

**THE INFLUENCE OF WHOLE BODY VIBRATION ON BONE  
MINERAL CONTENT AND BONE METABOLISM IN THE  
STALLED HORSE**

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A Thesis Submitted in Partial Fulfillment  
of the Requirements for the Degree of  
Master of Science in Horse Science  
Equine Physiology

Middle Tennessee State University  
May 2018

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## **ABSTRACT**

Whole body vibration (WBV), an emerging therapy, has shown positive effects on bone health in humans and horses. As stalling has shown deleterious effects on bone, this study aimed to evaluate the effect of WBV on bone parameters in stalled, healthy horses. Twelve mature mixed breed horses were randomly assigned to control (CON, n = 6) or vibration (VIB, n = 6). Radiographs were taken of the left third metacarpal for determination of bone mineral content (BMC), and serum was collected for determination of pyridinoline cross-links (PYD), a marker of bone resorption. No treatment effect was found for change in BMC in any cortex ( $P > 0.05$ ) or for PYD concentration ( $P = 0.85$ ). BMC unexpectedly increased across both groups in all cortices ( $P < 0.05$ ), yet PYD increased from day 0 to 14 ( $P = 0.01$ ). The results show no influence of WBV on BMC or bone metabolism in stalled, healthy horses if no stalling-related decrease in BMC is observed.

## TABLE OF CONTENTS

LIST OF FIGURES.....	iv
CHAPTER I: LITERATURE REVIEW.....	1
Introduction.....	1
Equine Performance and Preventing Injury.....	2
Stalling’s Impact on Equine Bone.....	7
Human Vibration Therapy.....	10
Animal Vibration Therapy.....	15
Conclusion.....	20
CHAPTER II: THE INFLUENCE OF WHOLE BODY VIBRATION ON BONE MINERAL CONTENT AND BONE METABOLISM IN THE STALLED HORSE.....	22
Materials and Methods.....	23
Results.....	25
Discussion.....	30
Summary and Conclusion.....	33
LITERATURE CITED.....	35.
APPENDIX.....	43
APPENDIX A: IACUC Approval.....	44

## LIST OF FIGURES

	<b>Page</b>
Figure 1. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the medial cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.....	26
Figure 2. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the lateral cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.....	27
Figure 3. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the dorsal cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.....	28
Figure 4. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the palmar cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.....	29

## CHAPTER I: LITERATURE REVIEW

### Introduction

Lameness is the most common factor inhibiting performance in the equine athlete (Jeffcott et al., 1982; Morris and Seeherman, 1991; USDA, 1998). Lameness can have multiple causes and be related to numerous injuries and disease states. Prevention of lameness and injury is the most efficient and effective way to keep the athletic horse healthy and able to perform its job well. However, if an injury has already presented itself, it must be treated, and treatment strategies may vary. In human medicine, whole body vibration (WBV) has gained popularity for both prevention and treatment of orthopedic maladies, especially as it relates to patients with osteoporosis (Rittweger, 2010; Cochrane, 2013). Whole body vibration has also recently gained popularity as a therapy tool in equine medicine, such as in acute or chronic musculoskeletal injuries (Halsberghe, 2017a).

Vibration therapy has shown promising results in many areas of human health, including warm-up, flexibility, range of motion, muscle soreness, postural sway, balance, and bone health (Rittweger, 2010; Aminian-Far et al., 2011; Cochrane, 2013). Limited research is available on the equine subject, but vibration plates have been used to increase healing and relieve pain of musculoskeletal ailments as well as to improve warm-up before competitions (Halsberghe, 2017a). Much of the use of WBV in horses is based on the use and results in human research (Rittweger, 2010). The vibration plates contain three key components including frequency, amplitude, and direction of movement (Lorenzen et al., 2009). Frequency refers to the number of Hz, or the number of up-and-

down cycles per second (Lorenzen et al., 2009). The vibration plate sends mechanical energy in the vertical and/or horizontal direction (Zepetnek et al., 2009).

Whole body vibration therapy is continuing to become more common in the horse industry following its rise in human medicine and athletics. With this increase in use, research should be completed to determine the efficacy of this therapy on the equine subject in various aspects of health. The major question and focus of this project is how vibration therapy affects bone density and metabolism in the stalled horse.

### **Equine Performance and Preventing Injury**

The equine industry has an important impact on the economy in the United States and across the world. Livestock as a whole in agricultural production accounts for about half of the total agricultural cash receipts in the United States, at above \$100 billion per year (Williams, 2011). A 2007 report indicated animal agriculture had an output of \$451 billion, which accounted for 3.4% of the GDP; earnings of \$83.6 billion; employment of 3.4 million jobs, which accounts for 2.3% of the United States jobs; and taxes of \$21.4 billion (Williams, 2011). The horse industry itself contributes about \$39 billion to the United States economy and accounts for 1.4 million full-time jobs (American Horse Council, 2005). When indirect and induced spending are added, this number increases to \$102 billion in economic impact to the United States (American Horse Council, 2005). There are a total of 9.2 million horses in the United States, and of this total, over 2.7 million are showing horses and over 800,000 are racing horses (American Horse Council, 2005). These numbers provide knowledge of the importance of the equine industry, especially the performance horse industry.

The ability of a horse to successfully perform at its high-quality level is negatively influenced by pain (Dyson, 2013). Athletic disciplines each require different forces to be inflicted on the physiology of the horse and can cause varying injuries of varying degrees (Murray et al., 2006; Parkes et al., 2013). The first predisposition to injury can occur at birth, where genetic and environment factors combine to determine the structure or conformation of the horse. Correct conformation has been shown to increase the athletic life of the horse (Jonsson et al., 2014). Jonsson et al. (2014) used 8,238 hunter-type Swedish Warmblood horses starting at ages 4 or 5 and followed their career through age 11 or 12. The results suggested ideal conformation in the horse prolongs its athletic career (Jonsson et al., 2014). This suggests that selecting for proper conformation, which in itself may differ slightly according to athletic discipline, can be a vital way to prevent future injuries in the horse.

Each discipline has unique demands for the horse and in such can cause injuries directly related to that discipline. Within the discipline of dressage, the most common causes of lameness include proximal sensory desmitis, suspensory branch injuries, degenerative joint disease, synovitis, tenosynovitis, palmar cortical fatigue fractures, and thoracolumbar and sacroiliac pain (Dyson, 2001). Of show jumping horses, the most common causes of lameness include desmitis, tendonitis, and tenosynovitis (Dyson, 2001). Event horses commonly display lameness as proximal suspensory desmitis, suspensory branch injury, superficial digital flexor tendonitis, exertional rhabdomyolysis, stifle trauma, foot soreness, over-reach, traumatic arthritis of the metacarpophalangeal and distal interphalangeal joints, degenerative joint disease of the centrodistal and

tarsometatarsal joints, tenosynovitis of the digital flexor tendon sheath, and thoracolumbar and sacroiliac pain (Dyson, 2001). Common lameness of cutting and reining horses include palmar heel pain/navicular syndrome, suspensory ligament desmitis, inflammation/arthritis of the distal tarsal joints, and stifle lameness (Jackman, 2001).

Horses involved in horse racing may experience the most serious performance-induced injury. In the racing industry, catastrophic musculoskeletal injury (CMI) is a factor in inhibiting performance and may be fatal (Beisser et al., 2011). In the 2011 study by Beisser et al., results found a rate of 1.48 CMIs per 1,000 race starts in Thoroughbred racing at Midwestern racetracks, which was consistent with previous studies in Kentucky (Peloso et al., 1994), California (Estburg et al., 1996), Florida (Hernandez et al., 2001), New York (Hill, 2003), and Ontario (Cruz et al., 2007) racetracks. Beisser et al. also found a rate of 1.36 CMIs per 1,000 race starts in Quarter Horse racing (Beisser et al., 2011). Related data for Quarter Horse racing shows 0.8 catastrophic injuries/1,000 race starts and 2.2 musculoskeletal injuries/1,000 race starts (Cohen et al., 1999).

The above examples highlight common lameness and injuries in a few disciplines. Any injury has a strong negative impact on the horse's ability to effectively perform at a high-quality level. As injuries present themselves, they must be treated accordingly. On another note, the ability to prevent new or worsening injury is a promising way to keep the horse and the industry as healthy as possible. Prevention of injury is important in any health management program as it is the most efficient way regarding economic and performance reasons to keep horses healthy and productive in performance ability.

Bone has a unique physiology in its daily maintenance and in its healing process. Wolff's law explains the system for which the bone manages itself as well as changes itself when change is warranted. Wolff's law states bone adapts to the mechanical stresses enforced upon it (Ahn and Grodzinsky, 2009). When less forces are applied, such as to astronauts in space, the bone density will be reduced, and when more forces are applied, such as during weightlifting, the bone density will be increased (Ahn and Grodzinsky, 2009). The cells making up bone and which remodel the bone include the osteocytes, osteoblasts, and osteoclasts (Chen et al., 2010). Osteocytes are known to be the mechanosensors, while the osteoblasts deposit bone matrix and osteoclasts resorb bone (Chen et al., 2010). Osteocytes occupy a fluid-filled matrix containing lacunae and are connected by canaliculi (Chen et al., 2010). When forces are applied to bone, pressure gradients are induced in the bone matrix, causing fluid flow and cellular processing among the canaliculi network (Piekarski and Munro, 1977). Loading on bone also produces a hydrostatic pressure among the lacunar-canalicular matrix, and this hydrostatic pressure has been suggested to be a factor in bone remodeling (Zhang et al., 1998). Regarding loading in horses, high intensity exercise, such as galloping and jumping, will promote higher bone matrix deposition and density than simply walking.

Change in bone density is significant in causing or reducing injuries in the horse and human. The density of bone ultimately determines the strength of the bone (Furst et al., 2008). In humans, osteoporosis, which is a rapid decrease in bone density, leads to the increase in random fractures occurring (Bartl and Bartl, 2004). Studies in horses indicated a decrease in bone density increased the risk of fracture (Siffert et al., 1996; Mosekilde,

2000; Warming et al., 2002). The decrease in bone density causes a decrease in the strength of the bone, and the alternate increase in bone density increases the strength of the bone. Maintaining the highest possible bone density is also maintaining high strength of the bone and aiding in prevention of injury.

Bone density in horses is often measured using radiographic bone aluminum equivalencies (RBAE) and biochemical markers. The RBAE method involves using radiographs of the third metacarpal, often of the left forelimb, and referencing an aluminum step wedge as a measure point (Meakim et al., 1981). The aluminum step wedge is compared to the area of mineralization in the bone to compute the mineralization of the bone. The bone mineral content (BMC) is directly related to bone density, higher BMC indicates higher bone density. Furthermore, biochemical markers are proteins released during bone metabolism, such as osteocalcin during deposition and deoxypyridinoline during resorption, which are measured using assays developed for measuring the human proteins but validated in the horse (Lepage et al., 2001). Pyridinoline cross-links (PYD) is a common biochemical marker used to measure bone resorption through serum or urine analysis (Lepage et al., 2001). PYD is released from the bone matrix during bone degradation processes (Lepage et al., 2001). PYD represent mature cross-linking amino acids, which function to stabilize the collagen molecules within the bone (Lepage et al., 2001). Use of this biochemical marker allows for determination of bone metabolism as it relates to resorption of the bone.

## **Stalling's Impact on Equine Bone**

Horses are often stalled for prolonged periods of time. Common reasons for prolonged stalling in the horse include preventing aggression from group turnout such as biting or kicking; preventing sun bleaching from outdoor turnout; maintenance of body condition, especially in a competing horse (Heleski et al., 2002); fitting in preparation for a sale (Bell et al., 2001); initiation of training (Hoekstra et al., 1999); and recovery from an injury (Porr et al., 1998). Performance horses are also kept in stalls during the active competition season, often to prevent injury on pasture (Haupt and McDonnell, 1993; Christensen et al., 2002; Werhahn et al., 2012). Based on the knowledge of bone physiology, however, prolonged stalling may have a detrimental impact on bone density as it limits the movement of the horse. Several research studies have examined the impact of stalling on bone health.

In a study by Porr et al. (1998) using 11 conditioned Arabians then deconditioned for 12 weeks in stall confinement, it was found that even with daily sessions on a mechanical exerciser at a walk, the horses lost bone mineral content (BMC) at a rate of 1.1 g/2 cm. The decrease in BMC shown in this study was unaffected by increased calcium intake, and the loss of BMC along with the walking time and increased calcium weakened the bone and increased risk of injury (Porr et al., 1998). This indicates that walking alone and increasing calcium intake would not properly be able to maintain the bone density in a stalled horse, in which the bone density is decreased with decreased activity.

A study using 18 Quarter Horse weanlings divided into group housing, confinement, and confinement with short-duration exercise, measured change in bone density with RBAE over the 56-day trial (Hiney et al., 2004a). The short-duration exercise involved the horses galloping down an 82-meter alleyway 5 days per week (Hiney et al., 2004a). The results provided evidence of increase in bone density in exercised weanlings versus the confined weanlings (Hiney et al., 2004a). This study indicates that while confinement negatively affects bone density, short-duration, high intensity exercise may be sufficient to increase the strength of the bone when stalling is required. Bell et al. (2001) also studied the effects of stalling on bone in weanlings and found higher cannon bone circumference in those on pasture or on partial pasture when compared to those stalled. This study used 17 Arabian weanling horses kept either on full-time pasture, 12-hour pasture and 12-hour stall, or full-time stalling and measured bone density every 28 days using RBAE as well as other parameters (Bell et al., 2001). The full-time stalling in this study resulted in the horses not being able to reach their properly maximum level of bone density during development (Bell et al., 2001). These studies may also imply the young horses are at less risk of injury and able to properly develop when raised with exercise on at minimum part-time pasture rather than full time stalling.

Hoekstra et al. (1999) studied yearlings raised on pasture compared to stall confinement using 16 Arabians age 16-19 months randomly selected for the different management groups. Of the 16 Arabians beginning the study, 6 from each group continued into a training phase of the study (Hoekstra et al., 1999). Bone mineral content

and bone metabolism was measured using RBAE, serum osteocalcin, and urinary deoxypyridinoline concentrations (Hoekstra et al., 1999). The yearlings kept on pasture increased in bone density, while the yearlings kept in stalls decreased in bone density through the study period (Hoekstra et al., 1999). This study further displays the stall confinement's detrimental effect on bone health and its contribution to risk of injury when beginning training, wherein the bone, already lower in density, is at greater risk of fracture during this high-stress period.

Cattle have also been used as a model for studying confinement and exercise effects on bone density and health. Hiney et al. (2004b) used eighteen 8-week-old Holstein bull calves to examine confinement versus exercise effects on bone measure with post-harvesting computed tomography and bending tests to failure to determine bone strength. Results of this study indicated higher bone density and greater fracture force in the exercised group when compared to the confined group (Hiney et al., 2004b). The confined cattle had decreased bone health, which would demonstrate a weaker skeletal system.

Further, Schenck et al. (2008) studied lack of exercise compared to low or high exercise's effect on bone in the swine model using gestating gilts. The low exercise group walked or ran 122 meters per day for 5 days per week, and the high exercise group walked or ran at increasing intensity, which by the end of the exercise period was 122 meters per day for 2 days and 427 meters per day for 3 days (Schenck et al., 2008). The gilts were harvested after weaning for bone and other analysis (Schenck et al., 2008). Results showed decreased bone density in the group with no exercise as compared to the

low exercise group and decreased breaking force in the group with no exercise compared to both exercise groups (Schenck et al., 2008). The swine model in this study also resulted in weakening bone with lack of exercise. The bovine and swine models in these studies had similar results to similar studies in the equine model, and the bovine and swine allow for post-harvest assessment of the bone strength or loss of strength.

Collectively, these studies on the impact of stalling horses and other animals on bone health reach the same basic conclusion. The results suggest that prolonged stalling and lack of exercise have a detrimental effect on the strength of the bone. The common conclusion shows the stalling and decreased exercise resulted in a decrease in bone density, which indicates a decrease in bone strength. Thus, prolonged stalling without any additional exercise may lead to a rise in the risk of injury, and caution should be taken with maintenance during stalling periods and commencement of exercise.

### **Human Vibration Therapy**

While WBV is still a more recent and emerging area of research, several studies have been conducted with human subjects on effects of WBV through various parameters. Factors examined include bone, muscle, attention, and other systems and their response to WBV. Bone, in particular, has been most commonly studied with human subjects and was a portion of the earliest studies examining WBV. Lai et al. (2013) examined the effect of WBV on bone mineral density (BMD) using 28 postmenopausal women divided into control or WBV groups, in which the WBV group stood on a vibration plate at 30 Hz for 5 min, 3 times per week for the study period of 6 months. The results demonstrated an increase in BMD in the lumbar spine in the WBV group when

compared to the control group (Lai et al., 2013). This study demonstrated a positive effect of WBV on increasing BMD and bone strength. A previous study by Verschueren et al. (2004) with postmenopausal women found similar results. This trial examined BMD change in 70 postmenopausal women divided into WBV group, resistance training group, and control group and found an increase in BMD in only the WBV group (Verschueren et al., 2004).

A separate study found conflicting results regarding WBV's effect on BMC. Torvinen et al. (2003) used 56 volunteer subjects divided into vibration or control group, where the vibration group experienced vibration for 4 min, 3-5 times per week at accelerating 25-45 Hz while performing a light exercise program. Results from this 8-month trial showed no effect on bone mass, structure, or strength; no change in bone biomarkers; an increase in vertical jump height; and no effect on other performance and balance tests (Torvinen et al., 2003). The results in this study conflict with other studies on WBV on bone but could be due to the study design having movement while standing on the platform, which may have altered results. Variability in the number of times per week using the platform may also have altered the results.

Humphries et al. (2009) compared groups of human subjects as control, WBV, and WBV with resistance training and the effect on BMD. The results of this study showed no difference between the WBV groups, but there was a difference between the control and both the WBV groups (Humphries et al., 2009). The WBV groups, both without and with resistance training, experienced an increase in adiponectin, transforming growth factor- $\beta$ 1, and nitric oxide and a decrease in osteopontin, interleukin-1 $\beta$ , and

tumor necrosis factor- $\alpha$  (Humphries et al., 2009). This related to an increase in bone deposition and a decrease in bone resorption leading to the increase in density of the bone through WBV.

Patients with Down syndrome (DS) have recently been a subject of WBV research as DS patients tend to show a lower bone strength than individuals without DS (Matute-Llorente et al., 2015). A study by Matute-Llorente et al. (2015) examined WBV's effect on bone in adolescents with DS and found an increase in BMD in those on the vibration platform at 25-30 Hz 3 times per week involving 10 repetitions of 30-60 seconds for 20 weeks. The DS patients were able to show improvement in BMD and BMC when subjected to WBV therapy for a prolonged term (Matute-Llorente et al., 2015). In a separate trial by Matute-Llorente et al. (2016), effects of WBV on BMD were examined in DS versus non-DS individuals. The results indicated an increase in BMD in both groups, but there was a greater increase in BMD in the non-DS group (Matute-Llorente et al., 2016). While WBV was able to increase BMD in both groups, it had less of an effect on individuals with DS than those without. Reasons for this discrepancy in response in DS patients compared to non-DS individuals is unclear as this is the first study of WBV comparing DS patients to non-DS individuals.

A recent study by El-Shamy (2017) examined the effect of WBV on individuals with hemophilia. A previous study found a decreased BMD in children with hemophilia compared to children without hemophilia (Tlacuilo-Parra et al., 2008). Thirty children, age 9-13 with hemophilia, were assigned to a physical therapy only group or a physical therapy with WBV group (El-Shamy, 2017). There was an increase in quadriceps

strength, BMD, and functional capacity in the vibration group (El-Shamy, 2017). The positive effects of WBV as shown here may be a potential therapeutic method for these children to reach peak performance. While there have been some studies with results that do not support WBV on bone or other parameters, there are numerous studies supporting the increases in BMD and bone strength with the use of WBV.

Muscle has further been studied with WBV in human subjects. Bogaerts et al. (2007) examined the effects of WBV on muscle strength and mass in men with a mean age of 67.3 years. The 97 men participating in the trial were divided into groups of WBV, which did various exercises on a vibration platform for 40 minutes; fitness, which performed the same exercises; and control, which did not change their regular routine (Bogaerts et al., 2007). The results of this study found increased muscle strength and mass in both the WBV and fitness group with no differences between those groups (Bogaerts et al., 2007). This leads to the possibility of WBV aiding in the maintenance of skeletal muscle in aged individuals at a level similar to fitness exercise programs.

The effect of WBV on muscle in individuals with chronic stroke was examined by Tankisheva et al. (2014) using 15 patients with chronic stroke divided into WBV, in which vibrations were experienced at 30-60 seconds with 5-17 repetitions at 35-40 Hz 3 times per week for 6 weeks, and control group, which continued their normal living. Findings of this study demonstrated an increase in the isometric knee extension strength, isokinetic knee extension strength, and postural control (Tankisheva et al., 2014). This study demonstrated potential for patients with chronic stroke to strengthen lower limb muscles and posture through WBV therapy.

Whole body vibration's effect on tendon has also been examined, and Rieder et al. (2016) using 55 subjects grouped into WBV, active control, or inactive control. The WBV in this study showed an increase in the patellar tendon cross-sectional area without affecting tendon stiffness or muscle architecture (Rieder et al., 2016). This study was the first to find improvement in tendon through WBV (Rieder et al., 2016). The results of these studies collectively give an example of the positive effect found in muscle and likewise in the tendon with WBV therapy.

Jump performance, grip strength, and flexibility have also been examined with WBV therapy. One study used 18 female elite field hockey players, whom each completed groups of WBV, control, and cycling (Cochrane and Stannard, 2005). Results from this study found improved arm countermovement, vertical jump, and flexibility after the WBV; no differences were found in grip strength in any of the groups (Cochrane and Stannard, 2005). This indicated WBV shows promise in improving jump and flexibility and may be a helpful therapy for elite athletes.

Further, the effect of WBV on attention was examined in a study using 83 healthy individuals and 17 individuals diagnosed with ADHD in which the participants sat in a chair on a vibrating platform (Fuermaier et al., 2014). Results demonstrated an increase in attention, measured with a color-word interference paradigm, for both groups of participants (Fuermaier et al., 2014). While the large difference in numbers between the 2 groups may have affected between-group differences, this study presents a possibility for the WBV to improve cognition in individuals.

The safety of WBV therapy use has been a research and discussion concern to ensure proper use while producing positive physiological effects. A recent study focused on examining the safety of and metabolic response to WBV therapy using 19 mechanically ventilated and immobilized intensive care unit (ICU) patients (Wollersheim et al., 2017). Results of this studied showed no change in vital signs or hemodynamics and an increase in energy expenditure with the WBV (Wollersheim et al., 2017). This study supported the safety of use of WBV in the ICU patients, in which a safety effect would be of great concern, as well as preventing some metabolic weakness that may be observed in these patients. Safety of any therapy is a concern, and there is importance in further validating the safety of WBV therapy.

### **Animal Vibration Therapy**

While the majority of WBV research has been conducted in humans, there have been studies using mice, rats, and more recently a few in horses. Research in mice and other animals can be a useful model for human research and application as well as a direct application to the animal as in horses. A study using mice examined the effect of WBV on mice injected with botulinum toxin, which normally results in bone degradation of the individual (Niehoff et al., 2014). This study used vibration of 25 Hz for 30 minutes per day 5 days per week for 4 weeks for the WBV groups, and results showed the WBV group had no difference in bone density from the group injected with a saline solution rather than the botulinum toxin (Niehoff et al., 2014). Results from this study kept in agreement with the human research and the positive effect of WBV on BMD.

The effect of WBV on bone in the growing skeleton was explored using mice divided into groups of baseline control, age-matched control, WBV at 45 Hz, and WBV at 45 Hz interrupted every second by 10 seconds of rest (Xie et al., 2006). This study resulted in a positive effect of the continuous WBV group on preventing bone resorption and maintaining high matrix quality (Xie et al., 2006). It seemed to indicate that continuous WBV must be used to aid in maintenance of bone strength and quality in growing individuals, which may benefit from the use of WBV in strengthening bone.

Ovariectomy of rats was examined to explore the effect of WBV on preventing the typical decreased bone density in ovariectomy individuals (Oxlund et al., 2003). Results of this study showed the 45 Hz of vibration brought increased bone formation and inhibited the resorption normally seen with ovariectomy (Oxlund et al., 2003). Whole body vibration further seemed to prove ability in maintaining and increasing bone density and strength in individuals with reduced ability to maintain bone health. The ovariectomy of these rats may also have implications with postmenopausal women, whom are lacking in normal hormone production from the ovaries similar to those with an ovariectomy.

Non-weight bearing mice were studied to model astronauts and other unloaded individuals, with mice undergoing WBV at 45 Hz for 20 min/d, 5 d /wk for 3 wk (Ozcivici et al., 2007). The study's results showed an attenuated decline in trabecular properties in the unloaded bone (Ozcivici et al., 2007). This indicates the possibility for WBV to assist in maintaining bone health in astronauts and other unloaded individuals, such as those bound to wheelchairs or bed rest.

An alternate perspective and result of WBV therapy was seen in a study examining the effect on fracture healing in mice by comparing non-fractured mice and fracture healing mice undergoing vibration at 35 or 45 Hz (Wehrle et al., 2014). Results found increased relative amount of bone at 35 Hz but no effect at 45 Hz in the non-fractured mice (Wehrle et al., 2014). In the fractured mice, no healing effect was observed at 35 Hz, but reduced bone formation was observed at 45 Hz (Wehrle et al., 2014). Results of this study suggest while WBV can have positive effects on bone strength and formation, caution should be taken when used on fracture healing individuals.

Additional exercise effects from WBV were examined in mice divided into two groups: control, fed a standard diet and treatment, fed a high-fat diet (Huang et al., 2014). Both groups underwent vibration therapy (Huang et al., 2014). The study's results found a trend of increased grip strength; decreased serum lactate; decreased ammonia; decreased CK levels; increased glucose levels after a swimming test; slightly decreased final body weight; decreased weight of fat pads; and decreased fasting serum levels of alanine aminotransferase, CK, glucose, total cholesterol, and triacylglycerol (Huang et al., 2014). These results indicate a possible positive effect of WBV on exercise and metabolism.

There has also been research using WBV on equine populations, though there is less available than in humans and mice. Whole body vibration's effect on bone density has been examined in the equine subject. Hulak et al. (2015) used 12 stalled horses divided into groups of control, which was exercised using a mechanical walker 60

minutes 6 days per week, and treatment, which underwent vibration therapy at 50 Hz for 45 minutes 5 days per week. Results of this study found an increase in total BMC in both groups but no difference between control or treatment groups (Hulak et al., 2015). Based on these results, there is an indication of WBV increasing bone density similarly to exercised horses.

Another recent study examined the effect of WBV on bone and other parameters in exercising horses using 11 horses randomly assigned to groups of control or WBV, where both groups were exercised on a mechanical exerciser for 1 hour 6 days per week, and WBV horses additionally stood on a vibrating platform at 50 Hz for 45 minutes 5 days per week (Maher et al., 2017). Results from this trial found no effect of WBV on bone density, a period effect of a decrease in BMC in the lateral cortex, a trend toward a decrease in total density with a probable cause of stalling, a trend toward decreased heart rate during the WBV as compared to the control group, and no difference in stride length between groups (Maher et al., 2017). Results in these horses seem to indicate a lack of further increase in bone density with WBV in already exercised horses, though a decrease in relative heart rate with WBV may demonstrate further positive results of this therapy.

Muscle has been another point of interest in WBV research in equine subjects. In a recent trial, the *m. multifidus* muscle was examined to determine the effects WBV therapy could have on this muscle (Halsberghe et al., 2017b). The 9 horses in the study were stood on a vibrating platform at 40 Hz twice per day 5 days per week for 30 minutes and resulted in an increase in *m. multifidus* cross-sectional area and symmetry

(Halsberghe et al., 2017b). This seems to suggest the ability of WBV to increase muscle amount and symmetry, even in the spinous region.

The acute effects of WBV on various parameters have also been examined. One study used 7 horses administered 10 minutes of vibration therapy at 15-21 Hz (Carstanjen et al., 2013). Researchers found results of decreased serum cortisol and creatine-kinase, while other parameters of hematology, fibrinogen, lactate, IGF-1, GGT, creatinine, myeloperoxidase activity and bone markers were not affected by the acute WBV (Carstanjen et al., 2013). This may demonstrate a positive and calming immediate effect of WBV, though the short time period of the acute study may not meet the needs to affect other values.

The effects of WBV on chronic lameness were examined in a recent trial using 8 chronically lame horses, all of whom underwent WBV therapy 5 days per week twice per day for 30 minutes at 40 Hz for 60 days (Halsberghe, 2017a). The results from this study showed a trend toward improvement in lameness during the first 30 days, though the inconclusive results in this study may be due to one horse becoming worse in lameness (Halsberghe, 2017a). These results do not seem to support use of this therapy in chronically lame horses, though an outlier horse may have skewed the results.

While there is a limited amount of research on WBV in equine subjects, the results of the studies that have been completed seem to agree with the positive results observed in human and other subjects. Animal subjects can often be an efficient model for human medicine as well as have direct application or use in that animal. The equine model may be used for research with application to both equine and human exercise.

With the increased use of WBV therapy, research is important to verify the use of this therapy for supporting quality health for both humans and horses.

### **Conclusion**

Vibration therapy is a relatively recent and emerging area of research and application in both human and animal medicine. Human research using WBV has indicated positive effects in several factors including bone health, metabolism, muscle, jump performance, flexibility, and cognition. Whole body vibration using rodent subjects has found improvement in various parameters including bone health, strength, and metabolism. Finally, a relatively small amount of research has been done using horses and WBV, but the results of the currently performed projects include limited and conflicting results in bone density, decreased heart rate, improved muscle, and decreased cortisol. Vibration therapy has seemed to provide positive but some conflicting results. These disparities may be due to the high variation in methods, such as frequency used. Care must be taken to maintain the improvement seen from WBV and find the safe and effective options with the use of this therapy. An issue well-established in equine health is the negative effect of stalling the horse on the bone health in that horse due to decreased activity and loading. Trials examining this effect have consistently found a decrease in bone health with prolonged stalling. While stalling has shown detrimental effects on bone density and strength, horses are often stalled for extended periods of time for multiple reasons. With this considered, a method of alleviating stalling related bone loss would be of value in the equine research and health industry. The further use of

research on horses using WBV could aid in finding a possible therapy for maintaining bone strength in horses.

## **CHAPTER II: THE INFLUENCE OF WHOLE BODY VIBRATION ON BONE MINERAL CONTENT AND BONE METABOLISM IN THE STALLED HORSE**

Safe, sound performance by the athletic horse is an important aspect of the horse industry. With the high physiological demand placed on the athletic horse, care must be taken to maintain or improve the health of these horses along with all equine populations. Lameness is the most common malady of the performing equine (Jeffcott et al., 1982; Morris and Seeherman, 1991; USDA, 1998). When lameness or any injury occurs in the horse, various treatment methods may be explored, which often includes prolonged stalling in an attempt to provide rest to the horse (Porr et al., 1998). Other reasons for long-term stalling include stalling during the weaning process to prevent biting, kicking, sun bleaching from outdoor turnout, and maintenance of quality body condition, especially in a competing horse (Heleski et al., 2002); several months of fitting in preparation for a sale (Bell et al., 2001); young performance horses beginning training (Hoekstra et al., 1999); and recovery from pregnancy (Mansell et al., 2001) and race or show season (Mansell et al., 2001). Performance horses are also often stalled during active competition seasons to prevent injury during turnout (Haupt and McDonnell, 1993; Christensen et al., 2002; Werhahn et al., 2012). However, considering these multiple reasons for long-term stalling of horses, previous research involving prolonged stalling has shown this to have a negative impact on bone health and strength (Porr et al., 1998; Hoekstra et al., 1999; Bell et al., 2001; Hiney et al., 2004a).

Prevention of injury is an important consideration in maintaining maximal health of the performing equine. One clinical method currently being investigated for its ability

to prevent and treat injuries in both humans and horses is whole body vibration (WBV), in which a vibration platform sends mechanical energy in the horizontal and/or vertical direction (Zepetnek et al., 2009; Rittweger, 2010; Cochrane, 2013; Halsberghe, 2017a) while the subject stands on the platform. This form of therapy has shown positive outcomes (Verschueren et al., 2004; Humphries et al., 2009; Lai et al., 2013) with some conflicting results (Torvinen et al., 2003) on human bone health. The use of WBV in equine medicine is based on the findings in human research and medicine, though little research has been conducted regarding its effect on bone health in the horse. The current study aims to evaluate the effect of WBV on bone mineral content and a bone degradation biochemical marker in stalled equines as a measure of the therapy's efficacy in maintaining bone strength.

### **Materials and Methods**

This study was approved by the MTSU Institutional Animal Care and Use Committee (Protocol #17-2017, Appendix A). Twelve healthy horses of mixed breed and age ( $18 \pm 1$  yr) were obtained from the Middle Tennessee State University (MTSU) teaching and research herd for use in the evaluation of WBV and its effects on bone strength. The 6 mares and 6 geldings were all under similar management conditions prior to the start of the study. All of the horses were turned out on pasture for 28 d to serve as a backgrounding period.

Horses were randomly assigned to either the control (CON, n=6) or treatment (VIB, n=6) group. During the experimental period, all horses were housed in 9.3 m<sup>2</sup> stalls for 28 d. All horses were fed prairie grass hay and a commercial pelleted concentrate

(Purina Strategy) twice daily to maintain body condition. The horses were provided *ad libitum* access to water throughout the study period. The weights of each horse were recorded on days 0, 14, and 28.

All horses were stalled for the duration of the 28-d experimental period. The VIB group was also placed on a whole body vibration platform (Equivibe, Malcom, Nebraska, USA) at 50 hertz for 45 minutes 5 d per week. The VIB horses were tied while standing on the vibration platform. The CON group was kept in their stalls throughout the experimental period, while the VIB group was stalled with their vibration therapy times. All horses were returned to the MTSU Horse Science Program following completion of the experimental period on day 28.

Digital dorsal-palmar and medial-lateral radiographs were taken of the left third metacarpal of all horses on days 0 and 28. The X-ray was set at 70 kV with an exposure of 0.16 seconds and a focal length of 90 cm. The cassettes for all radiographs had an aluminum stepwedge pentrometer attached. The radiographs were used to determine bone mineral content (BMC) for all cortices of the left third metacarpal for all horses using radiographic bone aluminum equivalence (RBAE). Measurements for cortical values were taken approximately 1 cm distal to the nutrient foramen. The radiographs were analyzed using BioRad Quantity One software, from which a regression model was formed using the known thickness of the aluminum stepwedge by the method presented by O'Connor-Robinson and Nielsen (2013). Measured values were recorded as mm Al.

Venous blood samples were collected from all horses on days 0, 14, and 28, via jugular venipuncture into 10 ml glass serum tubes. The serum tube samples were

coagulated after collection then centrifuged, aliquoted into microtubes, and stored frozen at  $-80^{\circ}\text{C}$  for later analysis. Serum pyridinoline cross-links (PYD) were analyzed using an enzyme immunoassay, previously validated in the horse, according to the manufacturer's instructions of the kit (MicroVue™ Serum PYD EIA Kit, Quidel Corporation, San Diego, CA).

Statistical analysis was performed using a mixed model ANOVA with repeated measures in SAS 9.4 (SAS Inst. Ver 9.4, Inc., Cary, NC). The changes in BMC and PYD were analyzed for day, treatment, and day by treatment interaction effects. A P-value of less than 0.05 was considered significant, and trends were considered with a P-value of less than 0.10.

## Results

BMC data were evaluated as changes from baseline values, reflecting day 0 to day 28. No treatment effect was found for change in BMC in the medial ( $P = 0.42$ ), lateral ( $P = 0.20$ ), dorsal ( $P = 0.07$ ), or palmar ( $P = 0.11$ ) cortices (Figures 1, 2, 3, and 4). Across both groups, an increase in BMC was found in the medial ( $+2.71 \pm 1.20$  mm Al,  $P = 0.03$ ), lateral ( $+2.96 \pm 1.04$  mm Al,  $P = 0.01$ ), dorsal ( $+1.72 \pm 0.62$  mm Al,  $P = 0.01$ ), and palmar ( $+1.85 \pm 0.55$  mm Al,  $P = 0.003$ ) cortices from day 0 to day 28 ( $P < 0.05$ ).

Concentration of PYD cross-links did not indicate a treatment effect ( $P = 0.85$ ) or a treatment by day effect ( $P = 0.26$ ). PYD concentrations showed a day difference ( $P = 0.03$ ), where both CON and VIB groups increased from day 0 to day 14 ( $+0.49 \pm 0.18$  nmol/L,  $P = 0.01$ ). There was no difference between day 0 and day 28 ( $P = 0.36$ ) or between day 14 and day 28 ( $P = 0.06$ ).

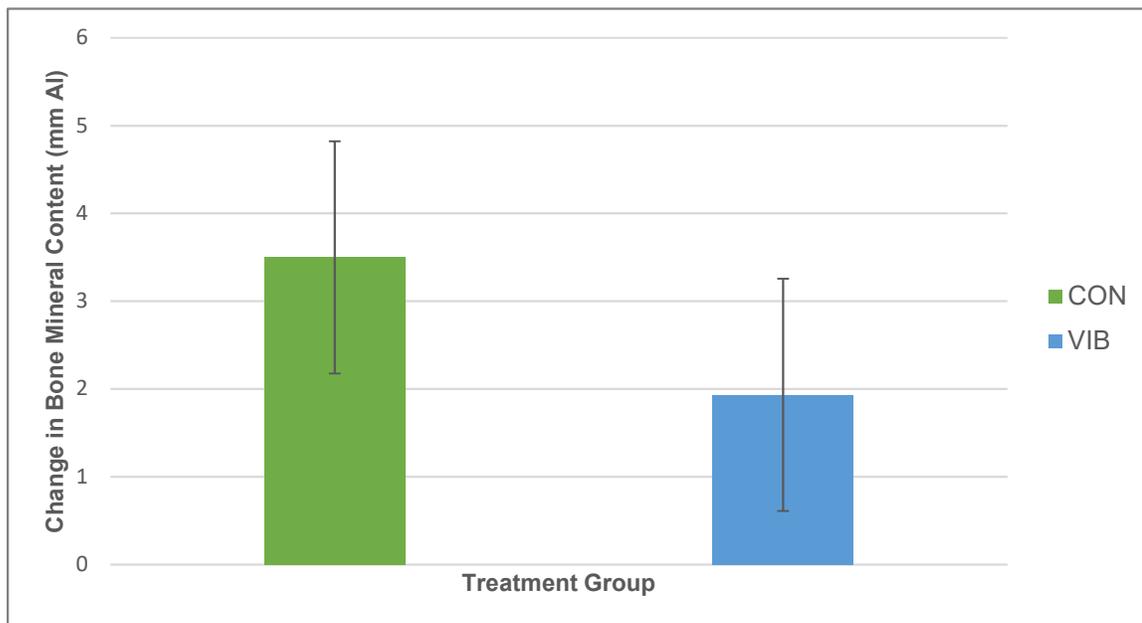


Figure 1. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the medial cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.

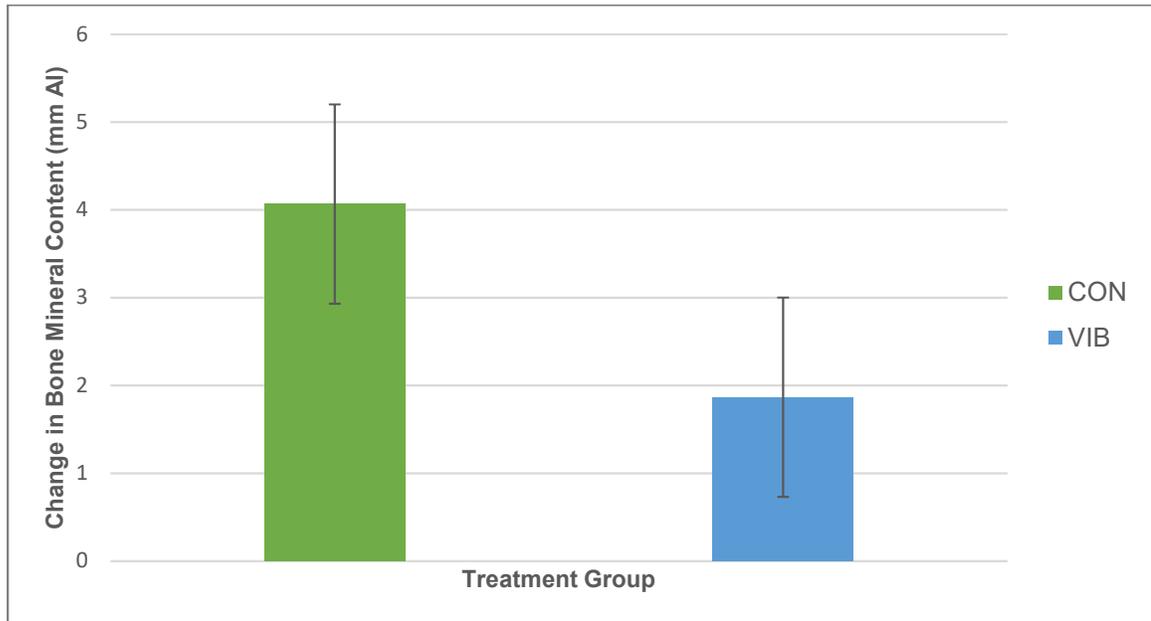


Figure 2. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the lateral cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.

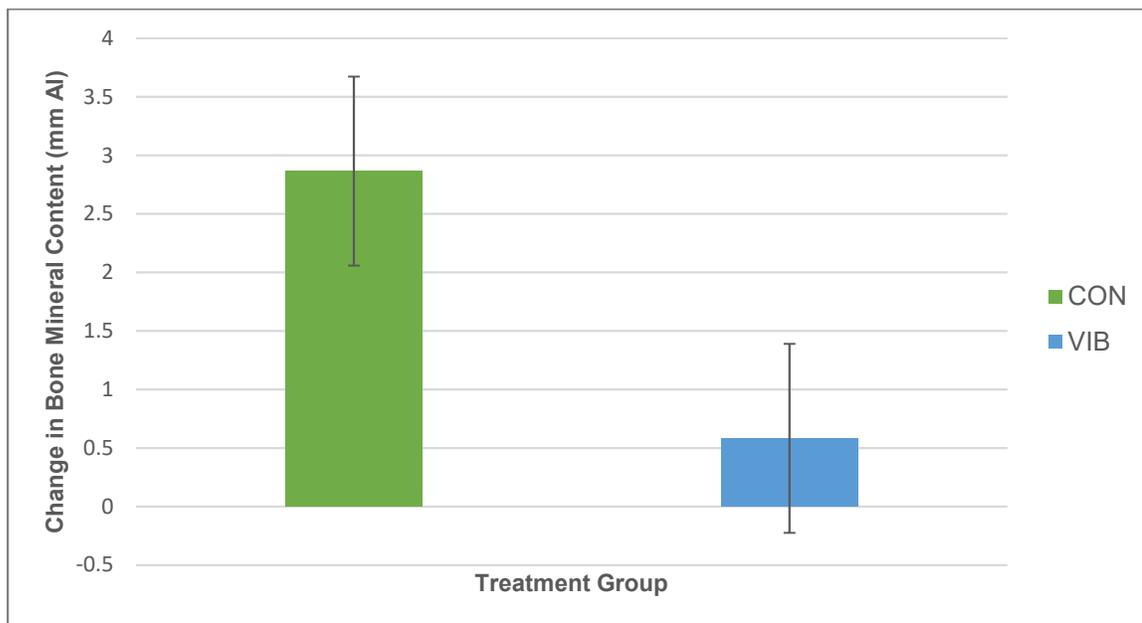


Figure 3. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the dorsal cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.

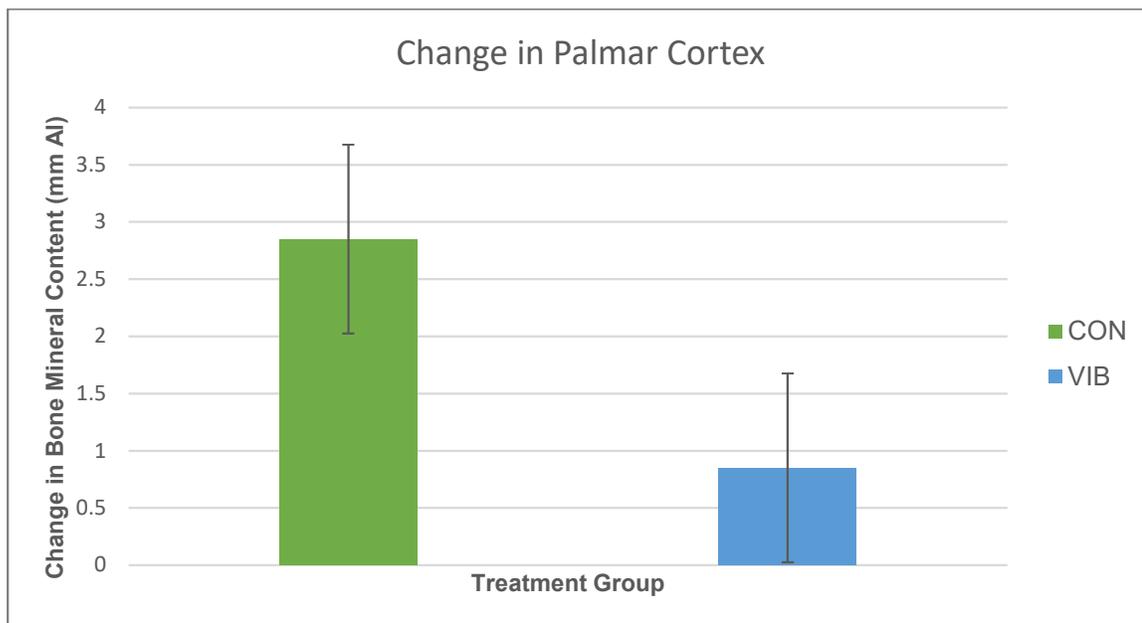


Figure 4. Mean change in bone mineral content (mm Al) as determined by radiographic bone aluminum equivalence (RBAE) in the palmar cortex of the equine left third metacarpal bone over a 28-day stalling period where VIB horses received whole body vibration therapy.

## Discussion

The focus of this study was to evaluate the effect WBV would have on bone parameters in horses stalled for 28 days. Previous research has shown a decrease in BMC when horses are stalled for a period of time (Porr et al., 1998; Hoekstra et al., 1999; Maher et al., 2017). However, the current study found an increase in BMC after 28 days of stalling across both the treatment and the control group. The reason for this increase rather than decrease is unclear as previous studies have found a change in as little as 14 days (Bell et al., 2001). Thus, a decrease in bone density through decreased BMC and increased bone degradation would have been expected for the horses stalled for 28 days in this study.

A study by Maher et al. (2017) evaluated the effect of WBV in horses stalled with forced exercise. The horses in the study were stalled for 28 days with both control and WBV horses exercised on a mechanical exerciser. Results showed a decrease in BMC and an increase in PYD concentration after 28 days of stalling (Maher et al., 2017). Contrary to the results from Maher et al. (2017), the current study found the opposite day effect of an increase in BMC during the 28-d stalling period. These unexpected findings are evident even with the stalling confinement without exercise as compared to stalling with exercise in Maher et al. (2017). However, the current study does show an increase in PYD concentration from day 0 to day 14, which is supported by the results in Maher et al. (2017). The similarities in the study designs suggest the current study should have seen a decrease in BMC, rather than the increase in BMC seen in both groups.

Bell et al. (2001) also examined the effect of stalling on bone, finding a negative effect within 28 days of confinement. With the research horses in the study divided into

pasture, partial pasture, and stall groups, results found a lower BMC in the stalled group as compared to the pasture and partial pasture groups within 28 days. The results provided by Bell et al. (2001) demonstrate the ability of bone to change within 28 days. As such, the 28-d stalling period would be expected to have sufficient time to demonstrate a decrease in BMC in the current study. However, the opposite effect, an increase in BMC, was seen in the stalled horses across both the CON and VIB groups. The reason for this increase in BMC remains unclear.

Little to no change in BMC was discovered in a study by Hiney et al. (2004a), which examined weanlings divided into group housed, confinement with no exercise, and confinement with exercise groups. Results from the study showed no change in BMC in the confinement with no exercise group, where a growth in bone in weanlings would be expected in those without confinement limitations. Behavior was recorded in the study and found excessive pawing behavior in the confinement with no exercise group. This pawing was suggested to have provided the high impact loading on the bone needed to maintain BMC (Hiney et al., 2004a). While behavior was not recorded in the current study, anecdotally, excessive pawing or other stereotypies were not observed. The current study utilized mature horses rather than weanlings used in the research by Hiney et al. (2004a), in which the weanlings would be undergoing more changes in the bone structure and may be more easily susceptible to adaptations. The reason for an increase rather than no change or a decrease in BMC remains unclear still.

The current study's results regarding PYD concentration are supported by previous research in stalling horses. Hoekstra et al. (1999) evaluated pasture-reared

yearlings in comparison to stall-reared yearlings. Results of the study found an increase in deoxypyridinoline concentration in the stalled horses over the horses on pasture on day 28, which then returned to baseline values for the remainder of the 140-d experimental period (Hoekstra et al., 1999). In the current study, PYD concentration increased to day 14 and then remained unchanged for the remaining experimental period. The increase in the PYD bone degradation marker is supported by previous research as in Hoekstra et al. (1999). The increase in PYD concentration from day 0 to day 14 in both the VIB and CON groups suggests bone degradation did occur in the first 14 days of the study. However, it is unclear why this increase in bone breakdown did not result in a decrease in BMC.

The backgrounding period before day 0 of the study may have an influence on the potential outcomes. As the purpose of the backgrounding period is to theoretically bring all horses to maximal BMC, the horses are typically turned out on pasture because the typical horse will sprint (load the bone) often enough to allow them to reach their maximum BMC. However, the less excitable, mature, school-type horses, such as those used for the current study, may not run often enough during turnout to reach this maximum BMC. Perhaps consideration should be given to using forced sprinting exercise as a method of reaching the highest level of BMC in the quieter horses whom may not reach this BMC level on pasture turnout alone. This maximum level BMC before day 0 gives the horse the ability to decrease or maintain BMC as needed. Though the turnout backgrounding period in this study may not have allowed horses to reach their maximal BMC, an increase in BMC would still not be expected with the stalling period. This

unusual increase in BMC does not allow for determination of the ability of WBV to maintain BMC in stalled horses, who would typically see a decline in bone density.

### **Summary and Conclusion**

The ability for the equine athlete to perform at a high-quality level is important for the welfare of the horse as well as success on the track or in the show ring. With the high physiological demand instilled upon these athletes, care must be taken to increase the health or maintain proper health of the horse. Keeping the horse at its peak health includes consideration of the care and housing methods used. Horses are often kept in stalls for a variety of reasons, which may include recovery from an injury, fitting for a sale, initiating training of young horses, and maintaining performance horses during competition season. However, previous research has discovered this prolonged stalling of horses has a detrimental impact on bone health. This negative effect on bone strength will have an undesirable influence on the overall health of the horse and put the horse at an increased risk of fracture. Methods for prevention of such injury are of great interest to the area of equine health. One new and emerging therapy in equine and human medicine is WBV, which has shown positive effects, with some conflicting results, in human research on bone health. There has been limited research on WBV using equine subjects, though, and current use in the horse industry is often based on human research. The current study sought to evaluate the influence of WBV on bone health in stalled horses, which typically see a decline in bone health. This study found no treatment effect between the WBV and CON groups. Results did show a day effect where both groups actually increased in BMC over the 28-d stalling period. PYD concentration also demonstrated a day effect, increasing across both groups from day 0 to day 14 and

remaining unchanged to day 28. The reason for the rise in BMC while undergoing an increase in bone degradation remains unclear. The results of this study suggest no influence of WBV on BMC or bone metabolism in stalled, healthy horses if no stalling-related loss in BMC is observed. Further research is needed to determine if WBV will have a positive impact on bone health in stalled horses because this study was unable to show an expected decrease in BMC. Future research should consider using forced exercise to reach maximal BMC during the backgrounding period if the horses used for the research will not reach this level on turnout alone. Keeping records of behavior should also be considered when performing future studies on stalled horses in order to determine whether any behavior is occurring that may influence the outcome of the research. Due to the positive effects of WBV shown through human research, future studies are needed to determine the influence of this therapy on equine bone health.

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**APPENDIX**

## APPENDIX A: IACUC Approval

### IACUC

INSTITUTIONAL ANIMAL CARE and USE COMMITTEE  
Office of Research Compliance,  
010A Sam Ingram Building,  
2269 Middle Tennessee Blvd  
Murfreesboro, TN 37129



### IACUCN001: PROTOCOL APPROVAL NOTICE

Friday, June 23, 2017

Principal Investigator: Holly Spooner  
Co-Investigator(s): Amy Berger, Seneca Sugg and John Haffner  
Investigator Email(s): *holly.spooner@mtsu.edu; ab5k@mtmail.mtsu.edu; sj3wr@mtmail.mtsu.edu; johnHaffner@mtsu.edu*  
Department/Unit: ABAS, CBAS

Protocol ID: **17-2017**  
Protocol Title: *Influence of vibration therapy on biomechanics and bone density in the stalled horse*

Dear Investigator(s),

The MTSU Institutional Animal Care and Use Committee has reviewed the animal use proposal identified above under the *Designated Member Review (DMR) mechanism* and has approved your protocol in accordance with PHS policy. A summary of the IACUC action(s) and other particulars of this protocol is tabulated as below:

IACUC Action	APPROVED for one year	
Date of Expiration	6/30/2018	
Number of Animals	12 (TWELVE)	
Approved Species	Equus caballus – MTSU Herd	
Category	<input type="checkbox"/> Teaching <input checked="" type="checkbox"/> Research	
Subclassifications	<input type="checkbox"/> Classroom <input checked="" type="checkbox"/> Laboratory <input type="checkbox"/> Field Research <input type="checkbox"/> Field Study <input type="checkbox"/> Laboratory <input checked="" type="checkbox"/> Handling/Manipulation <input type="checkbox"/> Observation	
	Comment: NONE	
Approved Site(s)	MTSU Horse Science Center	
Restrictions	Satisfy DMR requirements AND annual continuing review	
Comments	Student health screening completed to different IACUC protocol(s)	

This approval is effective for three (3) years from the date of this notice. This protocol expires on 6/30/2020. The investigator(s) MUST file a Progress Report annually regarding the status of this study. Refer to the schedule for Continuing Review shown below; NO REMINDERS WILL BE SENT. A continuation request (progress report) must be approved by the IACUC prior to 6/30/2018 for this protocol to be active for its full term. Once a protocol has expired, it cannot be continued and the investigators must request a fresh protocol.

#### Continuing Review Schedule:

Reporting Period	Requisition Deadline	IACUC Comments
First year report	4/30/2018	INCOMPLETE
Second year report	4/30/2019	INCOMPLETE
Final report	4/30/2020	INCOMPLETE

IACUC

Office of Compliance

MTSU

## Post-approval Protocol Amendments

Date	Amendment	IACUC Comments
NONE	NONE	NONE

The MTSU IACUC defines an "investigator" as someone who has contact with live or dead animals for research or teaching purposes. Anyone meeting this definition must be listed on your protocol and must complete appropriate training (refer below or [click here](#)). Adding new investigators requires submission of a specific addendum request. Changes to the approved protocol or adding new investigators must be approved by the IACUC before implementation.

Unanticipated harms to the research animals, adverse events or changes to the funding status of the protocol must be reported within 48 hours to the Office of Compliance at (615) 494-8918 and by email – [compliance@mtsu.edu](mailto:compliance@mtsu.edu).

All records pertaining to the animal care must be retained by the PI, or person in charge of the protocol, for at least three (3) years AFTER the study is completed. **Be advised that all IACUC approved protocols are subject to audit at any time and all animal facilities are subject to inspections at least biannually.** Furthermore, IACUC reserves the right to change, revoke or modify this approval without prior notice.

Sincerely,

MTSU Institutional Animal Care and Use Committee  
 Middle Tennessee State University  
 Tel: 615 494 8918  
 Email: [iacuc\\_information@mtsu.edu](mailto:iacuc_information@mtsu.edu) (for questions) and  
[iacuc\\_submissions@mtsu.edu](mailto:iacuc_submissions@mtsu.edu) (for sending documents)

IACUC Training - [http://www.mtsu.edu/iacuc/IACUC\\_Training.php](http://www.mtsu.edu/iacuc/IACUC_Training.php)  
 IACUC Forms -