# EFFECTS OF WHOLE BODY VIBRATION ON LAMENESS, STRIDE LENGTH, CORTISOL, AND OTHER PARAMETERS IN HEALTHY HORSES

by

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**ABSTRACT**

Whole body vibration (WBV) is a popular treatment modality in the horse industry anecdotally believed to improve performance, yet little research exists. This study examined the effect of WBV on stride and lameness parameters, as well as salivary cortisol and heart rate. Six treatment horses (VIB) were vibrated at 50 Hz for 45 minutes 6 days per week and compared to stalled controls (CON) (Aim 1). Further, VIB was analyzed for parameters at different time periods immediately before treatment (pre), after (post), and 1, 2, 4, and 24 hours after (Aim 2). For Aim 1, minimum pelvis displacement was lower in VIB versus CON ($P < .05$), suggesting more hind end stability. Both groups decreased in stride length from d 0 to d 14 or d 28 ($P < 0.01$). For Aim 2, no differences were found in lameness or stride length at any time point. However, salivary cortisol concentrations were lower after ($P < 0.05$) and heart rate showed a similar pattern ($P = 0.06$). Whole body vibration appears to have an acute relaxation effect in stalled, healthy horses.

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# CHAPTER 1. LITERATURE REVIEW

## Introduction

Many sports look for ways to improve the safety and enhance the performance of their athletes. Many equine enthusiasts, for example, aim to enhance the performance quality and welfare of the athletic horse. Therefore, many researchers have explored numerous possibilities for creating a better performing, healthier, and safer athlete.

Enhancing athletic performance quality and safety are subjects vastly researched in humans, as well as some other athletic species like equines (Jackson and Turner, 2003; Cormie et al., 2006; Colson et al., 2007; Kawanabe et al., 2007; Halsberghe et al., 2016), or in other animal models designed to mimic the human. There are several possible avenues of research, such as whole body vibration (WBV), within this realm.

Whole body vibration (WBV) platforms have been utilized by performing athletes across species in hopes of enhancing biomechanical, physiological, and other performance parameters. In humans, research on the effect of WBV on bone mineral density (BDM) is fairly developed and known to be beneficial (Torvinen et al., 2002; Verschueren et al., 2004; Xiang-yan et al., 2008; Matute-Llorente et al., 2015). It has also been observed to maintain BMD in stalled horses when compared to horses that received slight exercise (Hulak et al., 2015). Other than bone, the effects of WBV on muscular, biomechanical, or other parameters has been briefly observed in man and equines (Colson et al., 2010; Giorgos and Elias, 2007; Kawanabe et al., 2007; Halsberghe et al., 2016). WBV is a relatively new area of research and thus requires more validation on different parameters, such as biomechanics and performance.

**Overview of Biomechanics and Performance**

Stride length and lameness are some main parameters utilized in order to measure the quality of performance, and heart rate and cortisol concentrations are determinates of stress in athletic subjects. Stride length is a parameter used when looking at human biomechanics and performance (Kawanabee et al., 2007; Giorgos and Elias, 2007). In humans, stride length is important for many different reasons, such as the ability to maintain proper walking cadence, without limping, or being able to run with greater speed and agility. Athletes, especially, value the increase in stride length, as it should contribute to a faster run time, a higher pole vault jump height, a greater ability to maneuver around a soccer, or baseball, field, or a higher chance of accurately performing a gymnastics floor maneuver. Therefore any treatment, perhaps such as WBV, that may stimulate an increase in stride length will be valued among many different athletes.

Furthermore, and more importantly, the safety of human athletes is a large area of research and observation, mainly including ergogenic drugs and mechanical methods of preventing injury. Human athletes are drawn to performance, and body enhancing drugs, whether to improve their appearance and, or, their performance. However, research shows that many of these ergogenic drugs have harmful, and sometimes lethal, side effects (Reardon and Creado, 2014). Therefore, on the human side of athletic performance, these more harmful drugs have been banned from major athletic associations. Additionally, researchers have formulated ways to test for these drugs in the body. These tests can be utilized to mitigate the use of some of the harmful drugs (Reardon and Creado, 2014). Researchers have also studied a great deal on the possible ways to prevent injury, whether skeletal, muscular, or other. It is commonly known that stretching before athletic endeavors is beneficial for preventing injury to the muscles. Likewise, tools such as proper education of the specific sport, as well as adequate protective equipment worn during exercise, will greatly add to the prevention of unnecessary athletic injuries, such as fractured bones or torn ligaments and tendons. These are just some of the ways that researchers and human sports enthusiasts have endeavored to reduce the injury and harm that is so commonly associated with human athletes (Parkkari et al., 2001).

Heart rate is a common parameter seen in a variety of different research studies. Heart rate monitors are the standard technique for measuring heart rate, with slight variations in the type and methodology. It is commonly known that heart rate increases with the stimulation of the sympathetic nervous system, and decreases with the stimulation of the parasympathetic nervous system. Additionally it has been realized that, in man, heart rate recovery is quicker in more fit individuals (Daanen et al., 2012). Therefore, if a study aims to observe the effects of a treatment on stress response to that treatment or stimulus, heart rate can be a proper evaluator of stress or athletic ability.

Another measurement of stress can be collecting either cortisol and, or, adrenocorticotropic hormone concentrations via blood or saliva samples, analyzed with assays. Cortisol is a hormone released and regulated by the adrenocorticotropic hormone of the pituitary gland in the brain. The cortisol and adrenocorticotropic hormone concentration levels rise when there is some type of stress applied to the body. Free cortisol concentrations are generally analyzed via assays of blood serum, but salivary samples are also an accurate and noninvasive technique to measure unbound cortisol concentrations in many species including man and horses. This is due to the fact that salivary cortisol concentrations are directly proportional to, and accurately reflect, serum cortisol concentrations (Teruhisa et al., 1981; Hellhammer et al., 2009; Peeters et al., 2010). Furthermore, some human research would even suggest that the salivary cortisol methodology can be more efficient, as serum cortisol can be more time consuming to collect, as well as require more sample than the saliva. Additionally, a cortisol binding globulin binds to cortisol with high affinity, yet the concentration of this globulin can be affected by pregnancy or some oral drugs (Vining et al., 1983). Therefore, serum cortisol concentrations may be affected by some variable, whereas salivary cortisol samples appear to be unaffected by these variables. Not only this, but salivary collection may be preferred over serum cortisol collection, because salivary collections are minimally invasive and can be considered an easier methodology for the researchers. This may, however, be speculation and, or, preference. Nonetheless, both serum and salivary measurements of cortisol concentration remain viable methods for indicating stress in subjects of many species.

**Equine Biomechanical Enhancement and Performance Safety**

Just like human athletes, horse enthusiasts are constantly searching for ways to improve their show horse's performance, as well as safety, in training and in the show arena. These improvements may include the horse's stride length, stress level in training and at shows, or overall soundness. In equine athletes, a longer length of stride is indicative of a higher quality performer, according to official horse show judges. For events such as dressage, reining, cow classes, and etcetera, it is more beneficial to have a long-strided horse, in order to gain enough impulsion to perform the required maneuvers within each discipline. Additionally, it is obvious that for the speed events such as racehorses, barrel racing, polo, and etcetera, a longer stride will propel the horse more forward, and complete the timed event faster. This is because velocity is equal to stride length times the stride frequency. However, the stride length is the more variable of the two, and is therefore measured more often in research studies. Additionally, it is speculated that there could be many ways that stride length might be affected, such as the balance of a rider, the type of shoe that is worn, gait that the horse is traveling, or outside influences such as WBV. In current research, computer software programs, like OnTrack Equine Software, allow a video recording to be freeze-framed and analyzed for stride distance, angles, speed, and etcetera. This method has been used by some equine studies (Hulak et al., 2015; Hyatt et al., 2017). Because of the efficiency of this method, researchers can easily measure a large number of strides, in many different horses, via a simple videography test. This allows large data numbers to be analyzed more quickly and at different gaits.

Stress in horses may be induced from training, trailering, showing, or other outside stimuli. Therefore, it is important for researchers to analyze this parameter, especially when observing the effects of some influencing factor on performance of the horse. Stress can be measured through many different methods. Heart rate is a simple measurement to possibly indicate some stress either physiologically, mentally, or psychologically, and has been used in many different research studies to observe the subjects' reaction to different treatments (Clark et al., 1993; Padalino et al., 2012; Maher et al., 2016). It would seem that higher heart rate (beats per minute) would imply that the subject is stressed; whereas a decrease in heart rate should imply a relaxation effect. As far as cortisol as a performance parameter, it has been found to indicate some sort of physiological stress, whether mental, emotional, or physical, in man and other species. It is known that cortisol concentrations will rise with exercise and decrease back to the original levels during post-exercise (Linden et al., 1991). Of course, equine trainers and owners may wish to monitor, or even alter, their horse's stress levels, as they could affect the animal's performance quality and behavior. For example, a training system was developed in order to minimize the stress associated with the trailering process. The system involved separating the trailer loading processes into smaller sections that would allow the horse to be introduced in a slower and calmer manner, thus reducing the stress that is so often related to loading horses onto trailers (Shanahan, 2003). Another study indicated that horses previously exposed to horse shows exhibit less stress than those horses who have less experience at horse shows (Covalesky et al., 1992). We can, thusly, see that overstimulation could cause stress, and a desensitization could minimize stress, in equines. Not only this, but it appears highly likely that human-made influences, such as different training methods or WBV, could alter the stress of horses in many different ways.

Furthermore, equine lameness can also be easily analyzed. What was previously qualitative assessment from veterinarians has now become more quantitative with the addition of new technology. Lameness is defined as a change in locomotor pattern coupled with fatigue (Leach and Dagg, 1983), and is obviously undesirable in the athletic show horse. Many horses exhibit some degree of lameness, even if it is not obviously seen by a subjective observer. This idea can be observed objectively via technology, like the Lameness Locator. The Lameness Locator accurately and objectively measures the degree of lameness in each of the horse's legs via a commercial wireless inertial sensor system. It is comparative to other ways of measuring lameness, such as the stationary force plate, subjective lameness scoring systems, and hind end flexion tests (Keegan et al., 2012; Marshall et al., 2012; Mccracken et al., 2012). The Lameness Locator has since been used by several studies to measure degree of lameness in their subjects (Azevedo et al., 2015; Silva et al., 2015; Taintor et al., 2016). There are numerous studies looking for ways to help prevent or mitigate lameness, such as different track surfaces (Azevedo et al., 2015), the use of WBV (Halsberghe et al., 2017), and training and shoeing (Dyson, 2002).

**Development of Whole Body Vibration**

Whole body vibration was initially developed by a Russian scientist, Vladimir Nazarov, as a means of influencing the BMD of astronauts, who do not get the necessary forces for building stronger bones while in space (Vasile et al., 2013). This led to a springboard of research opportunities to validate the use of WBV for increasing BMD and influencing muscle fibers in humans.

Whole body vibration (WBV) is studied and used for a wide variety of purposes and species. Perhaps the most researched use for WBV still is bone health. Research strongly suggests that WBV is a valid method to help increase bone mineral density (BMD) and even improve the consequences of osteoarthritis in humans (Xiang-yan et al., 2008; Matute-Llorente et al., 2015). Xiang-yan and colleagues found evidence for this in over one hundred postmenopausal women, with osteoporosis, who showed an increase in BMD after six months of WBV; whereas the control group showed a decrease in BMD at the six month mark (2008). Similarly, another study produced findings that agree with this through their research of subjects with Down Syndrome, who are known to have consistently less BMD than those humans without Down Syndrome. With WBV, the twenty-five adolescents with Down Syndrome showed a significant increase of whole body BMD, lumbar spine BMD, tibia structure, and cortical thickness of radius with twenty weeks of WBV, three days per week (Matute-Llorente et al., 2015). These findings strongly indicate WBV is beneficial for bone health and remodeling in humans. More research, however, is needed on the effects of WBV on parameters other than the skeletal system.

Although there appears to be some contradictions in currently published studies, there is still valid evidence to pursue research on the effects of WBV on performance linked parameters, such as stride length, vertical jump height, and walking or running speed in humans. One such study researched two groups of elderly postmenopausal women: the WBV group at only four minutes per week combined with walking exercises of two times per week, versus walking exercises alone, for two months. This study found the WBV group, combined with the walking exercises, greatly increased step length, duration of standing on one leg, and walking speed. The walking exercise only group showed no change in these parameters (Kawanabe et al., 2007). These findings are in agreement with another study, which concluded six weeks of WBV training increased step length and speed, as well as countermovement jump height in healthy human athletes (Giorgos and Elias, 2007). There is definitive evidence to further research how WBV affects performance parameters, both long term and acute. The current scientific research on the effects of WBV on performance and biomechanical parameters, however, has conflicting results and therefore still requires further exploration. For example it was found, with three days per week for four weeks with twenty minute bouts, WBV increased the knee extensor resistance strength training exercise as well as vertical jump height. However, no change occurred in the ten-meter sprint time, countermovement jump distance, drop jump distance, or thirty-second rebound jump when WBV was undergone (Coloson et al., 2010). Additionally, on the basis that WBV might depress the motor neurons and therefore may possibly decrease motor performance, another study found thirty minutes of WBV caused a decrease in maximal force and rate in knee extension leg resistance strength exercises (Jackson and Turner, 2003). It is seen in even these human studies that more uniform results are necessary to validate the effects of WBV on biomechanical and other performance parameters.

Cortisol concentration has been used in countless studies in order to determine stress levels in a variety of different subjects. Furthermore, short-term effects of WBV on hormonal responses have been analyzed on different species. One human study found a mild trend over time that WBV would increase healthy men's cortisol concentration, but not enough to deem WBV anything more than physiologically safe (Erskine et al., 2007). Another study, however, looked at cortisol in piglets, as a stress response to vibrations, which simulated the conditions of transportation. They found that the subjects' cortisol remained elevated until one hour after vibration, concluding that transportation, or WBV, puts a certain level of stress on healthy piglets (Perremans et al., 2001). The differences in these studies on the effect of WBV on cortisol may be species specific and transportation specific.

As far as acute effects of WBV on performance, the research again has some differing results. One study suggests, with a thirty second bout of WBV, the human subjects had significant increases to their jump height and countermovement jump performance when compared with the group that did not receive WBV. However, other parameters such as the isometric squat, countermovement jump peak power, and electromyography did not change (Cormie et al., 2006). Another such study showed no change in maximal isometric knee extensor force production after a single bout of WBV. This study reported additional follow-up results and concluded that two weeks of WBV also resulted in no change from baseline maximal isometric knee extensor force production, inferring that WBV may not have an effect on measureable performance parameters (Ruiter et al., 2003). Despite these different findings, it appears that jump height tends to be enhanced with WBV. However, it is also evident that WBV research should continue in the area of performance, both for the acute and long-term effects.

**Whole Body Vibration in Equines**

Very few studies with WBV have been performed on equine subjects, as WBV is a relatively new field of study. Regarding bone health, one study found that BMD can be maintained to a similar level in that of exercised horses, when WBV is utilized in lieu of exercise. This deemed WBV a possible training alternative in order to maintain BMD in a horse on stall rest (Hulak et al., 2015). A similar study, however, found that WBV, when added to exercise, had no effect on BMD as compared to a control group. This study also observed a decrease in stride length over the length of treatment, which was attributed to different handlers who trotted the horses. Furthermore, they found a decreased heart rate (HR) in the WBV group and theorized that future studies could find that cortisol concentration may be lowered during WBV and cause a relaxation effect (Maher et al. 2016). Therefore, more research should be done to verify that WBV can truly increase BMD in equines.

The possibility of utilizing WBV as a warm-up was also explored by comparing the differences between ten minutes of standing (control), ten minutes of WBV, ten minutes lunging at the walk, and twelve minutes lunging at the walk and trot. It was concluded that there were no differences in the WBV group's surface temperature, electromyography, or other clinical parameters when compared to the control (Buchner et al., 2016). It would be interesting to further investigate these group comparisons when looking at biomechanical and hormonal parameters, as opposed to temperature and electromyography. It could also be beneficial for this study to have used longer time periods. Furthermore, Halsberghe (2017) evaluated the long-term and immediate effects of WBV on lameness. Although WBV seemed to have no long-term effects on lameness, a thirty-minute bout of WBV showed a significant increase in forelimb lameness in chronically lame horses. However, this result was caused by a single outlier in that group (2017). Additionally, when looking at the 30 day time point, there was a trend for an improvement in lameness for the WBV group. Nonetheless, it is obvious that more research is needed to see any effects of WBV on equine warm up, lameness, performance, and behavior.

In the show setting, many riders and trainers subjectively believe that WBV enhances their horse's performance. However the scientific research on WBV in equines is relatively new and therefore has much room for more exploration. The available studies include the effects of WBV on muscle circumference and symmetry, cortisol and stress, and bone density. One such study reported that, with two months of WBV, horses with thoracolumbar spine complications, like kissing spines, showed an increase in cross-sectional area of the m. multifidus muscle, as well as symmetry of that muscle left to right

(Halsberghe et al., 2016). This implicates that WBV could supplement dynamic mobilization exercises, and provide a stronger topline, in horses.

In horses, the only known research on the effects of WBV on cortisol seems to agree that plasma cortisol concentration will increase, on some level, when trailer transportation is used (Clark et al., 1993; Fazio et al., 2008; Padalino et al., 2012). To date however, no research has observed the effects of an actual WBV plate, outside of the realm of trailer transportation, on cortisol concentration in equines. Therefore, horses simply standing on an actual vibration plate in a stall may not incur the same rise of cortisol concentrations as seen in these trailer studies. It could be suggested that an increase in heart rate, while on the vibration plate, would indicate a stimulation of the sympathetic nervous system. However, if a decrease in heart rate occurred, while a horse was on the vibration plate, this would indicate that the parasympathetic system has been stimulated, as an effect of the WBV.

# CHAPTER 2. EFFECTS OF WHOLE BODY VIBRATION ON LAMENESS, STRIDE LENGTH, CORTISOL, AND OTHER PARAMETERS IN HEALTHY HORSES

## Introduction

Athletes seek to constantly improve their performance and overall health. Similarly, those involved with equine athletes constantly seek to improve their horses' health and performance. In the equine field, stride length, stress levels, and soundness are all major contributing factors to overall health and performance. Therefore, research studies have been done to enhance these parameters, such as WBV, a new area of research for humans, as well as equine, performance athletes.

Whole body vibration is a progressive topic of research that has the potential to benefit the equine community in many different ways. It is already known to have a beneficial effect on bone mineral density and bone health overall in humans (Matute-Llorente et al., 2015; Xiang-yan et al., 2008). In equines, research is more limited, but many people claim that WBV improves their horse's performance. One study found a trend for WBV to decrease stride length (Maher, 2016), a negative performance effect; however, no significant results were noted. Therefore, this current study was designed to research this question of whether WBV truly has an effect on performance parameters, such as degree of lameness, stride length, and stress indicators such as cortisol and heart rate. The study also aimed to identify the time course of such effect. It is hypothesized that, compared to controls, degree of lameness, salivary cortisol concentration, and heart rate would decrease with WBV, with changes observed immediately following treatment and returning to baseline over a 24-h period. Additionally, it is hypothesized that stride length would be maintained with WBV, whereas the CON stride length would decrease.

**Materials and Methods**

This study was approved by the Institutional Animal Care and Use Committee of Middle Tennessee State University (Protocol 17-2017, Appendix A).

*Horses*

Twelve healthy mature horses (18 ± 1 yr) of different breeds were obtained from the teaching and research herd of MTSU Horse Science and randomly allocated into control (CON=6) or treatment (VIB=6). The six mares and six geldings were turned out on pasture 28 days prior to the experiment, but brought in for stalling during the 28 d study. They were kept in a $9.3m^2$ stall and given *ad libitum* access to water, except during the vibration treatment. Twice daily, the horses were fed a commercial pelleted concentrate (Purina Strategy) and a prairie grass hay to maintain each horse's body condition.

*Experimental Design*

Horses from both the CON and VIB groups were randomly placed into two groups (GRP 1 and GRP 2) to allow a staggered start (by 1 day) to allow adequate time for evaluation and testing. The three horses from GRP 1 VIB were subject to the vibration plate (EquiVibe, Malcom, NE) for 45 minutes 5 d/wk, Monday through Friday. The three horses from GRP 2 VIB were subject to the vibration plate for 45 minutes 5 d/wk, Tuesday through Saturday. The vibration plate was set to 50 hertz, and the horses were tied and given hay during the treatment.

For Aim 1, all horses in both treatment groups were analyzed for heart rate, salivary cortisol concentrations, stride length, and degree of lameness, where VIB underwent the 45 min/d vibration treatment during the 28 day period and the CON remained in the stalls. For Aim 2, the same data was collected in VIB horses immediately before a vibration session (PRE), immediately after vibration (POST), one hour from the end of vibration (POST1), two hours after (POST2), four hours after (POST4), and twenty-four hours from the end of the treatment and before the start of the next treatment (POST24).

*Heart Rate*

Heart rates were taken on days 0, 1, 14, and 28. The heart rate monitors (Polar Equine RS800CX, Polar Electro Inc., Lake Success, NY) were placed on a surcingle with one lead fitted underneath the surcingle on the left side slightly below the withers, and the other just behind the left shoulder. Aloe gel and alcohol were used as conductants. The heart rate monitors were placed on the CON horses immediately before, during, and after their one time tracking each data collection period, on days 0 and 28 (Aim 1). The heart rate monitors were placed on the VIB horses immediately before each acute tracking, during each acute tracking (PRE, POST, POST1, POST2, POST4, and POST24), and during each vibration treatment, on days 0, 1, 14, and 28 (Aim 1 & 2).

*Cortisol*

Saliva samples were taken from the VIB groups on days 0, 1, 14, and 28. CON horses were swabbed just once on days 0 and 28 immediately prior to tracking (Aim 1). For Aim 2, all VIB horses were swabbed at each of the different time increments: PRE, POST, POST1, POST2, POST4, and POST24. Samples were later analyzed for cortisol concentrations using a salivary cortisol ELISA kit previously validated in the horse (Salimatrics, Carlsbad, CA).

*Stride Length*

For analyzing stride length, VIB horses, in no particular order, were hand trotted by the same handler in a straight line for 22.86 m on a standard clay/sand arena. CON horses were tracked just once on days 0 and 28 (Aim 1). Each tracking was recorded by a video camera that was placed approximately 18 m from the center of the tracking line so as to capture the entire duration of each horse's trot. On collection days (0, 1, 14, and 28), the VIB horses were tracked and videoed twice, for each of the acute time periods: PRE, POST, POST1, POST2, POST4, and POST24. All videos were analyzed via OnTrack Equine Software (OnTrack EQUINE, Lake Elmo, MN) motion analysis software to determine the stride length for each horse at the trot. The mean of three strides in the middle of the trot distance were extracted for each tracking period.

*Degree of Lameness*

The Lameness Locator™ (Equinosis, Columbia, MO) system utilizes three accelerometer sensors placed on the horse's poll, right forelimb pastern, and upmost part of the croup. Data are collected through the motion software remotely connected with the three sensors as the horses moved. The collection periods for this parameter were the same as the stride length, where the VIB group was hand tracked twice for degree of lameness on days 0, 1, 14, and 28, and the CON group was tracked on days 0 and 28 (Aim 1). VIB group lameness was tracked and analyzed twice, for each of the time periods in the same protocol as stride length (Aim 2).

*Statistical Analysis*

Statistical analyses were performed using a mixed model ANOVA with repeated measures in SAS 9.2, analyzing effect of treatment, day and treatment*day for Aim 1, and day, time, and treatment*time for Aim 2. Significance was defined as $P < 0.05$ and trends were considered at $P < 0.10$.

## Results

### *Aim 1*

*Heart Rate*

Heart rate demonstrated a day effect, showing an increase over the 28-d treatment period (P = 0.04), being higher on d 28 (87.2 ± 2.8 bpm) than on d 0 (77.6 ± 2.8 bpm). No difference was found attributable to treatment (P=0.13)

*Cortisol*

Cortisol was not different between VIB and CON (P = 0.41). Salivary cortisol concentration demonstrated a trend toward a day effect (P = 0.08), being lower on d 28 (0.15 ± 0.10 ug/dl) than day 0 (0.45 ± 0.10 ug/dl).

*Stride Length*

In the first tracking, there was a trend towards a day difference (P = 0.06) where both groups decreased from d 0 (2.58 ± 0.05 m) to d 28 (2.41 ± 0.05 m). In the second tracking, there was a day difference (P = 0.02) for stride length across both treatment groups for the second tracking, both groups being lower on d 28 (2.41 ± 0.05 m) than d 0 (2.58 ± 0.05 m). In the first tracking, there was no difference between the groups (P = 0.25). The second tracking showed a difference in the groups (P = 0.03) where CON (2.5

± 0.05 m) had a longer stride than VIB (2.39 ± 0.05). However no day*treatment effect was noted in either the first or second trackings (P = 0.75; 0.95).

*Degree of Lameness*

A treatment effect (P = 0.01) was found for the first tracking in minimum pelvis displacement, where VIB was less than CON (-1.99 ± 1.63 mm; 4.89 ± 1.63 mm; respectively). Similar results occurred for the second tracking in that VIB had less displacement than CON (-2.01 ± 1.34 mm; 2.17 ± 1.34 mm, respectively). This indicates there could be more hind end stability in the VIB group. However, no other markers of lameness were different due to treatment (P > 0.05). Lameness Locator data was also unchanged over time (P > 0.05).

***Aim 2***

*Heart Rate*

Heart rate increased over the 28-day treatment period (P = 0.01). Heart rate showed a trend toward a time effect (P = 0.06), being lower immediately post vibration period than any other time point (Figure 1).

*Cortisol*

Cortisol also decreased across the study period (P = 0.04), being lower on d 28 (0.10 ± 0.07 ug/dl) than d 0 (0.40 ± 0.07 ug/dl). There was also a time effect for cortisol concentrations (P = 0.049, Figure 2), where as it was lower immediately following vibration as well as at 2, 4, and 24-h post..

*Stride Length*

For the first tracking, stride length exhibited a day difference (P = 0.001), being shorter on d 28 (2.39 ± 0.05 m) and d 14 (2.42 ± 0.05 m) than on d 1 (2.73 ± 0.05 m). The second tracking exhibited similar results for a day effect (P = 0.003) where the stride length for both groups was shorter on d 28 (2.37 ± 0.05 m) and d 14 (2.36 ± 0.05) than on d 1 (2.76 ± 0.05). Time following vibration had no time effect on the first or second stride length tracking (P = 0.99; 0.92).

*Degree of Lameness*

No differences in lameness parameters were observed following WBV at any measured time point (P > 0.05).

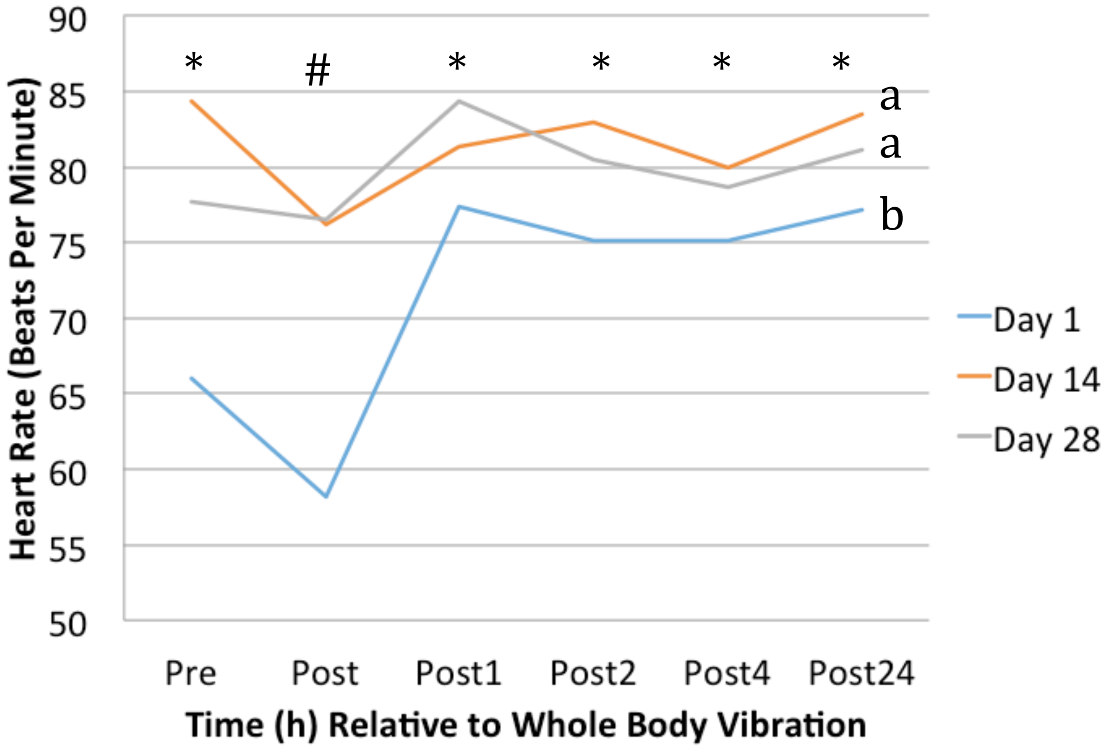


Figure 1- Heart rate in horses before and after 45-min of whole body vibration. Days and time-points not sharing a superscript are different (P<0.05).

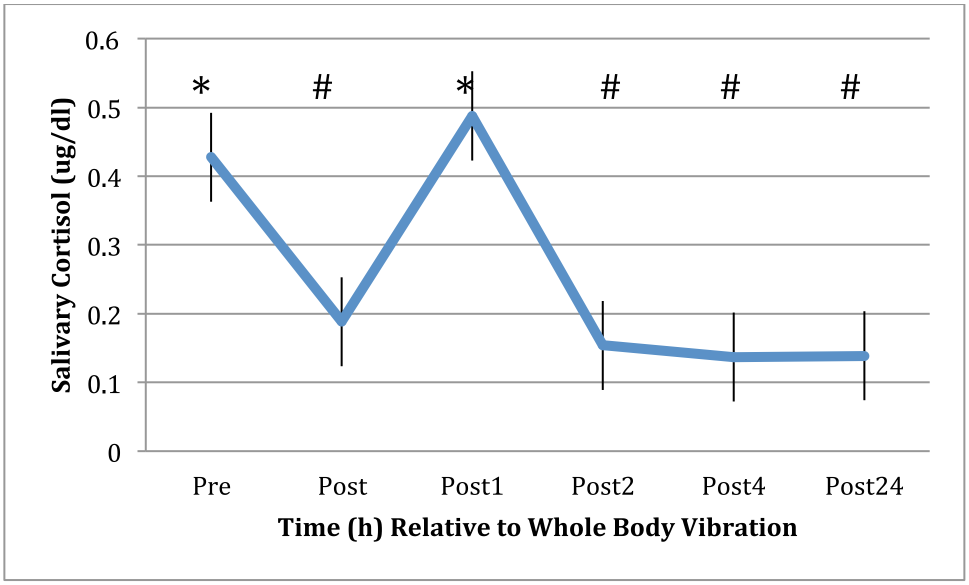


Figure 2- Salivary cortisol concentration in horses before and after 45-min of whole body vibration. Time-points not sharing a superscript are different ($P<0.05$).

**Discussion**

Until now there have been no published reports studying the effects of WBV across time periods or on cortisol concentrations, and little research on stride parameters. This current study suggests that WBV may have an acute relaxation effect on healthy horses, where heart rate and cortisol concentrations decreased immediately after the vibration sessions and when compared to the CON group. Interestingly, a similar effect has not been identified in human literature, where studies examining cortisol have indicated an increase in cortisol as a result of WBV (Perremans et al., 2001; Erskine et al., 2007). However, in those studies, participants were also participating in an exercise regime and thus the increase in cortisol may have been related to the stress of exercise.

No previous research has been done to study the effects of WBV on cortisol concentrations in horses. However, there has been some research on this effect in piglets and humans, both which found that WBV tends to increase cortisol concentrations in these two species (Perremans et al., 2001; Erskine et al., 2007). Yet it appears that, in horses, WBV has a relaxation effect thus causing cortisol concentrations to be lowered, differing from the human and piglet results. Anecdotal evidence would support the relaxation effect in the equine species.

Lameness and stride length exhibited less differences. Lameness only had an effect for the minimum pelvis displacement parameter when comparing VIB to CON over 28 days. Stride length decreased, however, in both VIB and CON groups across the 28 day treatment, indicating that WBV did not have a lengthening, or even mitigating, effect for stride as initially hypothesized. There is only one other published study observing the effects of WBV on lameness. Halsberghe (2017) observed an increase in lameness for chronically lame horses undergoing WBV. However this result was suspected by the authors to be attributable to an outlier in the data. More research is obviously needed to further observe the effects of WBV on lameness. It could be beneficial to utilize chronically lame horses and observe the effect of WBV across different time periods, such as in this current study. Further research, however, is necessary to observe the effect of WBV on lameness and stride length.

This study showed an increase in heart rate in both VIB and CON horses across the 28 day period. Yet there was an acute decrease in heart rate across the time periods for the VIB group, being lowered immediately after the vibration session but returning to baseline at POST1, POST2, POST4, and POST24. Maher and colleagues of 2016 also studied the effects of WBV on heart rate. They did not seek to investigate a time course, but found a decrease in heart rate for the VIB group when compared to the CON group when HR were measured in horses while on the vibration plate. The study by Maher and colleagues (2016) looked at exercising horses, who likely were less excitable than the horses herein due to the lack of turnout in this study.

Some studies suggest that WBV increases stride length in humans (Kawanabee et al., 2007; Giorgos and Elias, 2007). It is anecdotally known that stalled horses tend to be shorter in their stride due to the lack of stretch in the limbs, such as in a pasture turnout situation. Therefore it was the hypothesis of this study that the VIB horses' stride lengths would be maintained with WBV, whereas the CON stride length would decrease (Aim 1). However the results of this study suggest that WBV does not have an ability to mitigate the effects of stalling on stride length.

In this study many of the lameness parameters showed no difference when examined over a 28-d course of therapy (Aim 1) or acutely (Aim 2). There was a significant difference for the minimum pelvis displacement in the VIB group when compared to CON, where the VIB group seemed to be more even in their hind end movement. However the maximum pelvis displacement, maximum head displacement, minimum head displacement, or total head displacement did not exhibit any differences between treatment groups or across any of the time periods.

**Conclusion**

In summary this study suggests that WBV could have an acute relaxation effect, as heart rate and cortisol decrease with treatment, most specifically immediately following vibration treatment. Other studies have suggested that this may be the case, as well, demonstrating lower heart rate with vibration therapy. Since stride length decreased in both groups, WBV does not seem to have an effect on stride length in stalled horses. This study observed a decrease in the minimum pelvis displacement marker of lameness for the VIB group, indicating more hind end stability than controls. However, since no other marker of lameness showed a significant difference either over time or in an acute treatment, further research is needed. It could be useful to organize a similar study with clinically lame subjects, in order to see if there could be an acute or long term differences in those horses. Because WBV is being a popular anecdotal treatment for improving performance, more scientific evidence is needed to support these beliefs. For this study, WBV seems to incur acute and long term relaxation effects in healthy, stalled horses.

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# APPENDICES

## APPENDIX A: IACUC APPROVAL

# IACUC
**INSTITUTIONAL ANIMAL CARE and USE COMMITEE**
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: | ***Infleunce of vibration therapy on biometchanics 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 Subclassifications | ☐ **Teaching** | ☒ **Research** | | |
| | ☐ Classroom | ☒ Laboratory | ☐ Field Research | ☐ Field Study |
| | ☐ Laboratory | ☒ Handling/Manipulation | | ☐ 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 |

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.

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 (for questions) and
Iacuc_submissions@mtsu.edu (for sending documents)

IACUC Training - http://www.mtsu.edu/iacuc/IACUC_Training.php
IACUC Forms -