5-1-2020

Effect of vibration stimulation on muscle and bone parameters in mature stock-type horses on stall rest

Ashley L. Greene

Recommended Citation

Greene, Ashley L., "Effect of vibration stimulation on muscle and bone parameters in mature stock-type horses on stall rest" (2020). *Theses and Dissertations*. 1694.
https://scholarsjunction.msstate.edu/td/1694

Effect of vibration stimulation on muscle and bone parameters in mature stock-type horses on stall rest

By

Ashley Lorraine Greene

Approved by:

Clay A. Cavinder (Major Professor)
Cathleen A. Mochal-King
Caleb O. Lemley
Thu Dinh
Jamie Larson (Graduate Coordinator)
George M. Hopper (Dean, College of Agriculture and Life Sciences)

A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Agriculture
in the Department of Animal and Dairy Sciences

Mississippi State, Mississippi

May 2020

Name: Ashley Lorraine Greene

Date of Degree: May 1, 2020

Institution: Mississippi State University

Major Field: Agriculture

Major Professor: Clay A. Cavinder

Title of Study: Effect of vibration stimulation on muscle and bone parameters in mature stock-type horses on stall rest

Pages in Study: 48

Candidate for Degree of Master of Science

Current industry practices promote therapeutic use of pulsatile stimulation plates to increase muscle mass, strengthen bone density, reduce stress, and improve overall athletic performance of horses. The first objective of this study was to investigate the effect of vibration stimulation on muscle thickness of the *extensor carpi radialis*, *extensor digitorum longus*, *gluteus medius*, *longissimus lumborum*, *semitendinosus*, *supraspinatus*, and *longissimus thoracis*, as well as circumference and cross-sectional area of the *extensor carpi radialis* and *extensor digitorum longus*. The second objective was to evaluate changes in nutrient foramen thickness, circumference, and area, as well as dorsal cortical thickness of the left third metacarpal in response to vibration stimulation exposure over 56 d. Increases in thickness of the topline muscles and improvement of nutrient foramen parameters of treatment horses give evidence to conclude that vibration stimulation may be a viable therapeutic treatment for stock-type horses on stall rest.

ACKNOWLEDGMENTS

First, I would like to acknowledge my committee members, Drs. Clay Cavinder, Caleb Lemley, Cathleen Mochal-King, and Thu Dinh, for their guidance throughout my research project. Moreover, I'd like to thank Dr. Cavinder for taking a chance on me as a graduate student, trusting me with the MSU horse judging website, and allowing me to help with extension events. I will forever cherish the life skills you have taught me, and I am sincerely grateful for the time you spent pushing me to be the best possible version of myself. I would also like to thank Dr. Dinh for teaching me so many valuable skills in the lab, and for spending a great deal of time helping me understand my statistical analysis. Thank you to Dr. Lemley for patiently answering any and all of my physiology questions. And lastly, thank you Dr. Mochal-King for teaching me new skills that I hope to utilize in my future career, and for helping me learn more about the incredible animals that have been my passion for many years. Your guidance throughout the data collection of this project was truly invaluable.

Next, I must acknowledge my family who so patiently supported me throughout this endeavor. Without their endless encouragement and love throughout my time in graduate school, I would not be where I am today. I am truly blessed to have a family who supported my decision to move so far away from home and relentlessly encouraged me to pursue higher education. Lastly, I couldn't possibly thank my other half, Patrick, enough for never allowing me to doubt myself and for being my rock throughout this process. I truly would not have been able to finish this degree without your love and support, and I cherish each moment we have spent on this journey together.

Most importantly, I owe this thesis and a debt of gratitude to my savior, Jesus Christ, who has blessed me with this incredible opportunity to further my education. Without His love, faithfulness, and answered prayers, the completion of my research project and thesis would not have been possible.

TABLE OF CONTENTS

CHAPTER

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

In the performance horse industry, lameness is a major concern that can lead to a loss of training time. Many performance horses suffering from varying degrees of lameness are placed on stall rest which can ultimately lead to muscle atrophy thus increasing the time it takes to rehabilitate the injured athlete. As a result, many owners and trainers have been searching for ways to deal with these issues. While a period of stall rest is often necessary for the recovery of an injured horse, continuous immobilization or disuse will cause a decrease in the size, circumference, and maximum strength of muscles (Berg et al., 1997). The search for a well-rounded therapeutic option that can prevent as well as treat injuries, prepare horses for competition, and aid in training efforts is a focus in the industry. Thus, the use of vibration stimulation for equine athletes is growing in popularity. Performance horse injury diagnosis and prevention is a very active and costly business. It can become a financial and personal burden to horse owners when an equine athlete becomes injured and is sentenced to stall rest, thus resulting in loss of use, increased labor time, and veterinary consultation (Bailey et al., 1997; carrier et al., 1997). Additionally, it can become an even greater issue when selling a previously injured performance horse, even though a full recovery may have been made. Potential buyers may choose to have a pre-purchase exam performed and will likely negotiate the buying price or even pass up the opportunity of buying due to the common assumption that a previous injury may reoccur.

While the use of vibration plates is relatively new to the equine industry, it is being used with the intent to prevent injury, aid in the recovery of lameness and skeletal injuries, promote blood circulation, as a warm-up/cool-down for exercise, and other beneficial claims. TheraPlate® is a commercially available form of vibration therapy that is currently utilized in both humans and several species of animals for a variety of assumed whole-body benefits. The TheraPlate® uses a "proprietary technology" known as vortex wave circulation stimulation (VWC), which claims to use centrifugal force and oscillating internal movement to deliver zero-impact therapy. However, very little research has been done to investigate possible variations in simple vibration and claimed VWC. Still, there is speculation on the effects and potential benefits of whole-body vibration (WBV) in several species including humans and horses, but the primary focus has not been focused on the use of VWC.

With limited research present on the effect of vibration stimulation on muscle and bone parameters in horses, it is of interest to put scientific merit to the claims currently leading the therapeutic trends in the industry. Therefore, the objectives of this study are to measure the effect of vibration treatment on physiological changes in muscle thickness, muscle circumference, muscle cross-sectional area, nutrient foramen area, circumference, and thickness, and dorsal cortical thickness in mature, stock-type horses subjected to 8 wk of stall rest.

This manuscript has been formatted according to the Instructions for Authors for the Journal of Livestock Science.

CHAPTER II

LITERATURE REVIEW

Characteristics of Bone Growth and Remodeling

Bone is a connective tissue that is constantly changing in response to factors such as mechanical loading, endocrine, and paracrine cues (Sims and Gooi, 2008). During the turnover of bone, several steps must occur for the complete process to function well. Osteoclasts start by digesting old bone, a process also referred to as resorption. This is followed by the reversal step where mononuclear cells appear on the surface of the bone. Next, osteoblasts lay down newly formed bone until the area where resorption occurred is completely replaced, also known as the formation step (Lepage et al., 2001; Pagani et al., 2005). The architecture of a bone will be altered through this remodeling process in response to changes in loading or in order to achieve bone growth, and the remodeling can be described as the communication between the osteoblasts, osteoclasts, and osteocytes. This modeling process is common in animals that are young and growing during mechanical loading or the period of long bone growth, it includes changes in the shape, length, or size of bone (Price et al., 1995; Nielsen et al., 1997).

Matsuo and Irie (2008) described the 3 phases of bone remodeling as initiation, transition, and termination. Communication between the stages initiation and transition are opposites, and can occur through cell-cell contact, paracrine factors, and cell-bone matrix communication. This means that during the initiation phase communication flows from the osteoblasts to osteoclast precursor cells, and during the transition stage from osteoclasts to osteoblasts. The initiation phase can be better explained as the resorption stage where osteoclasts are signaled to digest old bone. Next, the transition phase can be explained as the step where the resorption of bone is inhibited, apoptosis of the osteoclasts occurs, and a signal is sent for the activation of osteoblasts. Lastly is the termination phase, during which new bone is formed (Matsuo and Irie, 2008). This process of bone reformation is considerably slower than the resorption of the old bone (Matsuo and Irie, 2008).

Damage can be a factor that can result in bone remodeling. More commonly, microdamages that occur in the matrix of the bone as a response to repetitive loading cycles beyond the threshold of the individual. But, according to Wolff's law, bone can be altered in response to the new stress being placed on it (Prisby et al., 2008). Bone remodeling in response to this stress can guarantee the integrity and mass of the bone through the rest of the animal's life (Price et al., 1995). However, if left unchecked too many occurrences of microdamages can lead to a complete fracture of the bone (Carrier et al., 1997; Nielsen et al., 1997). Therefore, it is of the utmost importance to know the effects of bone remodeling and microdamages in horses being exercised.

To compliment the knowledge of bone physiology, another important factor to the performance horse industry is knowing how different training methods provide various amounts of strain on a horse's bones. This insight can help forestall skeletal injuries or wasting in horses starting the training process (Carrier et al., 1997; Nielsen et al., 1997; Porr et al., 1998). Nielsen and colleagues (1997) studied changes in bone mineral content and frequency of bone injuries in young Quarter Horses entering race training. During this study, 53 Quarter Horses, at 18 mo of age, entered a race training program. The demineralization and remineralization of bone were characterized in the medial cortex of the third metacarpal via radiographs measuring bone density. The aim of the study was to determine the relationship between injuries and changes in bone integrity that happen during race training. Horses in the study were separated into 2 groups, those who did not sustain an injury during the training protocol and those who did. It was concluded that the overall radiographic bone aluminum equivalent (RBAE) was higher in the group that did not sustain an injury during race training as compared to the group that did receive an injury (Nielsen et al., 1997). These results illustrate that starting training subsequently initiated changes in bone remodeling and raised the amount of space where remodeling was happening.

A common anatomical area of study in the horse is the third metacarpal bone, also referred to as the cannon bone. The cannon bone is a long bone in the forelimb of the horse that is responsible for both weight-bearing and shock absorption (Garita, 2002; Couch and Nielsen, 2017). A common cause of lameness in racehorses, dorsal metacarpal disease, is commonly observed in the third metacarpal bone. Dorsal metacarpal disease is commonly known as bucked or sore shins amongst the equine performance horse industry. This ailment is often characterized by several signs or symptoms which include lameness, stride impairment (Setterbo, et al., 2008), swelling, with or without heat which can be palpated over the dorsal aspect of the third metacarpal (Nunamaker et al., 1990; Nunamaker et al., 1991; Verheyen et al., 2005), and a thickening of the dorsal aspect of the third metacarpal which can be seen via radiographs (Finsen and Andra, 1988). Norwood (1978) reported that approximately 70% of Thoroughbreds under the age of 2 yr that were in training had been diagnosed with dorsal metacarpal disease. Additionally, it was noted in young racehorses in Australia that up to 80% were affected by dorsal metacarpal disease (Buckingham and Jeffcott, 1990). Although bucked shins can be treated with rest or reduced exercise, they often leave the cannon bone more susceptible to future fracture at the same site where the dorsal metacarpal disease occurred.

A small, naturally occurring, yet critical factor about the cannon bone of horses, known as the nutrient foramen, has rarely been studied in equine science. Long bones are known to have a nutrient foramen near the "growing end" of the bone, meaning the bone that contributes the majority of the growth (Cunningham and Black, 2016), which provides passage for a nutrient artery. In the third metacarpal bone, the nutrient foramen serves as a portal that allows blood flow of the nutrient artery to reach the distal part of the horse's limb (Garita, 2002). As previously stated, the nutrient foramen is a small aspect of the bone and therefore has not been highly investigated in horses. However, Götzen et al. (2003) sought to understand structural stress surrounding the nutrient foramen in the equine third metacarpal and found that a reduced incidence of stress cracks or fractures around the nutrient foramen could be due to the presence of lamellar bone alongside the edge of the foramen. With limited research regarding the nutrient foramen in live horses, it is of interest to measure changes in the area, diameter, and circumference of the nutrient foramen in this study.

Muscle Physiology and Changes Associated with Training

The skeletal muscle system is quite complex and is responsible for locomotion (Hopkins, 2006). Skeletal, or striated, muscle is connected to bone via tendons that cross over joints and activate either flexion or extension of the joint following contraction or relaxation of the involved muscles (Zierath and Hawley, 2004). Skeletal muscle can be broken down into bundles of long muscle fibers which are the multinucleated cells fused together. Each muscle fiber contains myofibrils, which are the contractile components of muscle. Each myofibril is made up of a series of sarcomeres, which are microscopic contractile units that are comprised of actin (thin) and myosin (thick) filaments, I-bands, an A-band, and Z-discs. The central nervous system is responsible for controlling voluntary or conscious muscle movement by sending an action potential to the muscle fibers via motor neurons. Then, the motor neurons release acetylcholine which will cross the synaptic cleft of the muscle's motor endplate and cause a subsequent release of Ca from the sarcoplasmic reticulum resulting in muscle contraction. The release of this Ca into the muscle cell and its binding to troponin will allow for actin and myosin to adhere to each other. This leads to the binding and contraction of actin and myosin cross bridges which utilize ATP for energy. Next, ATP is resynthesized which allows actin and myosin to maintain their current bound state. Finally, relaxation of the muscle occurs when nerve stimulation ends and Ca returns to the sarcoplasmic reticulum or the muscle becomes depleted of energy sources (glycogen, ATP, etc; Hopkins, 2006).

Different fiber types are characterized by metabolism, speed or contraction, and time to fatigue. Muscle fiber type is known to influence performance of both human and equine athletes (Gollnick et al., 1972; Costill et al., 1976; Fink et al., 1977; Essen-Gustavsson et al., 1984; Rivero and Piercy, 2004). Slow-twitch, or type I, fibers are responsible for slow, consistent contractions which are often observed in endurance exercise or postural muscles. Type I fibers are known for their low myosin-ATPase activity, but high oxidative capacity, making them much less susceptible to exhaustion. Additionally, type I fibers utilize aerobic metabolism to produce ATP and they are said to be the most fatigue resistant of the muscle fiber types. Horses competing in endurance focused activities or long-distance runners are known for having an abundant amount of type I muscle fibers. Fast twitch, or type II, fibers can be broken down into type IIa (moderate-fast twitch) and type IIb (fast-twitch) categories. Type IIa muscle fibers contract more rapidly, are thicker, and become exhausted more quickly than slow-twitch fibers.

These fibers can use both aerobic and anaerobic metabolism to produce energy for work. They are most common in horses that compete in events such as jumping. Finally, type IIb muscle fibers are responsible for explosive, short bursts of activity. They are known for being the strongest of the fiber types, being activated when maximum exertion is reached, and are the quickest to reach exhaustion. Additionally, type IIb fibers use anaerobic metabolism to produce energy in the form of ATP which is produced during the breakdown of glucose and/or glycogen in the glycolysis cycle. While type of work, or discipline, can sway the ratio of type I to type II fibers (Pette and Staron, 1997; Rivero and Piercy, 2004), breed can also have an influence on this ratio (Stull and Albert, 1980). For example, Quarter Horses and Thoroughbreds have a lower percentage of type I fibers while breeds such as Arabians have a higher percentage of type I fibers (Lopez-Rivero et al., 1991). This makes Quarter Horses and Thoroughbreds more predisposed to high-intensity, short term activities such as cutting and speed events. Comparatively, Arabians utilize their type I fibers to successfully complete endurance races, which involve aerobic, submaximal intensity exercise.

Two types of muscle growth occur during the life of an equine athlete (Gunn, 1978a; Gunn, 1978b). The first, hyperplasia is growth of the muscle where there is an increase in the number of cells present, like during fetal growth stages. The second type of muscle growth is called hypertrophy, and it is the increase in the size of the present muscle cells. Hypertrophy occurs when protein synthesis is increased, exercise causes micro-tears in the muscle, and satellite cells are activated. Moreover, the diameter of the muscle fiber increases during hypertrophy when the muscle is stretched, causing splitting to occur at the Z-discs of the sarcomere. Undifferentiated muscle stem cells, more commonly referred to as satellite cells, are activated by cytokines and growth factors. Insulin-like growth factor is responsible for the differentiation and proliferation of the satellite cells while fibroblast growth factor-2 is only responsible for proliferation of satellite cells (La Vigne et al., 2015).

Muscle atrophy is the decrease in muscle size and ability to generate force (Berg et al., 1997) which can be associated with an injury or immobilization that leads to the disuse of a muscle or group of muscles. Atrophy associated with the disuse of muscles is commonly seen in patients on long term bed rest for health complications that require hospitalization or following a surgical operation (Berg et al., 1997; Dhert et al., 1988). In humans, it has been documented that muscle disuse or immobilization can result in a notable decrease in muscle mass, circumference, and strength (Narici and de Boer, 2011). An approximately 14% decrease in cross-sectional area of the knee extensor muscle was observed in human ICU patients on bed rest for a period of 6 wk (Berg et al., 1997). This study states the reduced muscle cross-sectional area was not attributed to a change in the muscle fiber type, but from the disuse of the muscle. A study by Ferrando et al. (1995) documented a 3% loss of thigh muscle volume in humans exposed to a strict 7-d bed rest period.

As previously stated, breed can influence the ratio of muscle fiber types, as noted with Standardbred foals who will see a decrease in the glycolytic capacity of their muscles as they age and train (Roneus et al., 1992). Comparatively, adolescent Thoroughbreds will see an increase in the percentage of type IIa fibers and a decrease in type IIb fibers in response to training (Yamano et al., 2002). Changes in glycolytic and oxidative capacity in addition to shifts in the ratios of type I to type II fibers can be credited to gender, breed, genetics, and the increase in proprioception (which is the horse’s awareness of its body position and movements, including limb and hoof placement) and coordination seen as foals age (Roneus et al., 1992).

When horses begin training, an increase in the type IIa and type IIb as well as an increase in muscle fiber size is observed (Roneus et al., 1992). However, the aerobic exercise for endurance and long-distance racehorses will not lead to an increase in muscle fiber size because this type of training favors and increase in the presence of type IIa fibers, which are smaller in cross-sectional area. This increase in type IIa fiber presence is crucial for long-duration, low intensity exercise because the lesser muscle fiber cross-sectional favors a more rapid oxygen transport and quicker waste removal from the muscle tissue being utilized. Within the first several months of training, an increase in of the oxidative enzyme activity of skeletal muscle as well as increases in the number of oxidative fibers (type IIa) and the overall oxidative capacity of the muscle will be observed. Additionally, as training continues, type IIa fibers will be converted to type IIb fibers (López-Rivero et al., 1992).

Effect of Stall Rest on Horses

It is quite common for horses to be stalled for long periods of time. Several reasons for stalling horses include injury recovery (Porr et al., 1998); maintenance of body condition (Heleski et al., 2002); fitting and sale preparation (Bell et al., 2001); and preventing injury from pasture mates. “Wastage,” a term commonly used in the Thoroughbred racing industry, refers to the time a horse spends absent from training because of a medical predicament or injury (Bailey et al., 1997). Wastage includes both physiological and financial factors, money spent on treating the injury or ailment and rehabilitation, and money lost as a result of removing a horse from competition. The treatment of the medical issue or injury is often stall rest, a halt in training, and often very limited exercise. The length of time spent on stall rest is heavily dependent on the injury sustained by the individual. For example, a lesser injury may only require a wk of stall confinement whereas a tendon injury would require 6 to 8 mo of strict stall rest. While stalling horses for long periods of time is popular within the industry, it has been documented that long-term stall rest can be detrimental to bone density and muscle mass in equids (Bell et al., 2001; Hiney et al., 2004).

As previously stated, if bone is not able to adapt to its mechanical environment, it will be more susceptible to injury. While most bone remodeling happens while animals are young, bone growth in horses can be influenced by their environment. Skeletal loading has been associated with loss of bone in horses (Bell et al., 2001; Nielsen et al., 1997; Porr et al., 1998). Such bone loss occurs because of a greater amount of resorption than formation. Currently, a leading loss of training time in performance horses is lameness (Rossdale et al., 1985), which can ultimately lead to a period of stall rest and consequently a loss of bone mineral content. In a study conducted by Porr et al. (1998), a weekly decrease of 0.45% bone mineral content in the third metacarpal was observed during a 12-wk deconditioning period. Such a loss in bone mineral content has the potential to lead to injuries of the skeleton and become more concerning if the horse is rushed back into intense work.

It is widely known that many horses in the racing industry sustain life ending injuries and that many casualties in the Thoroughbred racing industry of California are a result of severe skeletal injuries (Carrier et al., 1997). Many of these skeletal injuries of Thoroughbred racehorses in California are fractures. Carrier et al. (1997) found that horses who returned to training after they were laid-up for 60 days following a humeral fracture were at a greater risk for receiving another humeral fracture. With bone being more adaptable in younger horses, it is crucial that proper bone development is prioritized at an early age in order to prevent potential injuries to the skeleton later in life (Hoekstra et al., 1999). A previous study has shown that access to pasture every day will increase bone mineral content in weanlings as opposed to those kept in a stall (Bell et al., 2001). Bell et al. (2001) also noted that weanlings given 12 and 24 h on pasture had greater RBAE than weanlings kept in a stall. Access to free choice and deliberate exercise, even short duration, can help prevent the negative effects of stall rest (Hiney et al., 2004). Hiney et al. (2004) hypothesized that short-duration exercise would improve the observed decrease in bone mass associated with stall rest. The study used 18 Quarter Horse weanlings that were subjected to stall confinement and randomly assigned to 1 of 3 treatment groups: group housed, stall rest with exercise, and stall rest without exercise. Following the 8 wk trial, researchers concluded that short-term exercise could be effective in combatting the negative effects of stall rest seen in bone parameters of weanlings (Hiney et al., 2004). Utilization of the knowledge of how stall rest can affect bone is crucial for performance horse handlers when resuming training following prolonged stall rest in order to prevent potential injury associated with decreased bone strength or density.

Vibration Therapy

The use of vibration stimulation as a therapy in humans is a common practice as it is easily applied in a low impact manner. The therapy is applied to the individual via a plate that delivers pulsatile stimulation via vibration or oscillation. This makes it especially appealing for those who struggle with poor muscle strength, mobility, or other physical impairments (such as diseased patients or the elderly) preventing their ability to use higher impact treatments or therapies such as weightlifting. It has been observed in humans, sheep, and rats, that vibration stimulation increases quantity of bone, improves balance, and has several other positive effects on health parameters. In a study of children with cerebral palsy, vibration treatment for 4.4 min per d for 60 d increased bone mineral density in the proximal tibia by 6%, while the non-vibration group showed a decrease of 12% over the same period (Ward et al., 2004).

Verschueren et al. (2004) studied the use of vibration stimulation to influence bone density and prevent osteoporosis in postmenopausal women and reported an increase in bone mineral density in the hip by 0.93%.

Additionally, bone mineral density improvements were found in both sheep and rats that underwent WBV treatments (Rubin et al., 2002; Oxlund et al., 2002; Stuermer et al., 2014; Wei et al., 2014). Rubin and colleagues (2002) studied the effect of WBV on the quantity and quality of trabecular bone of the femur of sheep. To do so, they stimulated the hind limb of the sheep with vertical oscillation for 20 min per d, 5 d per week at an intensity of 30 Hz. There was an observed increase in the quantity of bone because of both an increasing number of trabeculae and an increase in the thickness of the pre-existing trabeculae (Rubin et al., 2002).

While WBV has been thoroughly researched in humans, very little research has examined the effects of WBV on horses. A study by Hulak (2015) sought to determine the effect of WBV on bone mineral content in horses. The treatment group was stalled and received WBV treatment whereas control horses were stalled and subjected to light exercise. It was reported that the utilized level of WBV did not cause an increase in bone mineral content, but it did maintain a similar baseline to that of the control, light exercising horses (Hulak, 2015). Additionally, a study by Maher et al. (2016) exercised horses 6 d per wk for 1 h to stimulate low impact exercise. The treatment horses were then exposed to WBV 5 d per wk for 45 min at 50 Hz for 28 days. However, it was concluded that WBV had little impact on bone mineral content and no effect on the circumference of the gaskin and forearm muscles.

There has also been evidence of increased muscle strength due to WBV therapy. Runge et al. (2000) found that 2 mo of WBV exercise on a tilting plate reduced chair rise time in 11 elderly women by 18%. Static and dynamic WBV exercise on an oscillating vibration plate improved both isometric and dynamic muscle strength (15% and 16%, respectively) in postmenopausal women (Verschueren et al., 2004). Halsberghe et al. (2017) studied the effect of vertical WBV on the symmetry and cross-sectional area of the *m. multifidus* muscle of the thoracolumbar spine in equines. They utilized 9 horses that were subjected to 40 Hz of WBV for 30 min, 2 times per d, 5 d per wk for 60 d. Both the left and right *m. multifidus* were measured for cross-sectional area and symmetry at 4 places along the spine (T15-T16, T16-T17, T18-L1, and L1-L2) via ultrasonography. The authors reported a significant improvement in both symmetry and cross-sectional area at all 4 locations both at d 30 and d 60 (Halsberghe et al., 2017).

Currently, WBV is used by horse owners as a means to warm-up performance horse. However, Buchner et al. (2016) utilized electromyography (EMG) to observe muscle vibration in horses subjected to 10 min of horizontal WBV. These researchers reported no additional EMG activity in response to the WBV and therefore concluded that WBV is not an acceptable method of warming-up or exercising horses.

Lameness and musculoskeletal injuries are major concerns in the performance horse industry, which often result in major losses as far as training time and money (Rossdale et al., 1985). It is not uncommon for these injuries to be associated with a period of stall confinement which can lead to secondary issues such as muscle atrophy and decreased bone mineral content. Studies have shown the adverse effects of stall rest on muscle and bone characteristics in performance horses (Carrier et al., 1997; Porr et al., 1998; Nielsen et al., 1997; Bell et al., 2001). However, with the promising results of human studies which report a positive influence of vibration stimulation it is of interest to the performance horse industry to quantify changes in bone and muscle parameters in response to WBV. Moreover, with previous equine studies utilizing the Equivibe® which delivers WBV and reporting no effect of treatment on bone mineral content, it was of interest to the authors to investigate changes in dorsal cortical thickness, nutrient foramen measurements, and muscle thickness of mature , stock-type horses exposed to VWC.

CHAPTER III

EFFECT OF VIBRATION STIMULATION ON MUSCLE THICKNESS OF MATURE, STOCK TYPE HORSES ON STALL REST

Introduction

Equine sports medicine is an ever-growing industry where different therapeutic tools are being discovered daily. With the pressures from performance horse enthusiasts, trainers, owners, and healthcare professionals, it is crucial that therapeutic tools help heal injuries, improve performance and competitiveness, and are proven to do what they claim. Whole body vibration (WBV), and vortex wave circulation stimulation (VWC) are two similar therapies that are provided to the horse through stationary plates. Interestingly, WBV is a popular treatment in human medicine, and has been known to improve muscle strength in post-menopausal women (Verschueren et al., 2004), improve chair rise time in elderly women (Runge et al., 2000), and prevent osteoporosis in post-menopausal women (Verschueren et al., 2004). However, a paucity of research has proven these claims of WBV and VWC benefits to horses to be true. A study by Maher (2016) questioned the efficacy of WBV on bone mineral content (BMC) and circumference of the gaskin and forearm muscles in the exercising horse but concluded that no treatment effects were observed following 45 min of WBV at 50 Hz over a 28 d treatment period. The commercially available TheraPlate® remits VWC through 2 plates that form one unit to deliver equally distributed pulsatile stimulation to all 4 limbs of the horse while standing on it. The TheraPlate® is said to improve circulation, increase muscle mass, and decrease healing time, amongst many other claims. While bone development and improved density is important for performance horses, another concern amongst trainers and owners in the performance horse industry is muscle atrophy due to long periods of stall rest or immobilization. This stall rest is usually practiced when an injury of some kind is sustained by the equine athlete. It is hypothesized that vibration stimulation will maintain muscle mass in mature, stock type horses placed on stall rest. Therefore, the objective of this study was to determine the effect of VWC on muscle parameters in mature, stock-type horses subjected to stall rest.

Materials and Methods

This study was conducted under an approved Mississippi State University Institutional Animal Care and Use Committee protocol (study #17-493).

Animals

Stock types horses (n = 8; 5 mares, 3 geldings) between the ages of 3 and 14 yr were utilized. Horses were stratified by BW and age then divided into control (CON; n = 4) and treatment (VWC; n = 4) groups. A 45-d (d -45 to -1) backgrounding and exercise period occurred prior to the experimental period. During this time, horses were subjected to a moderate intensity exercise protocol derived from requirements outlined by the National Research Council (NRC, 2007). This exercise protocol consisted of lunging 4 d per wk for 1 h per d at 30% walk, 55% trot, and 15% canter (Table 3.1). Horses were lunged in alternating round pens (7.64 m and 12.19 m) with a lunge line attached to the halter to better control gait. Additionally, each horse wore a surcingle and heart rate (HR) monitor during exercise. After each gait, HR was recorded to quantify exercise and to ensure a minimum average HR of 90 bpm (NRC, 2007) was met for the entirety of the exercise session. During the exercise protocol horses were maintained on a native grass pasture and had *ad libitum* access to water. From d -14 to -1, the VWC group was acclimated to the TheraPlate® in a protocol depicted in Table 3.2. The experimental period began on d 0 and continued until d 56. During this time horses were housed individually in stalls (3.65 m^2) at the Mississippi State University Horse Unit. While on stall rest, horses had *ad libitum* access to water and were fed Tifton 44 hay at 2% BW/d, which was split between 2 feedings at 0600 and 1700. During the 8-wk stalling period, CON horses remained on stall rest, while VWC horses were subjected to VWC 5 d per wk, 2 sessions per d, for 15 min each session (30 min total/d) at 50% plate output in addition to the stall rest.

Data Collection

On d 0, 14, 28, 42, and 56, the thickness of *extensor carpi radialis*, *extensor digitorum longus*, *gluteus medius*, *longissimus lumborum*, *semitendinosus*, *supraspinatus*, and *longissimus thoracis* muscles were measured via ultrasound as described by Lindner et al. (2010). The thickness, circumference, and cross-sectional area on d 0 were used as the baseline; whereas the thickness on other days were analyzed as repeated measures and percentage of change in muscle thickness from d 0 was calculated as follows:

$$Percentage\ of\ change\ in\ muscle\ thickness = \frac{thickness - d0\ thickness}{d0\ thickness} \times 100\% \qquad (1.1)$$

The *extensor carpi radialis* was measured at approximately 30 cm distal to the tuber radii, at the point of transition from muscle to tendon. The *supraspinatus* was measured cranially to the spine of the scapula so that the spine of the scapula could be seen in the ultrasound image and used as a reference point. The *longissimus thoracis* was measured at the point which was 30-cm caudal to the spine of the scapula and 30 cm off the midline, with the ultrasound probe placed in a way that the image was centered over a rib. The *longissimus lumborum* was measured 20-cm cranially to the tuber sacrale of the ilium and 3 cm off the midline. The *gluteus medius* was measured midway between the tuber coxae and first coccygeal vertebrae. The *semitendinosus* was measured over the ischial tuber, so that the ultrasound image was centered over the bone. Lastly, the *extensor digitorum longus* was measured 30 cm distal to the tibial tuberosity. Each muscle was measured in triplicate by the same individual and averaged to a single data point. All ultrasound measurements were taken while each horse stood square in stocks and was held by another individual in order to minimize the horse's movement and subsequent error in measurement. In addition to thickness, circumference and cross-sectional area measurements were taken for the *extensor carpi radialis* and *extensor digitorum longus*. The cross-sectional area measurement was taken with the probe placed parallel to the horses' leg, at the point where the muscle meets the tendon. A set of reference ultrasound images recorded by a licensed veterinarian was used to ensure the accuracy and precision of measurement locations. To ensure good contact with the ultrasound probe and uniformity in the measurement locations, the hair was clipped with a surgical prep clipper set. For optimal probe contact and signal penetration, the area of interest was sprayed with 90% alcohol and scrubbed and a water soluble, ultrasound transmission gel (Parker Laboratories, Fairfield, NJ) was applied. A SonoSite (SonoSite, inc., Bothell, WA) portable ultrasonographic instrument with a transrectal probe was used.

Statistical Analysis

Percentages of changes in muscle thickness, circumference, and cross-sectional area were analyzed as a randomized block design with repeated measures. Analysis of variance was performed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) in a generalized linear mixed model, with treatment, time, their interaction as fixed effects and age as random effect. Actual probability values of statistical significance were reported.

Results

There was no treatment × time interaction ($P > 0.136$). Thickness of the *extensor carpi radialis* was increased in VWC horses when compared with d 0 (30.9%; $P = 0.005$); whereas no change was reported in CON horses (-0.40%; $P = 0.945$), which led to a 31.3% greater increase observed in VWC horses ($P = 0.096$; Table 3.3). As the thickness was normalized to d 0, there was an overall treatment effect on the *longissimus thoracis* muscle, with VWC horses having an 8.9% increase ($P = 0.008$); whereas CON horses had an 8.4% decrease ($P = 0.010$), which led to a total of 17.5% difference between the two groups Compared with d 0, the thickness of the *longissimus lumborum* muscle had a 7.7% increase in VWC horses ($P < 0.001$); whereas that in CON horses was increased by 2.9% ($P = 0.011$), which led to a 4.8% greater increase observed in VWC horses ($P = 0.068$; Table 3.3).

There was no treatment effect on the thickness of the *supraspinatus, gluteus medius*, *semitendinosus*, and *extensor digitorum longus* muscles ($P \geq 0.261$). Similarly, no treatment effect was observed in circumference ($P \geq 0.260$; Table 3.4) and cross-sectional area ($P \geq 0.138$; Table 3.5) of the *extensor carpi radialis* and the *extensor digitorum longus* muscles. Lastly, no effect of time was observed for thickness of the *supraspinatus* ($P = 0.482$; Table 3.6), *longissimus thoracis* ($P = 0.578$; Table 3.6), and *longissimus lumborum* ($P = 0.182$; Table 3.6). However, a significant effect of time was recorded for thickness of the *extensor carpi radialis* ($P = 0.054$; Table 3.6), *extensor digitorum longus* ($P < 0.0001$; Table 3.6), *gluteus medius* ($P = 0.051$; Table 3.6), and *semitendinosus* ($P = 0.001$; Table 3.6) muscles. There was also a significant effect of time on the circumference of the *extensor carpi radialis* ($P < 0.0001$; Table

3.6) and *extensor digitorum longus* ($P < 0.0001$; Table 3.6) muscles and cross-sectional area of the *extensor carpi radialis* ($P = 0.000$; Table 3.6) and *extensor digitorum longus* ($P < 0.0001$; Table 3.6) muscles.

Discussion

The recorded improvements of the topline muscles, *longissimus lumborum* and *longissimus thoracis*, in VWC horses could be attributed to the stimulation or encouragement of the horse to activate these muscles or stand with a more athletic posture during the VWC exposure. A study by Halsberghe et al. (2017) measured changes in cross-sectional area of the *m. multifidus* muscle, found in the thoracolumbar spine of horses. Authors concluded that WBV did increase overall cross-sectional area of the *m. multifidus*, which is indicative of hypertrophy, or increase in muscle fiber size, occurring. The response to training observed in muscles is dependent on the function of that muscle as well as its composition. In horses, the *m. multifidus* has an equal ratio of Type I: Type II muscle fibers and function is both postural and locomotive in nature (Hyytiäinen et al., 2014). The results reported by Halsberghe et al. (2017) are similar to those seen in the present study where topline muscles (*longissimus thoracis* and *longissimus lumborum*) were improved in response to VWC exposure. A horse standing quietly, with minimal movement, on a vibration plate is described as a static exercise, alternative to dynamic or dynamic mobilization exercises (Stubbs et al., 2011). This seems to be supported by Verschueren et al. (2004) that demonstrated an advantage to WBV over low intensity resistance training in regard to improved muscle strength in postmenopausal women exposed to WBV therapy. Additional support for this can be recognized in studies utilizing mice and rats being exposed to a similar type of WBV plate which found that WBV can influence changes in muscle fibers (Xie et al., 2008; Lochyński et al., 2013), denoting a greater muscle activation due to WBV rather than resistance training.

Maher (2016) noted that when horses were exposed to vibration therapy the circumference of the forearm muscle did not change; however, in the current study an improvement in thickness of the *extensor carpi radialis* did occur. Maher (2016) used a standard soft measuring tape to record the circumference (cm) of the forearm muscle at a consistent, shaved location. Maher (2016) related the reported lack of change to the absence of muscle hypertrophy due to WBV exposure. However, the current study presents evidence to support the claim that vibration treatment does lead to hypertrophy of muscle, specifically in the current study the *extensor carpi radialis*, *longissimus thoracis*, and *longissimus lumborum* muscles. Vibration stimulation may be a therapeutic treatment option in terms of maintaining and improving muscle mass or thickness of certain and specific muscle groups.

Conclusion

Participants in the performance horse industry are constantly searching for effective, economical, and efficient injury prevention and therapeutic technologies. However, it is not uncommon in the performance horse industry to see popular “therapies” that are used at an exponential rate that lack sound evidence to prove their effectiveness. Companies claim that vibration stimulation plates can increase muscle mass, improve bone strength and density, increase blood flow. However, to date there has been limited research testing these claims and the studies that have been done have had minimal validation of success. This study mirrored industry practices of placing fit horses on stall rest for an extended period and measure changes in muscle parameters in response to the proposed vibration therapy. The present study found vibration stimulation to positively influence the forearm and topline muscles, providing evidence that it is a viable option for mitigating muscle atrophy in performance horses subjected to stall rest.

Table 3.1 Protocol to meet moderate exercise requirements[1]

Time (min)	Gait
8	Trot
3	Canter
4	Walk
8	Trot
2	Canter
5	Walk
Change directions and repeat	

[1] Exercise to meet average heart rate of 90 bpm for moderately exercised horses (NRC, 2007)

Table 3.2 Acclimation schedule for treatment (VWC) group to the TheraPlate®.

Day	Machine Output (%)	Time (min)
-14	0, machine off	Walk on/off 5x
-12	15	1
-10	15	5
-8	30	5
-6	30	10
-4	50	12
-2	50	15

Table 3.3 Overall mean change in muscle thickness (%) for treatment (VWC; standing on TheraPlate® for 5 d per wk, twice a d, for 15 min each period, and at 50% plate output vibration frequency; n = 4) and control (CON; stall-resting without vibration; n = 4) over a 56 d period.

Muscle	Mean (%)		SE	*P*
	CON	VWC		
Extensor carpi radialis	-0.40^{b}	30.92^{a}	11.22	0.096
Supraspinatus	-0.60	3.20	4.69	0.451
Longissimus thoracis	-8.40^{b}	8.90^{a}	5.00	0.020
Longissimus lumborum	2.88^{b}	7.73^{a}	1.54	0.068
Gluteus medius	3.90	14.46	6.03	0.262
Semitendinosus	2.51	7.13	2.92	0.306
Extensor digitorum longus	24.24	38.37	11.86	0.432

^{a,b} Rows not sharing a superscript are different.

Table 3.4 Overall mean change in circumference (%) of the *extensor carpi radialis* and *extensor digitorum longus* for treatment (VWC; standing on TheraPlate® for 5 d per wk, twice a d, for 15 min each period, and at 50% plate output vibration frequency; n = 4) and control (CON; stall-resting without vibration; n = 4) over a 56 d period.

Muscle	Mean (%)		SE	*P*
	CON	VWC		
Extensor carpi radialis	7.37	19.13	6.69	0.260
Extensor digitorum longus	4.77	8.27	5.82	0.686

Table 3.5 Change in cross-sectional area (%) of the *extensor carpi radialis* and *extensor digitorum longus* for treatment (VWC; standing on TheraPlate® for 5 d per wk, twice a d, for 15 min each period, and at 50% plate output vibration frequency; n = 4) and control (CON; stall-resting without vibration; n = 4).

Muscle	Mean (%)		SE	*P*
	CON	VWC		
Extensor carpi radialis	16.41	50.37	19.67	0.138
Extensor digitorum longus	17.99	21.78	10.67	0.810

Table 3.6 Effect of time on circumference, cross-sectional area, and thickness averaged across for treatment (VWC; standing on TheraPlate® for 5 d per wk, twice a d, for 15 min each period, and at 50% plate output vibration frequency; n = 4) and control (CON; stall-resting without vibration; n = 4).

Measurement (%)	Muscle	Day					SE	*P*
		0	14	28	42	56		
Circumference	*Extensor carpi radialis*	0.00^{c}	-0.50^{c}	12.99^{b}	7.84^{bc}	32.68^{a}	5.58	0.0002
	Extensor digitorum longus	0.00^{c}	1.72^{c}	7.92^{b}	-5.52^{bc}	21.97^{a}	4.35	<0.0001
Cross-sectional area	*Extensor carpi radialis*	0.00^{c}	-1.45^{c}	31.53^{b}	21.52^{bc}	84.84^{a}	16.71	<0.0001
	Extensor digitorum longus	0.00^{c}	4.28^{bc}	21.33^{b}	-8.195^{c}	62.14^{a}	9.05	<0.0001
Thickness	*Extensor carpi radialis*	0.00^{b}	4.62^{b}	38.43^{a}	12.97^{b}	5.02^{b}	9.99	0.0314
	Supraspinatus	0.00	-2.69	0.60	3.60	3.09	4.17	0.5748
	Longissimus thoracis	0.00	-3.67	-0.12	1.05	4.14	4.71	0.6591
	Longissimus lumborum	0.00^{c}	4.26^{ab}	6.29^{ab}	3.66^{b}	7.01^{a}	1.34	0.002
	Gluteus medius	0.00^{b}	-0.41^{b}	12.93^{a}	8.84^{ab}	15.36^{a}	4.87	0.0218
	Semitendinosus	0.00^{b}	-0.67^{b}	10.23^{a}	7.83^{a}	1.90^{b}	2.30	0.0005
	Extensor digitorum longus	0.00^{d}	18.48^{c}	25.60^{bc}	35.36^{ab}	45.77^{a}	8.02	<0.0001

$^{a, b, c, d}$ Rows not sharing a superscript are different.

CHAPTER IV

EFFECT OF VIBRATION STIMULATION ON BONE MEASUREMENTS OF MATRUE, STOCK-TYPE HORSES ON STALL REST

Introduction

Lameness and loss of training time are major concerns in the performance horse industry today. Skeletal injuries are a common cause of both mortality and morbidity in performance horses (Price et al., 1995). Unfortunately, injuries to bones are hard to foresee and often are found after they occur. Consequently, the use of vibration stimulation plates has been introduced to the equine industry as a means to prevent bone injuries to equine athletes. It is thought by producers, trainers, and owners that the use of whole-body vibration (WBV) technology will strengthen bone and therefore prevent said skeletal injuries. The TheraPlate® is claims to use a form of vibration stimulation known as vortex wave circulation stimulation (VWC). Both WBV and VWC therapies are delivered via plates that provide pulsatile stimulation to the individual standing on them. Previous research has found that WBV can help prevent osteoporosis and improve bone density in postmenopausal women (Verschueren et al., 2004). Additionally, Rubin et al. (2002) found that mechanical stimulation improved the quality and quantity of the trabecular bone in hind limbs of female sheep. However, studies by both Maher (2016) and Hulak (2015) determined WBV had no effect on the bone mineral content of either exercising or stall-rested horses. The third metacarpal is a site of common injury in the performance horse. Dorsal metacarpal disease occurs in horses undergoing training and is characterized by a significant thickening of the dorsal aspect of the cannon bone. Potential therapies to help decrease the overthickening of the third metacarpal bone are of interest to the performance horse industry with the intent of preventing the occurrence of dorsal metacarpal disease. Another aspect of bone, the nutrient foramen, is crucial for nutrient blood flow to the distal limb of the equine. The nutrient foramen is a hole that naturally occurs in the third metacarpal bone of horses and can also be found in the long bones of other species (Henderson, 1978; Garita, 2002; Craig et al., 2003). The nutrient foramen houses a nutrient vessel, also known as the nutrient artery, in the third metacarpal of the horse which supplies the bone and distal limb with blood flow and nutrients (Garita, 2002). However, the nutrient foramen has seldom been the focus of research in the horse in terms of manipulating its size via exercise or treatment. It is hypothesized that vibration stimulation will improve nutrient foramen measurements as well as decrease dorsal cortical thickness. Therefore, the objective of this study was to determine the effect of vibration stimulation on nutrient foramen diameter, circumference, area, as well as dorsal cortical thickness in mature, stock-type horses on stall rest.

Materials and Methods

This study was conducted under an approved Mississippi State University Institutional Animal Care and Use Committee protocol (study #17-493).

Animals

Stock types horses (n = 8; 5 mares, 3 geldings) between the ages of 3 and 14 yr were utilized in this study. Horses were blocked by age group (old or young) then divided into control (CON; n = 4) and treatment (VWC; n = 4) groups. A 45-day (day -45 to -1) backgrounding and exercise period occurred prior to the experimental period. During this time, horses were subjected to a moderate-intensity exercise protocol derived from requirements outlined by the National Research Council (NRC, 2007). This exercise protocol consisted of lunging 4 d per wk for 1 hr per d at a walk (30%), trot (55%), and canter (15%; Table 4.1). Horses were lunged in alternating round pens (7.64 m and 12.19 m) with a lunge line attached to the halter to better control gait. Additionally, each horse wore a surcingle and heart rate (HR) monitor during exercise. After each gait, HR was recorded to quantify exercise and to ensure a minimum average HR of 90 bpm (NRC, 2007) was met for the entirety of the exercise session. During the exercise protocol horses were maintained on native grass pasture and had *ad libitum* access to water. From day -14 to -1, the VWC group was acclimated to the TheraPlate® (Table 4.2). The experimental period began on day 0 and continued until day 56. During this time horses were housed individually in stalls (3.65 m^2) at the Mississippi State University MAFES Horse Unit. While stalled, horses had *ad libitum* access to water and were fed Tifton 44 hay at 2% BW per d, which was split between 2 feedings at 0600 and 1700. During the 8-week stalling period, CON horses remained on stall rest, while VWC horses were subjected to VWC 5 d per wk (Monday - Friday), 2 times per d, for 15 min per session (30 min per day total) at 50% plate output in addition to the stall rest.

Data Collection

On days 0, 14, 28, 42, and 56, radiographs of the left third metacarpal were taken by veterinarians in the Radiology department at the Mississippi State University College of Veterinary Medicine. Each radiograph was analyzed for nutrient foramen area, nutrient foramen circumference, nutrient foramen diameter, and dorsal cortical thickness by the same individual to ensure consistency and minimize error. Measurements were collected using a Bio Rad veterinary radiology software. Nutrient foramen circumference was found by tracing around the foramen.

Nutrient foramen diameter was measured from top to bottom across the center of the nutrient foramen on a vertical, straight line. Lastly, the area of the nutrient foramen was given after collecting the circumference and diameter measurements in the radiology software. Dorsal cortical thickness was measured at the thickest part on the lateral view of the left third metacarpal, on a horizontal and straight line. Actual measurements were reported for nutrient foramen area, circumference, diameter, and dorsal cortical thickness. Nutrient foramen and dorsal cortical thickness measurements collected on d 0 were considered the baseline for this study and were used to calculate the percentage of change as follows:

$$Percentage\ of\ change\ bone\ parameter = \frac{measurement - d0\ measurement}{d0\ measurement} \times 100\% \qquad (2.1)$$

Statistical Analysis

Actual values and percentages of changes in nutrient foramen area, circumference, and diameter were analyzed as a randomized block design with repeated measures. Analysis of variance was performed using the GLIMMIX procedure of SAS 9.4 (SAS Institute Inc., Cary, NC) in a generalized linear mixed model, with treatment, time, their interaction as fixed effects and age as random effects. The actual probability values of statistical significance were reported.

Results

Treatment horses had an overall greater nutrient foramen diameter (4.9 vs 2.9 ± 0.4; *P* = 0.021; Table 4.3) and nutrient foramen circumference (16.0 vs 12.0 ± 1.4; *P* = 0.094; Table 4.3) when compared to CON. A two-way treatment × time interaction was observed (*P* = 0.040; Figure 4.2), in which nutrient foramen area of CON and VWC were initially similar (d 0 and 14; *P* ≥ 0.226), but VWC horses subsequently had greater nutrient foramen area on d 28 (19.6 vs 7.6

± 2.3; *P* = 0.001), d 42 (15.1 vs 8.0 ± 2.3; *P* = 0.038), and d 56 (16.2 vs 8.0 ± 2.3; *P* = 0.019) as compared to CON. Similarly, a two-way treatment × time interaction (*P* = 0.051; Figure 4.3) was found for percentage change nutrient foramen area, in which nutrient foramen area of VWC remained the same throughout (*P* ≥ 0.163) while CON decreased 30.0% from d 0 to d 28 (*P* = 0.079), d 42 (*P* = 0.014), and d 56 (*P* = 0.006). No difference was observed in dorsal cortical thickness (*P* = 0.248; Table 4.3). However, VWC horses saw a decrease in percentage change dorsal cortical thickness which was different from the increase noted in CON horses (-1.10 vs 1.52 ± 0.97; *P* = 0.104; Table 4.4).

Discussion

Bone is a critical component in the body as it provides protection, support, and locomotion and is a peculiar type of tissue that is constantly changing and responding to mechanical loading or stress (Ahn and Grodzinsky, 2009; Chen et al., 2010). This presents a slight challenge when trying to assess bone strength and condition. Often, loss of training occurs because of bone-related injuries such as a fracture, break, or even multiple micro damages that over time become greater issues (Carrier and Estberg, 1997; Nielsen et al., 1997).

The nutrient foramen is a naturally occurring hole in the third metacarpal bone of the equine. Not only is the nutrient foramen present on the palmar aspect of the major weight bearing bone of the forelimb (Garita, 2002), but it also provides passage for the nutrient artery which supplies blood and nutrients to the distal part of the horse's forelimb. Craig et al. (2003) speculated that, in humans, the nutrient foramen could be an area of weakness in the bone. Moreover, it is possible that when under excess stress (physical activity or decreased bone density), the nutrient foramen could potentially allow a fracture to occur (Craig et al., 2003). A study by Garita (2002) sought to quantify the occurrence of damage to the equine third metacarpus near the nutrient foramen when compared with a drilled hole. Following the observation that damage around the nutrient foramen was less than that near the drilled hole, the author concluded that the nutrient foramen is in fact a strengthening factor about the long bone (Garita, 2002). It was also noted in a study by Götzen et al. (2003) that the nutrient foramen is an area of stress reduction due to the variations in the microstructure surrounding the nutrient foramen. While the previously mentioned studies questioned the integrity of the nutrient foramen and the bone that surrounds it, the current study sought to quantify changes to the size of the nutrient foramen in the equine. Changes in the size of the nutrient foramen are of particular interest to the performance horse because changes, or more specifically the enlargement of the nutrient foramen noted in the current study, could potentially affect the size of the nutrient artery which is housed by the nutrient foramen, Therefore, it could be speculated that as the increase in the area of the nutrient foramen reported in the current study could allow for both an increase in size of the nutrient artery as well as an increase in blood flow to the distal limb of the horse in response to vibration stimulation exposure.

Dorsal cortical thickness has not been previously studied in the horse in response to vibration stimulation. Rajao et al. (2019) measured change in cortical bone density of the thoracic and pelvic limbs of endurance horses. To begin the study, control horses were untrained and housed in a pasture. Whereas the treatment horses had a previous training of at least 4 yr and had been competing in 120 and 160 km endurance races. Control horses were exercised 5 d per wk, with the exercise protocol consisting of 1 h and 30 min walking and 2 d per wk galloping between 15 and 30 km. Treatment horses were exercised either 60 or 80 km 4 wk prior to race. Results stated that in the left third metacarpal, cortical thickness was increased by 18% in the exercising group (Rajao et al., 2019). However, it has been well documented that racehorses are affected by dorsal metacarpal disease, which primarily occurs in the forelimb of the horse (Couch and Nielsen, 2017). With the knowledge that dorsal metacarpal disease is characterized by a thickening of the dorsal aspect of the third metacarpal, it could be concluded from the present study that vibration stimulation may be a way to combat thickening of the third metacarpal. Therefore, it would be of interest to study the effect of vibration stimulation on dorsal cortical thickness in young racehorses in training.

Previous studies have investigated the effect of vibration plate therapy on bone mineral content and have so far found no change in response to WBV exposure (Hulak, 2015; Maher, 2016). Additionally, there have been no studies measuring changes in dorsal cortical thickness in response to vibration stimulation. Therefore, it was of interest to the authors of this study to investigate potential changes to the nutrient foramen which may in theory reflect possible changes in the area of passage for vasculature to the distal limb of horses on stall rest in response to vibration stimulation. This study aimed to mimic industry practices of placing fit horses on stall rest for an extended period and measure changes in nutrient foramen parameters as well as dorsal cortical thickness in response to the proposed vibration therapy. The observed enlargement of the nutrient foramen could provide a greater area of passage for vasculature, which may enhance nutrient blood flow to the distal limb. It would be of interest to measure actual change in blood flow in response to vibration stimulation in future studies. Additionally, it would be beneficial to measure changes in dorsal cortical thickness over a longer period of time in response to vibration stimulation.

Conclusion

With the current search for well-rounded therapeutic options in the performance horse industry, it is not uncommon for participants to be mesmerized by the “magic” effects of popular therapies. Claims by companies who produce vibration stimulation plates include increased bone density and strength, improved muscle mass, greater blood flow, etc. However, there has been a lack of previous research to investigate these claims, and minimal validation in the studies who have examined the effects of vibration stimulation in the horse. Therefore, the current study sought to emulate current industry practices of placing horses on stall rest for an extended period of time. Improvements to nutrient foramen measurements seen in treatment horses could be indicative of changes to bone remodeling in response to vibration stimulation. Additionally, vibration stimulation may be a viable therapeutic option for influencing the nutrient foramen, and subsequently the vessel it houses in the distal limb of the horse in performance horses during stall rest. Vibration stimulation may also be a means of combating thickening of the dorsal aspect of the third metacarpal bone in horses and therefore could potentially be used to prevent dorsal metacarpal disease.

Table 4.1 Protocol to meet moderate exercise requirements[1]

Time (min)	Gait
8	Trot
3	Canter
4	Walk
8	Trot
2	Canter
5	Walk
Change directions and repeat	

[1] Exercise to meet average heart rate of 90 bpm for moderately exercised horses (NRC, 2007)

Table 4.2 Acclimation schedule for treatment (VWC) group to the TheraPlate®.

Day	Machine Output (%)	Time (min)
-14	0, machine off	Walk on/off 5x
-12	15	1
-10	15	5
-8	30	5
-6	30	10
-4	50	12
-2	50	15

Table 4.3 Average change in bone parameters (mm) across 56 d compared with d 0, treatment (standing on TheraPlate® for 5 d per wk, twice a d, for 15 min each period, and at 50% plate output vibration frequency; VWC; n = 4) and control (stall-resting without vibration; CON; n = 4).

Measurement (mm)	Mean		SE	*P*
	CON	VWC		
Dorsal cortical thickness	11.54	12.60	0.59	0.248
Nutrient foramen diameter	2.95[b]	4.90[a]	0.45	0.021
Nutrient foramen circumference	11.98[b]	15.96[a]	1.42	0.094

[a,b] Rows not sharing a superscript are different.

Table 4.4 Average change (%) in bone parameters across 56 d compared with d 0, treatment (standing on TheraPlate® for 5 d per wk, twice a d, for 15 min each period, and at 50% plate output vibration frequency; VWC; n = 4) and control (stall-resting without vibration; CON; n = 4).

Measurement (%)	Mean		SE	*P*
	CON	VWC		
Dorsal cortical thickness	1.52	-1.10	0.97	0.104
Nutrient foramen diameter	-1.64	13.38	11.99	0.410
Nutrient foramen circumference	-3.19	22.97	12.41	0.187

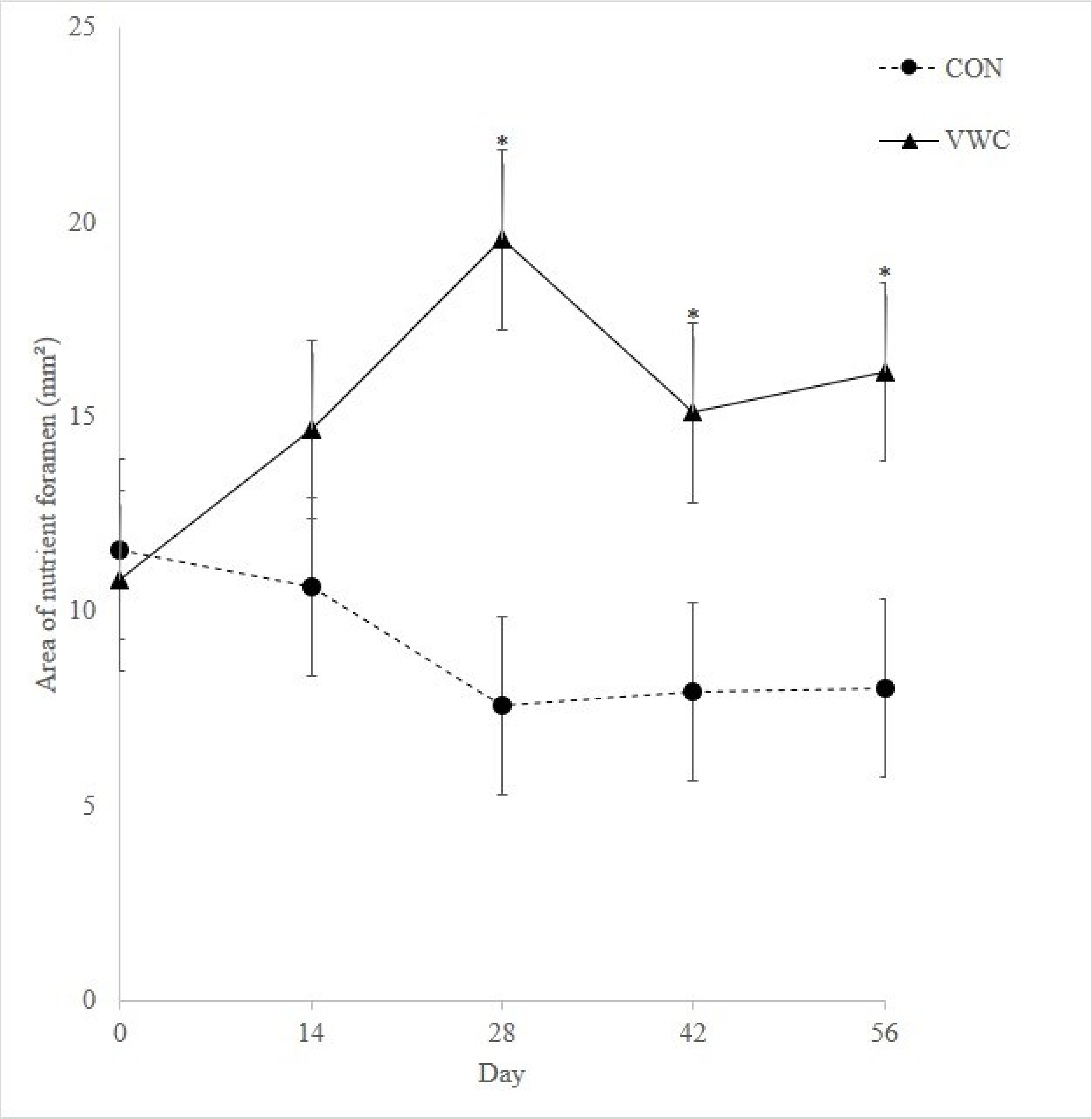

Figure 4.1 Treatment × time interaction for change in nutrient foramen area.

*Treatments and timepoints not sharing a superscript are different ($P \leq 0.100$).

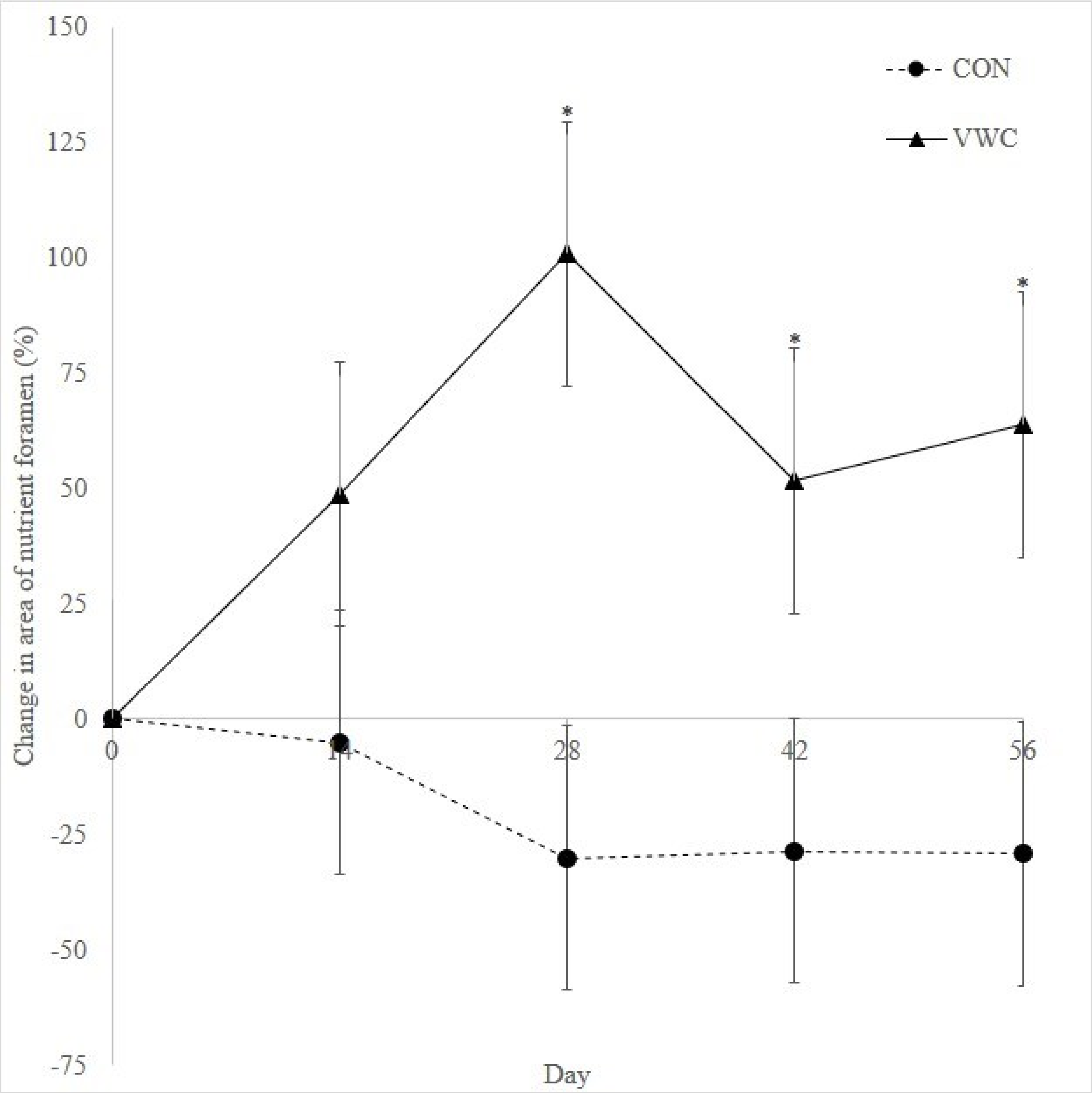

Figure 4.2 Treatment × time interaction for % change in nutrient foramen area.

*Treatments and timepoints not sharing a superscript are different ($P \leq 0.100$).

REFERENCES

Ahn, A. C., and A. J. Grodzinsky. 2009. Medical Engineering & Physics Relevance of collagen piezoelectricity to “Wolff's Law”: A critical review. Mech. Eng. Phys. 31:733–741.

Bailey, C. J., R. J. Rose, S. W. J. Reid, and D. R. Hodgson. 1997. Wastage in the Australian Thoroughbred racing industry: a survey of Sydney trainers. Aust. Vet. J. 75:64-66.

Bell, R. A., B. D. Nielsen, K. Waite, D. Rosenstein, and M. Orth. 2001. Daily access to pasture turnout prevents loss of mineral in the third metacarpus of Arabian weanlings. J. Anim. Sci. 79:1142–1150.

Berg, H. E., L. Larsson, and P. A. Teschi. 1997. Lower limb skeletal muscle function after 6 wk of bed rest. J. Appl. Physiol. 82:182-188.

Buchner, H. H. F., L. Zimmer, L. Haase, J. Perrier, and C. Peham. 2016. Effects of whole body vibration (WBV) on the horse: actual vibration, muscle activity, warm up, and rideability. Equine Vet. J. 48:7-39.

Buckingham, S. H. W., and L. B. Jeffcott. 1990. Shin soreness: a survey of Thoroughbred trainers and racetrack veterinarians. Austr Equine Vet. 8:148–153

Carrier, T. K., and L. Estberg. 1997. Lay-Up Is Associated with Complete Humeral but Not Pelvic Fracture in Lay-Up Is Associated with Complete Humeral but Not Pelvic Fracture in California Thoroughbred Racehorses. AAEP Proc. 43:271–272.

Chen, J., C. Liu, L. You, and C. A. Simmons. 2010. Boning up on Wolff's Law: Mechanical regulation of the cells that make and maintain bone. J. Biomech. 43:108–118.

Costill, D. L., J. Daniels. W. Evans, W. Fink, G. Krahenbuhl, and B. Saltin. 1976. Skeletal muscle enzymes and fiber composition in male and female track athletes. J. Appl. Physiol. 40:149-154.

Couch, S., and B. D. Nielsen. 2017. A review of dorsal metacarpal disease (bucked shins) in the flat racing horse: prevalence, diagnosis, pathogenesis, and associated factors. J Dairy Vet Anim Res. 5(6):228–236.

Craig, Joseph G, D. WIldman, and M. van Holsbeeck. 2003. Longitudinal stress fracture : patterns of edema and the importance of the nutrient foramen. Skeletal Radiol. 32:22–27.

Cunningham, C., L. Scheuer, S. Black. 2016. Developmental Juvenile Osteology. 2nd ed. Academic Press Inc. Cambridge, MA.

Dhert, W. J., S. W. O'Driscoll, B. J. van ROyen, R. B. Salter. 1988. Effects of immobilization and continuous passive motion on postoperative muscle atrophy in mature rabbits. Canadian Journal of Surgyer. 31:185-188.

Essen-Gustavsson, B, K. Karlstrom, and A. Lindholm. 1984. Fibre types, enzyme activities and substrate utilisation in skeletal muscles of horses competing in endurance rides. Equine Vet. J. 16:197–202.

Ferrando, A. A., C. A. Stuart, D. G. Brunder, and G. R. Hillman. 1995. Magnetic resonance imaging quantification of changes in muscle volume during 7 days of strict bed rest. Aviat. Space Environ. Med. 66(10):976-981.

Finsen, V., and S. Andra. 1988. Accuracy of visually estimated bone mineralisation in routine radiographs of the lower extremity. Skel Rad. 17(4):270-275.

Garita, B. 2002. Foramen Is Tougher Than Drilled Hole in Equine Third. University of Florida.

Götzen, N., A. R. Cross, P. G. Ifju, and A. J. Rapoff. 2003. Understanding stress concentration about a nutrient foramen. J. Biomech. 36:1511–1521.

Halsberghe, B. T., P. Gordon-Ross, and R. Peterson. 2017. Whole body vibration affects the cross-sectional area and symmetry of the m . multi fi dus of the thoracolumbar spine in the horse. Equine Vet. Educ. 29:493–499.

Heleski, C. R., A. C. Shelle, B. D. Nielsen, and A. J. Zanella. 2002. Influence of housing on weanling horse behavior and subsequent welfare. Appl. Anim. Behav. Sci. 78:291-301.

Henderson, R. G. 1978. The position of the nutrient foramen in the growing tibia and femur of the rat. J. Anat. 125:593–599.

Hiney, K. M., B. D. Nielsen, and D. Rosenstein. 2004. Short-duration exercise and confinement alters bone mineral content and shape in weanling horses. J. Anim. Sci. 82:2313-2320.

Hoekstra, K. E., B. D. Nielsen, M. W. Orth, D. S. Rosenstein, H. C. Schott II, and J. E. Shelle. 1999. Comparison of bone mineral content and biochemical markers of bone metabolism in stall- vs. pasture-reared horses. Equine Vet. J. 30:601-604.

Hopkins, P. M. 2006. Skeletal muscle physiology. Continuing Education in Anaesthesia Critical Care & Pain. 6 (1):1-6.

Hulak, E. S. 2015. Influence of whole body vibration on bone density in the stalled horse. MS Thesis. Middle Tennessee State Univ., Murfreesboro.

Hyatt, C. S. 2017. Effects of vertical whole-body vibration on select biochemical markers of muscle turnover in yearling horses. MS Thesis. Texas A&M Univ., College Station.

Hyytiäinen, H.K., A. K. Mykkänen, A. K. Hielm-Björkman, N. C. Stubbs, and C. M. McGowan. 2014. Muscle fibre type distribution of the thoracolumbar and hindlimb regions of horses: relating fibre type and functional role. Acta Vet. Scand. 56:8.

La Vigne, E. K., A. K. Jones, A. Sanchez Londoño, A. S. Schauer, D. F. Patterson, J. A. Nadeau, and S. A. Reed. 2015. Muscle growth in young horses: Effects of age, cytokines, and growth factors. J. Anim. Sci. 93:5672-5680.

Lepage, O. M., B. Carstanjen, and D. Uebelhart. 2001. Non-invasive assessment of equine bone: An update. Vet. J. 161: 10-23.

Lindner, A., R. Signorini, J. Vassallo, F. Tomatis, F. M. Flores, M. E. Gagliano, J. Curiotti, and E. Terragona. 2010. Reproducibility and repeatability of equine muscle thickness measurements with ultrasound. J. Equine Vet. Sci. 30:635–640.

Lochyński, D., D. Kaczmarek, M. J. Redowicz, J. Celichowski, and P. Krutki. 2013. Long-term effects of whole-body vibration on motor unit contractile function and myosin heavy chain composition in the rat medial gastrocnemius. J. Musculoskelet. Neuronal Interact. 13:430-441.

López-Rivero, J. L., E. Agüera, J. G. Monterde, J Vivo, M. V. Rodríguez-Barbudo. 1992. Skeletal muscle fiber size in untrained and endurance-trained horses. American Journal of Veterinary Research. 53(5):847-850.

Maher, K. E. 2016. The effect of whole body vibration on bone density and other parameters in the exercising horse. MS Thesis. Middle Tennessee State Univ., Murfreesboro.

Matsuo, K., and N. Irie. 2008. Osteoclast-osteoblast communication. Arch. Biochem. Biophys. 473:201–209.

Narici, M. V. and M. D. de Boer. 2011. Disuse of the musculo-skeletal system in space and on earth. Eur. J. Appl. Physiol. 111:403-420.

Nielsen, B. D., G. D. Potter, E. L. Morris, T. W. Odom, D. M. Senor, J. A. Reynolds, W. B. Smith, and M. T. Martin. 1997. Changes in the third metacarpal bone and frequency of bone injuries in young quarter horses during race training - Observations and theoretical considerations. J. Equine Vet. Sci. 17:541–549.

Norwood G. The bucked shin complex in Thoroughbreds. Proceedings of the 24th Convention of the American Association of Equine Practice. St. Louis MO, USA; 1978. p. 319-335.

NRC. 2007. Nutrient Requirements of Horses. 6th ed. The National Academies Press, Washington, D. C.

Nunamaker, D. M., D. M. Butterweck, and M. T. Provost. 1990. Fatique fractures in Thoroughbred racehorses: relationship with age, peak bone strain and training. J Orthop Res. 8(4):604-611.

Nunamaker, D. M., D. M. Butterweck, and J. Black. 1991. *In vitro* comparison of Thoroughbred and Standardbred racehorses with regard to local fatigue failure of the third metacarpal bone. Amer J Vet Res. 52:97-100.

Oxlund, B. S., G. Ortoft, T. T. Andreassen, and H. Oxlund. 2002. Low-intensity, high frequency vibration appears to prevent the decrease in strength of the femur and tibia associated with ovarieectomy of adult rats. Bone 32:69–77.

Pagani, F., C. M. Francucci, and L. Moro. 2005. Markers of bone turnover: biochemical and clinical perspectives. J. Endocrinol. Invest. 28:8–13.

Porr, C. A., D. S. Kronfeld, L. A. Lawrence, R. S. Pleasant, and P. A. Harris. 1998. Deconditioning Reduces Mineral Content of the Third Metacarpal Bone in Horses. J. Anim. Sci. 76:1875–1879.

Price, J. S., B. Jackson, R. Eastell, A. M. Wilson, R. G. G. Russell, L. E. Lanyon, and A. E. Goodship. 1995. The response of the skeleton to physical training: a biochemical study in horses. Bone. 17:221–227.

Prisby, R. D., M. H. Lafage-Proust, L. Malaval, A. Belli, and L. Vico. 2008. Effects of whole body vibration on the skeleton and other organ systems in man and animal models: what we know and what we need to know. Ageing Res. Rev. 7:319–329.

Rajao, M. D., C. S. Leite, K. Nogueira, R. F. Godoy, and E. M. M. Lima. 2019. The bone response in endurance long distance horse. Open Vet J. 9(1):58-64.

Rivero, J. L. L. and R. J. Piercy. 2004. Muscle physiology: responses to exercise and training. In: K. W. Hinchliff, A. J. Kaneps, and R. J. Geor, editors, Equine Sports Medicine and Surgery. Saunders, London. p. 45–76.

Roneus, M., B. Essen-Gustavsson, A. Lindholm, and S. G. B. Persson. 1992. Skeletal muscle characteristics in young trained and untrained Standardbred trotters. Equine Vet. J. 24:292-294.

Rossdale, P. D., R. Hopes, N. J. Digby, and K. Offord. 1985. Epidemiological study of wastage among racehorses 1982 and 1983. Vet. Rec. 116:66–69.

Rubin, C., A. S. Turner, R. Müller, E. Mittra, K. McLeod, W. Lin, and Y.-X. Qin. 2002. Quantity and Quality of Trabecular Bone in the Femur Are Enhanced by a Strongly Anabolic , Noninvasive Mechanical Intervention. J. Bone Miner. Res. 17:349–357.

Runge, M., G. Rehfeld, and E. Resnicek. 2000. Balance training and exercise in geriatric patients. J Musculoskel Interact. 1:54–58.

Setterbo, J. J., T. Garcia, I. Campbell, S. Kim, M. Hubbard, and S. Stover. 2008. Forelimb kinematics of galloping Thoroughbred racehorses measured on dirt, synthetic, and turf track surfaces. In: The Engineering of Sport 7, Eds: M. Estivalet, and P. Brisson, Springer, Paris. pp 437-446.

Sims, N. A., and J. H. Gooi. 2008. Bone remodeling: multiple cellular interactions required for coupling of bone formation and resorption. Semin. Cell. Dev. Biol. 19:444–451.

Stubbs, N. C., L. J. Kaiser, J. Hauptman, and M. Clayton. 2011. Dynamic mobilisation exercises increase cross sectional area of musculus multifidus. Equine Vet J. 43(5):522-529.

Stuermer, E. K., M. Komrakova, S. Sehmisch, M. Tezval, C. Dullin, N. Schaefer, J. Hallecker, and K. M. Stuermer. 2014. Whole body vibration during fracture healing intensifies the effects of estradiol and raloxifene in estrogen-deficient rats. Bone 64:187–194.

Stull, C. L. and W. W. Albert. 1980. Comparison of muscle fiber types from 2-year-old fillies of the Belgian Standardbred, Thoroughbred, Quarter horse and Welsh breeds. J. Anim. Sci. 51: 340–343.

Verheyen, K. L. P., W. E. Henley, J. S. Price, and J. L. Wood. 2005. Training-related factors associated with dorsometacarpal disease in young Thoroughbred racehorses in the UK. Equine Vet J. 37(5):442-448.

Verschueren, S. M., M. Roelants, C. Delecluse, S. Swinnen, D. Vanderschueren, and S. Boonen. 2004. Effect of 6-Month Whole Body Vibration Training on Hip Density, Muscle Strength, and Postural Control in Postmenopausal Women: A Randomized Controlled Pilot Study. J. Bone Miner. Res. 19:352–359.

Ward, K., C. Alsop, J. Caulton, C. Rubin, J. Adams, and Z. Mughal. 2004. Low magnitude mechanical loading is osteogenic in children with disabling conditions. J Bone Miner Res. 19:360–369.

Wei, Q. S., L. Huang, X. Chen, H. Wang, W. Sun, S. Huo, Z. Li, and M. Deng. 2014. Effect of whole body vibration therapy on circulating serotonin levels in an ovariectomized rat model of osteoporosis. Iran. J. Basic Med. Sci. 17:62–68.

Yamano, S., D. Eto, T. Sugiura, M. Kai, A. Hiraga, M. Tokuriki, and H. Miyata. 2002. Effect of growth and training on muscle adaptation in Thoroughbred horses. American Journal of Veterinary Research. 63:1408-1412.

Xie, L., J. M. Jacobson, E. S. Choi, B. Busa, L. R. Donahue, L. M. Miller, C. T. Rubin, and S. Judex. 2006. Low-level mechanical vibrations can influence bone resorption and bone formation in the growing skeleton. Bone. 39:1059-1066.

Zierath, J. R., and J. A. Hawley. 2004. Skeletal Muscle Fiber Type: Influence on Contractile and Metabolic Properties. PLoS Biol 2:337-348.