. All assays were completed to the

specifications of the kits (Quidel Corporation, San Diego, CA).

Heart Rate

On day 23 of the study, after daily exercise, heart rate monitors were placed on 8

of the horses, 4 control (CON) and 4 treatment (VIB). At 30 min prior to the treatment,

equine heart rate monitors (Polar Equine RS800CX, Polar Electro Inc., Lake Success,

NY) were placed on both a control horse and an experimental horse. The heart rate

monitors were attached with vet wrap on the left side of the horse with one lead slightly

below the withers and the other behind the left shoulder; the leads were placed on the

horse using water and gel as a conductant. Horses remained loose in stalls for a 30 min

period to allow heart rate to return to baseline. The VIB horse was then placed on the

vibration plate and the CON horse paired to it was tied in an empty stall directly next to

the vibration plate stall to standardize other environmental conditions with the exception

of vibration. Both horses were provided access to prairie grass hay. Heart rate monitors

continued to record throughout the duration of the 45-min treatment. After the

completion of the vibration treatment both horses were lead back to their stalls and the

heart rate monitors were left on for 30 min post vibration.

Muscle Measurement

On days 0, 7, 14, 21, 28 forearm and gaskin circumference measurements (cm)

were taken using a standard soft measuring tape, with the location of the measurement

standardized by clipping hair to assure consistency among dates.

Stride Length Tracking

Stride length was determined on days 0 and 28 of the study. All horses, in no

particular order, were hand trotted in a straight line over a 15 m track within a sand/clay

arena. A video camera was placed approximately 18 m from the center mark to allow

video recording of the entire track. After the end of the study all videos were analyzed

using OnTrack EQUINE Software (OnTrack EQUINE, Lake Elmo, MN) movement

analysis software to measure stride length of each horse. Using the values provided from

the OnTrack software the first four strides for each horse were then averaged for each

date.

Statistical Analysis

Statistical analysis was completed using SAS 9.2 (SAS Stat Inc., Cary, NC). An ANOVA mixed model procedure with repeated measures was used with a Tukey-Kramer adjustment when necessary. The data were also analyzed with a covariate to examine any changes from baseline. The statistical model included day, treatment, and day by treatment interaction effects.

Results

Gaskin/Forearm

No differences were observed in forearm measurements attributable to day, treatment, or day by treatment interaction (P = 0.20, P = 0.28, P = 0.54, respectively; Table 1). There was a day by treatment interaction observed on the gaskin measurements that trended toward significance (P = 0.067; Table 1). There was no day (P = 0.12) or treatment (P = 0.99) effect on gaskin measurements.

Table 1- Gaskin and Forearm values (cm ± SEM) of horses subjected to whole body vibration and light exercise (VIB) or light exercise (CON). Values not sharing a common superscript tend to differ (p < 0.067), while values with no superscripts are not different (P > 0.20).

	Day 0		Day 28	
	Control	Vibration	Control	Vibration
Gaskin	$45.9^b \pm 0.6$	$45.4^{ab} \pm 0.6$	$44.9^a \pm 0.6$	$45.5^{ab} \pm 0.6$
Forearm	44.7 ± 1.5	46.4 ± 1.4	43.2 ± 1.5	45.9 ± 1.4

Bodyweight

Body weight showed no influence of day, treatment, or date by treatment interaction (P = 0.28, P = 0.92, P = 0.68; respectively). Mean body weight was 525 ± 24 kg on day 0 and 515 ± 24 kg on day 28.

Heart Rate

A trend for a treatment effect was observed between the CON and the VIB group (P =0.065; Figure 1). Across all time points, CON increased on average 3.04 ± 2.8 bpm, while VIB decreased -4.7 ± 2.8 bpm. There was no time effect or treatment by time interaction (P = 0.94, P = 0.70, respectively).

Stride Length

Stride length at the trot exhibited a difference by day (P = 0.0002). On day 0, stride length averaged 2.91 ± 0.08 m, where on day 28 average stride length decreased to 2.44 ± 0.08 m. There was no treatment or date by treatment interaction observed (P = 0.88, P = 0.39).

Serum PYD and OC

A day effect was observed for serum PYD (P < 0.0001). On day 0 the mean for serum PYD levels was 1.41 ± 0.16 ng/ml, compared to day 28 where the mean levels of serum PYD increased to 2.48 ± 0.16 ng/ml. No differences were attributable to treatment and no day by treatment interaction was found (P = 0.95, P = 0.15). Plasma OC showed no difference by day, treatment, or day by treatment (P = 0.75, P = 0.70, P = 0.83).
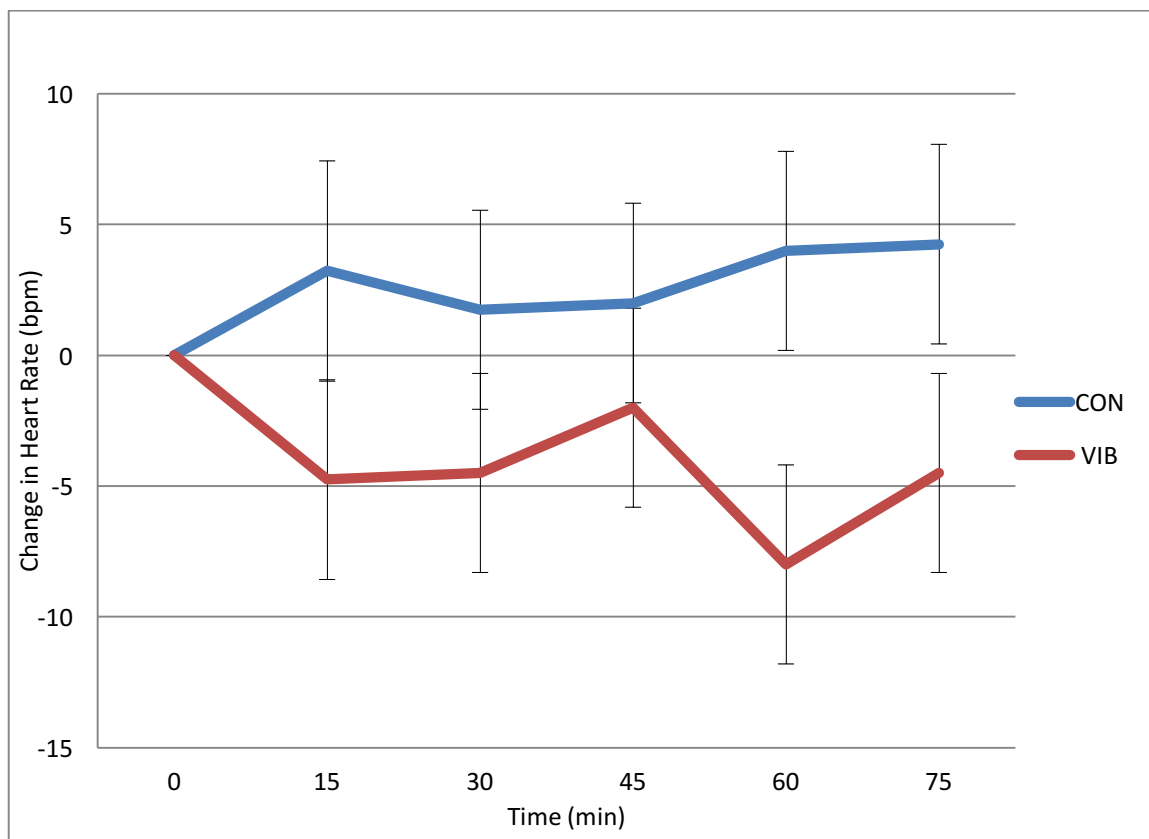
Figure 1- Change in heart rate (bpm ± SEM) of horses subjected to whole body vibration (VIB) or control (CON) over a 45-min treatment period and a 30-min recovery. A trend for a treatment difference was identified across all times (P = 0.065).

RBAE

Data for RBAE values were analyzed for all cortices for two periods to compare pasture turnout and stalling of all horses as well as for a treatment effect once the treatments were initiated. Period 1 represented the pasture turnout for all horses and period 2 represented when horses were stalled for the 28 d treatment period. No period effect was seen in BMC in the medial cortex (P = 0.60). A period effect was observed in the lateral cortex (P = 0.011), where during period one the mean change RBAE value was 1.87 ± 0.65 mmAl, whereas period 2 showed a decline of -1.24 ± 0.65 mmAl. No period effect was observed in the dorsal or palmar cortices (P = 0.39 and P = 0.12, respectively). A period effect was also identified in total BMC (P = 0.059), where during period 1 BMC was higher (321.08 ± 833.8 mm^2-Al) compared to period 2 (-2529.1 ± 833.8 mm^2-Al).

During the treatment period days 0-28 there was no difference in BMC in the medial cortex attributable to day, treatment, or interaction (P = 0.34, P = 0.99, P = 0.14, respectively). Similarly, the effect of day, treatment and day by treatment was not different in the dorsal cortex (P = 0.15, P = 0.48, P = 0.73, respectively). A trend toward significance was seen in lateral BMC for a day effect (P = 0.095); day 0 mean for BMC was 30.97 ± 0.58 mmAl when compared to day 28 which decreased to 29.72 ± 0.58 mmAl. Further, the palmar cortex had a day effect (P = 0.001), where day 0 had an average BMC of 25.29 ± 0.79 mmAl and day 28 decreased to 23.06 ± 0.79 mmAl. No difference was attributable to treatment or date by treatment interaction (P = 0.45, P =

0.92). Finally, total BMC exhibited a day effect (P = 0.002); total BMC on day 0 averaged 14357 ± 720 mm^2-Al which decreased on day 28 to 11828 ± 720 mm^2-Al.

Discussion

Until now there have been no published reports on the effects of WBV in the exercising horse. The results of the current study show the effects of WBV on forearm and gaskin circumference, heart rate, stride length, OC and PYD concentrations and BMC. Application of WBV was deemed safe and no adverse effects were detected throughout the present study.

WBV did not have an effect on forearm circumference as a measure of muscle hypertrophy. There was a day by treatment interaction observed on the gaskin measurement but no other interaction was seen. This could be contributed to human error and the accuracy of each measurement taken. This would contribute to high variability, which is reflected in the high SEM. There is no evidence to date that WBV has an effect on muscle circumference, the current study did not find anything contrary to past research. Previous research has shown an increase in muscle strength due to vibration-induced muscle activity. Verschueren et al. (2004) found an increase in dynamic strength in the group that performed static and dynamic exercises while on a vibration platform. While vibration may have an effect on muscle strength and performance it did not directly affect circumference. It may have proved interesting to look at muscle composition as an alternative to circumference as such may be a better indicator of dynamic strength in the horse, where tests to directly measure strength prove difficult to administer and assess.

In this current study body weight was measured but was not expected to change during the treatment period. Body weights were recorded at the beginning and end of the study to make sure that a body weight necessary for maintenance was maintained. Since no difference was observed throughout the study and the horse's diets remained constant no effect would be expected.

No previous research has been conducted to date on the effects of WBV on heart rate or other cardiovascular effects in the horse. The current study found a trend for a treatment effect between the control and vibration groups. Average heart rate decreased in the vibration group compared to the control group. This is contrary to research conducted in humans. Robbins et al. 2014 examined the effects of WBV on the central and peripheral cardiovascular system. Human participants stood on a vibration plate set at 40 Hz. Vibration exposure totaled 5 min in 1 min increments and a 5 min recovery between each testing period. No changes in heart rate, blood pressure or peripheral skin temperature were observed. Robbins et al. (2014) did note a change in blood flow velocity, this was suggested to be due to a high level of sensitivity of the peripheral vascular system to vibration exposure. Another study conducted on humans found the opposite effect, that WBV increased heart rate although the increase was modest (Liao et al., 2015). In the horse, it might be suggested, horses in the treatment group could have experienced a reduction in stress while receiving treatment. Experiencing the vibration treatment may have a relaxation effect on the horses lowering cortisol levels and therefore reducing the heart rate. Anecdotal behavior observations would support this suggestion as horses appeared relaxed throughout treatment.

The current study hypothesized WBV would increase the stride length in the horse. Stride length at the trot did exhibit a difference by day, where the average stride length decreased from day 0 to day 28. This is likely attributable to the fact that a different person hand trotted the horses on the two testing days. Unfortunately, using the same person for both days was unavoidable. Although only one person hand trotted all horses each day, the size and stride of the person may have had an effect on the movement of the horses. The current study found no treatment or date by treatment interaction. Further research should be done to evaluate the effect of WBV on movement in the horse.

The use of bone markers has not been researched in regards to the effect of WBV. Both osteocalcin (OC) and serum PYD levels were examined in this study. A day effect was observed for serum PYD. The mean level of serum PYD increased from day 0 to day 28. When bone or cartilage collagen is degraded, PYD is released into circulation. This can be explained by a stalling effect. The horses had been turned out on pasture until the initiation of the study and then stalled for the 28 d treatment portion. Skeletal unloading has been associated with loss of bone mass and decreased bone formation. Since the horses in this study were experiencing light exercise this was not as detrimental as if they had been on strict stall rest. Bell et al. (2001) researched the effects of daily access to pasture on prevention of bone mineral content loss in Arabian weanlings. It was determined that pasture turnout or 12 h/d of turnout is beneficial to maintaining and increasing BMC. Hoekstra et al. (1999) also found an increase in BMC in pasture-reared horses compared to stalled horses. Medial and lateral BMC were consistently higher in

horses kept on pasture for the 56 d study. Similarly, in the current study total BMC decreased in horses from a period of turnout to initiation of the current study when stalling began.

Plasma osteocalcin showed no difference by day, treatment, or day by treatment interaction. Osteocalcin makes up a portion of the non-collagenous protein in bone. Plasma OC is used as a biochemical indicator of bone turnover. Increases in OC indicate an increase in bone turnover and vice versa. Although horses were receiving light exercise in the current study, having the horses stalled did not allow for enough loading to occur to cause microcracks. Also seen in previous studies, mature horses are seen to have lower levels of OC when compared to younger horses (Lepage et al., 1990). This could explain the lack of difference in OC levels seen between initiation of the current study and the end.

BMC values were evaluated for two periods to compare pasture turn out and stalling as well as for a treatment effect once the study began. Period 1 was the time frame where horses were out on pasture receiving free choice exercise. Period 2 was the start of the treatment portion of the study and when stalling began. A period effect was observed in the lateral cortex and total BMC. In both cases period 2 had a decrease in RBAE values, which could once again be attributed to a stalling effect. During the treatment period (days 0-28), a trend toward significance was seen in the lateral cortex for a day effect. A similar day effect was also observed in the palmar cortex as well as total BMC. In all instances mean BMC had decreased from day 0 to day 28. This could potentially be explained by the fact that horses during the treatment period were

experiencing only light exercise yet were subjected to stalling. Although horses were receiving light exercise 6 d/wk it was not at a level of loading likely equivalent to horses out on pasture.

Conclusion

In summary the results of this study found that bone density was not increased due to the addition of WBV on the exercising horse. Heart rates were lower in the treatment group, possibly due to a relaxation effect. No differences were seen in stride length, gaskin and forearm circumference, or markers for bone metabolism. WBV is becoming more widespread throughout the equine industry. In all research to date, across the various species, there are many discrepancies about the application of WBV. There is a great deal of variation in duration of treatment, length of each individual treatment and strength of vibration. Further research needs to be done in the horse. Studies in other species had longer durations, and since no significant change was seen in our 28 d period a future direction would be to increase the duration of the study. Another possible study should explore the strength of vibration used in horses, which could possibly lead to different results. Since the horse is the largest animal WBV has been tested on then perhaps a higher frequency should be used to see significant results. Since there was a trend toward significance for decreased heart rate in the treatment group, perhaps a future study should be done examining cortisol levels in relation to heart rate of horses receiving whole body vibration to determine if it is a stress relieving activity.

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Appendices

Appendix A: IACUC 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