# ROLE OF CIRCULAR EXERCISE ON FORELIMB LOADING AND ACCOMPANYING SKELETAL AND JOINT ADAPTATIONS

By

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ABSTRACT

ROLE OF CIRCULAR EXERCISE ON FORELIMB LOADING AND ACCOMPANYING SKELETAL AND JOINT ADAPTATIONS

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Circular exercise is used frequently in equine exercise and competition, but little is known of the impact circular diameter and gait have to the joint and bone health of the forelimb. The first study evaluated the impact of circle diameter (10-m and 15-m) and gait to the forelimb solar outputs of average surface area, vertical force, and average pressure. Nine horses exercised in a straight line and in a round pen while wearing the Tekscan Hoof System™ on both front hooves with a glue-on shoe, a method of adherence which was determined to be reliable when measurements were recorded within one session. Gait, and not circle diameter, impacted forelimb outputs, with the average loaded area of the outside hoof while circling, being greatest at the canter ($P = 0.001$). While exercising on both a large and small circle, the outside hoof had greater vertical force at the canter than the trot ($P = 0.01$). A second study utilizing calves as a model for juvenile horses allowed the determination of physiological responses to circular exercise. Calves were assigned to small circle exercise (12 m), large circle exercise (18 m), treadmill exercise, or non-exercised control treatments ($n = 6$). Computed tomography and biomarkers were evaluated to determine impacts to bone and joint health. The inside leg of the small circular exercise group had larger dorsopalmar external diameter than the outside ($P = 0.05$). The medial proximal phalanx had greater mediolateral diameter than the lateral proximal phalanx of the small circle group ($P = 0.01$). Cartilage glycosaminoglycan concentration was greater in the outside leg of the small circle exercise treatment than the inside leg ($P = 0.03$). Combined, both of these studies suggest that circular exercise diameter and gait can impact animal health and should be considered when performing circular exercise.

This thesis is dedicated to all who have supported me in my academic and equine careers.

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# CHAPTER 1: Review of Literature

## BACKGROUND OF CIRCULAR EXERCISE

The use of circular exercise is frequent in equine populations, both under-saddle and on the ground via lunging or a round pen. During early training, horses are often exercised in a circular manner in a round pen or by lunging [1,2]. Some disciplines of riding, such as dressage, reining, and barrel racing use circular exercise during training and competition throughout a horse's career. Thoroughbred and Standardbred racehorses also perform circular exercise as they travel around a turn while racing as well as training. When surveyed, 50% of Thoroughbred trainers in Victoria, Australia indicated the use of a mechanical horse walker as an alternative exercise method to overground workouts [3]. Lunging with and without lunging aids, and the use of walkers are also found in many rehabilitation protocols [4,5].

In lameness evaluations, a higher proportion of lameness can be found while utilizing lunging on hard or soft surfaces compared to straight lines [6]. In fact, it may be difficult to discriminate between lameness, and inherent gait asymmetry caused by trotting in a tight circle during lameness evaluations [7,8]. While circular exercise is commonly used, most of the industry is unaware of potential negative impacts it can have on joint health. Anecdotal evidence suggests a connection between circular exercise and lameness. When being exercised in a circle, horses will lean into the continuous turns up to 20° to maintain balance [9,10]. As speed increases and radius decreases, lean angle will also become more severe [11]. Due to the reduced surface area of the hoof that is loaded, uneven forces may be placed on joints and bones of the fore and hind limbs during circular exercise [9]. These uneven forces exerted on a smaller surface area have the potential to lead to a higher risk for joint injury and osteoarthritis.

## CIRCULAR EXERCISE IN HUMANS AND QUADRUPEDS

There are limited equine studies evaluating circular exercise. However, human exercise studies evaluating running on a curve are plentiful. When running around a curve, the inside and outside limbs of humans are not biomechanically symmetrical, and have different functions [12,13]. When running around an un-banked curve the inside leg has lower peak ground reaction force than the outside leg, which has increased ground reaction force. Speed is found to be slower when sprinting around a curve compared to sprinting in a straight line [12]. In both male and female athletes, race times for the 200-m sprint are slower on indoor tracks than outdoor tracks. When straight aways and curves of indoor and outdoor tracks were evaluated, it was found that athletes were slower on curves of the indoor tracks compared to outdoor tracks. The authors attribute this difference in race times to the notably smaller curve radii of indoor tracks [14].

In humans, the outside leg plays an important role in changing the direction of travel while running through a curve, as the outside leg generates larger centripetal force, thus pushing the runner through the direction change [13]. Within a cohort of college students, those with larger cross-sectional area to the psoas major of the outside leg compared to the inside leg were able to run faster around a circle of 23-m radius [15]. Not only does the presence of a curve impact runners, so does the sharpness of a curve. Running on a sharply curved track (5-m radius) leads to greater torsion on the inside tibia compared to running on a gently curved track (15-m) or a straight line [16]. As circle radius decreases, peak resultant ground reaction forces to the inside leg decrease compared to the outside leg [12].

Quadrupeds, such as horses, may be at an advantage during curve running, as they can redistribute weight to multiple stance legs within a stride. It has been found that while cantering in a 10-m circle, horses will have greater peak ground reaction force on the outside forelimb compared to the inside forelimb [17]. At the trot, a two beat diagonal gait, horses have been found to have decreased loading when the inside foreleg and outside hindleg are in stance, compared to a push-off pattern for the outer limbs [18]. While traveling around a curve, Thoroughbred race horses experience greater strain to the outside forelimb, which is increased as speed increases [19]. In both humans and horses, the outside limb while sprinting through a curve is known to generate more vertical and lateral force than the inside limb, to serve as a push-off limb [11].

When speed is held constant between a straight line and a curve, stride duration and subsequently stride length, are seen to increase when horses travel around a curve compared to a straight line. With training, this increase in stride duration around a curve is seen to decrease, potentially through familiarity via training and neuromuscular adaptation [20]. In humans, step frequency will decrease as the radius of a curve decreases, leading to a decreased velocity. Given this, during track races the individuals running in the innermost lane may be at a disadvantage as they are running a tighter radius [21].

As travel around a curve is performed, forces are exerted in many directions, not just the vertical force of the limb interacting with the area loaded directly underneath the foot or hoof [22]. Lateral forces are responsible for propulsion, while transverse forces are responsible for turning. Vertical, lateral, and transverse ground reaction forces are frequently captured with a force-plate embedded into a surface and kinematic data captured with a camera system [23–25]. The use of an embedded force plate is difficult for gait analysis on a curve, especially at high speeds such as while racing. Compared to bipedal sprinters, such as humans, quadrupeds are estimated to be superior at curve running due to their ability to have more than one stance leg or push-off leg during a stride.

Another quadruped which is known for track racing is the greyhound. The majority of racing injuries in New Zealand racing greyhounds are localized to the limbs (83%), with overall injury rate increasing with age. Nearly 50% of injuries occur while the greyhound is running on a bend, with 17% occurring on a straight section of track [26]. Track design is a significant factor to greyhound injury rate, dogs are more likely to injure themselves in the first turn of an oval track, due to congestion and contact between dogs, as well as high speed of travel while exiting the straight-away and entering the turn [26,27]. Some tracks closely resemble a circular track and do not have dramatic curves like a traditional oval track with a long straight-away. These circular tracks are predicted to have a lower injury rate due to the lack of a sharp curve between a straight-away and the start of a turn [28]. While greyhound racing and horse racing have unique characteristics, it is worth noting that the presence of running on a curve leads to injuries in both species.

It has been hypothesized that horses handle traveling in a circle similar to the adjustments they make in response to lameness on a straight line; horses counteract uncomfortable limb loading with asymmetrical movement which redistributes limb loading [7]. Stride length of the inside leg is shorter than the outside leg at both the walk and canter when lunging on a 10-m circle [29]. Horses will lean into the circle in which they are traveling, with the lean increasing with speed and smaller radii. Body lean may also be increased when a lame forelimb is on the inside of the circle, with lameness score also increasing [8,30]. Level of training can also impact lean angle, as a well-trained horse acclimated to traveling on a circle can travel more upright than a horse which is not acclimated to traveling in a circle or bend. This has especially been noted in dressage, where trained horses are able to travel “on the bit”, while an inexperienced horse may not be able to maintain bit contact and engage the topline and hindlimb musculature through a circle [8].

For dressage horses, lunging has been found as “protective” against lameness, while time on a walker is associated with lameness [31]. In dressage, lunging is typically done with a horse in a bridle and surcingle, allowing a skilled handler to encourage a horse to travel on a lunge line in a more-upright position and in larger circles. These animals frequently exercise under saddle in the same size and speeds as they are exercised on the lunge line, so for dressage horses, lunging is often similar to ridden exercise. Horses exercised freely on a mechanical walker may have more lean than those lunged in surcingle, the walker may have a relatively smaller radius, and the horses are often unsupervised on a walker. Walkers are also often used during injury rehabilitation and recovery, which may be a confounding factor in the association between walker use and lameness.

## CIRCLE RADIUS AND GAITS

Horses can utilize many gaits to travel, most common are the walk, trot, canter, and gallop. The walk is a symmetrical gait with a lateral sequence of footfalls having no suspension. The trot is a symmetrical, two-beat diagonal gait with suspension. Unlike the walk and trot, the canter is an asymmetrical gait characterized by three beats with a suspension phase after lift-off of the leading forelimb [32]. At the canter, horses will move with a protracted leading limb through greater flexion of the elbow and hip joints [33]. When canter speed increases to the gallop, the diagonal pairs become dissociated, leading to a four-beat gait [32]. While galloping, a horse will propel itself over the single lead limb, with the lead limb sustaining a greater load than at a symmetrical gait, such as the trot [34]. A four-beat lope has also been noted in western-style horses [35]. This style of lope is much slower than that of a four beat gallop is not currently preferable in performance, but is found frequently among the western stock-horse population, especially during lunging.

In both human and equine runners, the radius of a curve has been found to impact movement characteristics. When trotting in a small circle with a radius of 1.8 m, horses can travel with a lean-in angle of up to 20°. Overall, decreasing circle radius and increasing speed will lead to an increased body lean-in angle [9,36]. During Thoroughbred racing, injuries are most likely to occur when running around a curve than on a straight section of track [11]. Dirt tracks with tighter turn radii have a higher risk for right (outside) forelimb fractures, while the left (inside) forelimb is at a lower risk for fracture while racing at the tightest radii on dirt tracks, potentially due to the increased centripetal force on the outside limb providing propulsive and turning forces during a tight turn at high speed [37].

Yearling Quarter Horses in training for lunge-line classes typically travel at slower speeds than that of English-style lunging. A study evaluating the response in serum osteocalcin and keratin sulfate of lunging in a large circle while preparing for lunge-line classes found that lunging did not significantly alter osteocalcin or keratin sulfate concentrations compared to animals exercised on a mechanical walker [38]. Unfortunately, this study did not include a straight-line exercise group for comparison. The characteristic slow speeds and large circle size during preparation for a stock-horse lunge-line class may not have elicited changes in the studied markers, but other non-studied markers or bone characteristics determinable by radiographs may have been impacted. Alternatively, higher speeds and smaller circle size would be expected to lead to greater lean-in angle during travel, potentially leading to bone and joint health biomarker responses suggesting joint damage.

Combining both variability in circle diameter as well as gait of travel, can lead to unique conditions of circular exercise. For example, lean-in angle had been found to be 12° on average at the trot and 19° at the canter [39]. Lean-in angle most likely occurs to prevent a horse from falling over during circular exercise, and to align the limbs with resultant forces to avoid excessive torque to joints [39]. Understanding that decreased circle diameter and increased speed lead to greater body lean-in angle, can aid in clarity towards conditions which may be detrimental to the horse [9,36].

## CIRCULAR EXERCISE UNDER SADDLE

Movement asymmetry has been found, even in sound horses, when exercising in a circular motion at the rising trot. The technique of the posting trot should be taken into account, as one half of the stride the rider is sitting down in the saddle, while the other half they are posting out of the saddle [18]. When riding the trot in a sitting position, the rider's weight is parallel between the two diagonals. However, when posting the trot, the horse's back is unloaded during the rising part of the post. During the sitting part of the post, weight is distributed very similar to the sitting trot [32]. It is worth noting that the experience, balance, and confidence of the rider could impact an observed asymmetry during the posting trot on a circle.

Unpublished data in the Spartan Equine Research lab found that when ridden by a professional rider, a horse has more consistent force output of the forelimbs and lower average force output compared to being worked in-hand (Figure 1 and 2). This has been noted both on a straight line and in circular exercise. The lower force output of the forelimbs with the presence of a professional rider may be due to the fact that a professional rider is able encourage a horse to transfer weight to the haunches [32]. A study evaluating sacroiliac joint pain in horses found that when ridden, horses were less likely to canter on the incorrect lead in the hindlimbs than when lunged, potentially because a rider can to encourage the horse to stay balanced and maintain its lead better than lunging [40]. A study evaluating movement symmetry differences at the trot between ridden and non-ridden reining Quarter Horses found that with an experienced rider, wither and pelvic parameters were more symmetrical when ridden. The more-movement for western riders may be due to the fact that the western jog is ridden in a sitting position, with no asymmetrical posting [41].

Figure 1: Tekscan™ outputs of Force vs. Time with a horse trotting in a straight line
*Bright green (left) and red (right) values represent the forelimb forces while worked in-hand. Dark green (left) and purple (right) values represent the forelimb forces while ridden by a professional. Each "peak" represents one step with the associated limb.*

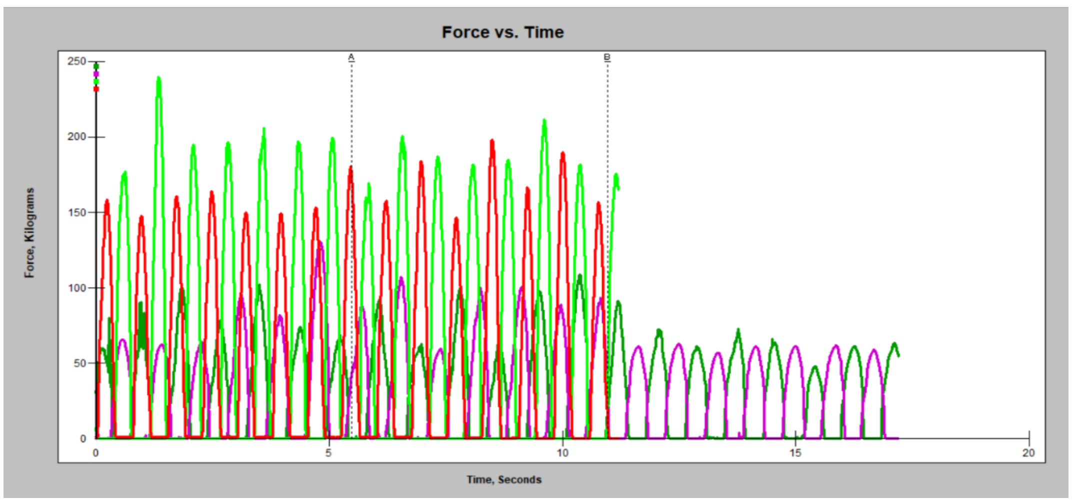
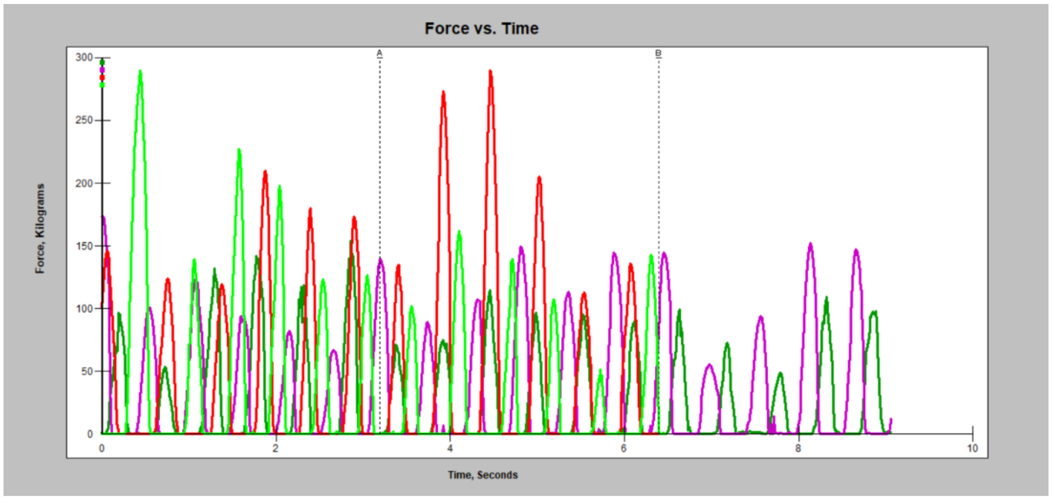


Figure 2: Tekscan™ outputs of Force vs. Time with a horse cantering to the left in a 10-m circle
*Bright green (left) and red (right) values represent the forelimb forces while worked in-hand. Dark green (left) and purple (right) values represent the forelimb forces while ridden by a professional. Each "peak" represents one step with the associated limb.*




When experienced riders rode non-lame horses at the rising trot with one stirrup 5 cm shorter than the other, more rotation and bending of the thoracolumbar spine of the horse was seen when the shortened stirrup was on the inside compared to the outside. This asymmetry is attributed to the fact that riders tilted towards the shortened stirrup. Riding with the shortened stirrup on the outside also lead to greater inside limb fetlock extension [42]. While riding at the sitting trot, collapse of one hip increases the force on the opposite side, while tilt of the rider's upper body in one direction leads to greater pressure on that same side [43].

A study evaluating horses trotted in-hand and under saddle found that hind limb lameness increased when ridden by a dressage rider compared to unridden [44]. Another study found that a professional rider has been found to decrease gait velocity and acceleration variability at the working trot, compared to the unridden trot [45]. It is important to note the impact that a rider can have on horse locomotion, with both the potential to increase asymmetry and variability, or decrease in the case of the professional rider. Conditions of circular exercise, such as the presence and experience of a rider need further evaluation to determine if there is a consistent impact to equine bone and joint health.

## INJURIES

Osteoarthritis and joint injuries have been reported as a leading cause of lost training days and horse wastage, yet little has bridged that gap between circular exercise and joint damage. With up to 60% of lameness being related to osteoarthritis, this gap in research greatly affects the equine community [46,47]. A 10 – year retrospective study of western performance horses found the location of the majority of lameness to be the distal forelimb [48]. Within western performance, all-around horses (western pleasure, western riding, western horsemanship, and trail) and reining horses both experienced lameness that was most frequently in the distal forelimb. Reining horses experience greater speeds while traveling and turning compared to all-around horses. While the discipline of reining rarely utilizes lunging, reining horses are loped in circles extensively, as circles are a required maneuver in reining patterns and lateral flexibility is highly desired in these horses. Quarter Horses are the predominant breed used for reining, and often succumb to osteoarthritis [49]. In a survey across the United States, Quarter Horses and Thoroughbreds were found to make up over half of the population affected by osteoarthritis [50]. A dearth of research utilizing performance horses as the subject of research exists, especially given the high incidence of osteoarthritis in Quarter Horses and popularity of these horses across the United States.

Bone is able to adapt to the strains it is placed under, or a lack of strains. Based on the difference in training regimens they commonly experience, racing Thoroughbred and Standardbred horses can have different bone shape to the third metacarpal [34]. Racing Thoroughbreds travel short distances at the gallop and Standardbreds travel longer distances at the trot or pace. Another study found that when turning counter-clockwise, a small cohort of Thoroughbred horses had increased strain measured on the lateral surface of the left (inside) third metacarpal and medial surface of the right (outside) third metacarpal when walking on a bitumen surface [51]. When exercising on a straight, flat surface, the lateral cortex of the third metacarpal is not significantly loaded like it is loaded when running on a turn [51]. This may put the lateral cortex at higher risk of fracture when running into a curve at high speeds. Catastrophic fractures in older Thoroughbred racehorses frequently occur in the lateral cortex of the left third metacarpal, most likely due to the high strains experienced to the leading front leg during turning [52].

At North American Thoroughbred racetracks, from January 2009 – December 2014, horses euthanized due to fracture within 72 hours of race start, were most frequently found to have forelimb fractures compared to hindlimb [37]. In the majority of North American tracks, track shape is an oval, and races are run counterclockwise, leading to horses traveling with their left lead around a turn, then switching to their right lead on a straightaway to prevent fatigue of traveling on one lead the entire race. A study of Midwestern racetracks found that in Thoroughbreds the left forelimb was the most common site for injuries (56% of CMIs) [53]. As a result of greater impulse on the left forelimb while galloping around the turn of a race track, Thoroughbreds are at greater risk for injury to the left forelimb while traveling around a turn [54]. In Quarter Horses, the right forelimb is most commonly involved in catastrophic musculoskeletal injuries (CMIs), with the left forelimb following second (57% and 24% of CMIs, respectively). This may be due to the preference of the right lead in racing Quarter Horses [46,53]. However, the presence of motor laterality within the equine population is questionable and may not exist outside of horses in race training, as it was not found in Quarter Horses trained for cutting [55].

This may seem contradictory, as previous sections in this review have discussed increased forces to the outside front limb during turning both in training at slower gaits and racing at the gallop. However, during turning, the outside front limb has a pushing function, and while a horse is galloping, the weight of the horse will be pushed forward and laterally onto the lead forelimb [34]. The risk of injury to inside or outside forelimb appears to be dependent on curve radius, with the tightest of curve radii being higher risk to the outside forelimb [37]. Curve radius and the potential for catastrophic injury can also put jockey's at risk; a retrospective study of risk factors for jockeys in Japanese Thoroughbred racing found smaller tracks to have a greater risk of injury [56].

## IMPROVEMENTS

The transition from running along a straight portion of a track into a curve can be drastic and cause a horse to lose coordination. To mitigate this, transitional curves, such as a gradual curve that slowly decreases in radius, can be implemented at tracks [57]. One way to rid tracks of the problem of curve design is to race on straight tracks. This is how racing started, however, is not conducive to spectators. A completely circular track could also be used, but also comes with its own set of issues such as increased space, ideal speed, and safe starting [57]. The Hanshin Racecourse in Japan which was found to have geometric and surface issues was restructured, and horse injuries were evaluated after this restructuring. This racetrack was nearly flat, and triangular shaped (turns 1 and 2 were nearly seamless, with turns 3 and 4 as the base of a triangle), producing quick times, as well as higher fracture risk. After many years of racing, the surface had become concerningly hard. Restructuring resulted in more gradual corners, better surface cushioning, and an upgrade slope before the finishing stretch. After restructuring, severe injuries were significantly reduced, including fracture and non-fracture injuries. The track renovations did effectively decrease horse speeds, leading to slower racing times caused by slower times around the 3rd and 4th corners and the home stretch [58].

The increase in banking at tracks for Standardbred racehorses has led to a decrease in injuries. Standardbred racetracks are often smaller than those used for Thoroughbreds, necessitating banked turns to prevent injuries [19,59]. Thoroughbred racetracks do not include banking as dramatic as Standardbred racetracks. The lack of banking causes a horse to need to change how their limbs interact with the ground underneath of them. Horses running at high speeds through a curve utilize friction and place their feet sideways to stay on the flat, curved path. The inclusion of a greater degree of banking and increasing the radius of corners for Thoroughbred racehorses may reduce injuries [19,57].

Even at speeds slower than the racing gaits of Thoroughbreds and Standardbreds, banking has been found to allow horses to travel around a curve in a similar fashion to a straight line. When traveling on a 10-m circle that is flat, horses will perform greater inside forelimb adduction (bringing closer to the midline of the body) compared to a banked surface at the walk, trot, and canter [29]. When lunging on a flat circle, horses may need more forelimb adduction to properly balance as they lean into the curve, and allow their mass to travel over the stance leg. Body lean is also greater in a flat lunge circle than a banked lunge circle. As the body leans into the curve, the limbs also “tilt” and are potentially loaded unevenly. However, the use of banking deceases relative body lean angle, and therefore reduces uneven loading of the limbs [29].

Acclimation to traveling around a curve may be needed for young horses. Given that young horses in race training can become acclimated to running through a curve, an adjustment period should be considered in training regimens [20]. Speed work in young horses should not be avoided, as it is beneficial for bone formation [60–63], and should be permitted in the straight portions of a track while animals are acclimating to traveling around a curve at high speeds.

# CHAPTER 2: Evaluation of within- and between- session reliability of the Tekscan™ Hoof System with a glue-on shoe

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A current trend in equine research is technology development to minimize the subjective nature of gait analysis. One such technology is the Tekscan Hoof System, which records force and area loaded by the hooves during motion. The objective of this study was to determine the test-retest reliability of the Tekscan Hoof System between two sessions, and the recordings within those sessions. Four mature Standardbred geldings wore Tekscan Hoof System sensors on both front hooves, secured by glue-on shoes (SoundHorse Technologies). Horses were exercised in AM and PM sessions. In each session, horses walked and trotted for three recordings of at least 10 steps. Statistical analysis was performed in SAS 9.4 with fixed effects of gait, horse, leg, and recording nested within session (significance at $P \leq .05$). Intraclass Correlation Coefficients (ICC; 3,k) and confidence intervals between AM and PM sessions and recordings were calculated with SPSS. Average force and area were higher in AM sessions than PM sessions ($P < .0001$). Between AM and PM sessions, ICC for the walk had good reliability (0.96, 95% CI = 0.80 – 0.99) and excellent reliability at the trot (0.98, 95% CI = 0.91–0.99). Within the AM and PM sessions, reliability was excellent at both the walk, and trot (ICCs > 0.96). The Tekscan Hoof System has been found to have excellent reliability within sessions. Caution should be taken when comparing between sessions, as the system is found to have lower force and area output during later sessions due to potential sensor damage.

## Chapter 3: Impact of gait and diameter during circular exercise on front hoof area, vertical force, and pressure in mature horses

Logan, A. A.; B.D. Nielsen, C. I. Robison, D.B. Hallock, J.M. Manfredi, K.M. Hiney, D.D. Buskirk, J.M. Popovich Jr. *Animals* **2021**, 11, 12. https://doi.org/10.3390/ ani11123581

SIMPLE SUMMARY

Circular exercise is used frequently to exercise, train, and evaluate horses both under saddle and with lunging. However, little is known of the impacts this type of repetitive exercise has on the front limbs of horses. Nine mature horses wore Tekscan$^{TM}$ Hoof Sensors on their front hooves to determine if changing the circle size and gait at which the horse is traveling impacts the area, vertical force, or pressure output. Sensor data were collected while horses travelled in a straight line at the walk and trot and in small and large counterclockwise circles at the walk, trot, and canter. Gait was found to be a driving factor for differences in outputs, with mean area, mean vertical force, and mean pressure being greater at the walk in a straight line, and the area being greater at the canter when circling. When traveling in a counterclockwise circle, the mean area of the outside front leg was highest at the canter. This study shows gait is an important factor when evaluating exercise in a circle or straight line. Horse owners may choose to perform circular exercise at slower gaits or minimize unnecessary circular exercise to decrease differences between limbs and potentially reduce injury.

ABSTRACT

Circular exercise can be used at varying gaits and diameters to exercise horses, with repeated use anecdotally relating to increased lameness. This work sought to characterize mean area, mean vertical force, and mean pressure of the front hooves while exercising in a straight line at the walk and trot, and small (10-m diameter) and large circles (15-m diameter) at the walk, trot, and canter. Nine mature horses wore Tekscan$^{TM}$ Hoof Sensors on their forelimbs

adhered with a glue-on shoe. Statistical analysis was performed in SAS 9.4 with fixed effects of leg, gait, and exercise type (PROC GLIMMIX) and $p < 0.05$ as significant. For all exercise types, the walk had greater mean pressure than the trot ($p < 0.01$). At the walk, the straight line had greater mean area loaded than the large circle ($p = 0.01$), and both circle sizes had lower mean vertical force than the straight line ($p = 0.003$). During circular exercise at the canter, the outside front limb had greater mean area loaded than at the walk and trot ($p = 0.001$). This study found that gait is an important factor when evaluating circular exercise and should be considered when exercising horses to prevent injury.

## INTRODUCTION

The use of circular exercise is frequent in equine training, both under-saddle and in-hand via lunging, and anecdotally has the potential to contribute to lameness. During early training, horses are often exercised in a circular manner through lunging or in a round pen. Some riding disciplines, such as dressage, reining, and barrel racing, use circular exercise during training and competition throughout a horse's career. Often, the circles performed in these disciplines are on a small radius with high speed and are utilized frequently within a training session. Thoroughbred racehorses also experience circular forces as they lean into a bend at high speeds [11,20]. Lunging with and without lunging aids, and the use of mechanical horse walkers is found in many rehabilitation protocols [4,5]. When surveyed, 50% of Thoroughbred trainers in Victoria, Australia indicated the use of a mechanical walker as an alternative exercise method to track work [3]. In lameness evaluations, a higher proportion of lameness can be found while utilizing lunging on hard or soft surfaces compared to straight lines [6]. While circular exercise is commonly used, many in the industry are unaware of the potential negative impacts it can have on joint health. While being exercised in a circle, horses will lean into the continuous turns up to 20 degrees to maintain balance. As speed and curve increase, the lean angle will also increase [8–11,29]. Greater tilt on a flat curved surface has also been found compared to a banked curve surface [29]. Due to the possibility of a reduced loaded area, uneven vertical forces may be placed on joints and bones of the limbs during circular exercise.

Quadrupeds, such as horses, may be at an advantage during curve running, as they can redistribute weight to multiple stance legs within a stride [11]. It has been found that, while cantering in a 10-m circle, horses will have greater peak ground reaction force on the outside forelimb compared to the inside forelimb. This difference in vertical forces between limbs was not evident at slower speeds and may be due to the presence of a lead and non-lead forelimb and hindlimb during the canter. In both humans and horses, the outside limb while sprinting through a curve is known to generate more vertical and lateral force than the inside limb [11]. When speed is held constant between a straight line and a curve, stride duration is seen to increase when horses travel around a curve compared to a straight line. With training, this increase in stride duration around a curve is seen to decrease, potentially through familiarity via training or neuromuscular adaptation [20].

There are few equine studies evaluating circular exercise; however, human exercise studies evaluating running on a curve are abundant. It has been found that in humans, when running around an unbanked curve, the inside leg has a lower peak ground reaction force than the outside leg. Peak ground reaction forces of both legs and speed are also lower when sprinting around a curve compared to in a straight line [12]. Not only does the presence of a curve impact runners, so does the sharpness of a curve. Running on a sharply curved track (5-m radius) led to greater torsion on the inside tibia compared to running on a gently curved track (15 m) or a straight line [16]. Both the speed and the radius of a circle will impact the gait asymmetry of horses circling at the trot [8].

A retrospective study of risk factors for jockeys in Japanese Thoroughbred racing found smaller tracks to have a greater risk of injury [56].

In racing Quarter Horses, who race on straight portions of tracks, the right forelimb is most commonly involved in catastrophic musculoskeletal injuries (CMIs), with the left forelimb following (57% and 24% of CMIs, respectively). This may be due to the preference of the right lead in racing Quarter Horses [46]. However, the presence of motor laterality within the equine population is questionable and may not exist outside of horses in race training, as it was not found in Quarter Horses trained for cutting [55]. Thoroughbreds typically race counterclockwise on an oval track in North America, with the left front leg as the leading leg while traveling around a curve in the track. A study of Midwestern U.S. racetracks found that the left forelimb was the most common site for injuries (56% of CMIs) in Thoroughbreds, while the right forelimb was the most common (60% of CMIs) in Quarter Horses [53]. As a result of greater impulse on the left forelimb while galloping around the turn of a race track, Thoroughbred racehorses are at greater risk for injury to the left forelimb while traveling around a turn [54].

It has been hypothesized that horses handle traveling in a circle similar to the adjustments they make in response to lameness on a straight line; horses counteract uncomfortable limb loading with asymmetrical movement, which redistributes limb loading [7]. Horses will lean into the circle in which they are traveling, with the lean increasing with speed and smaller radii [8]. The level of training can also impact lean angle, as a horse acclimated to tracking in a circle can travel more upright than a horse that is not acclimated to tracking in a circle or bend. This has especially been noted in dressage, where older, trained horses are able to travel while engaging their neck, back, and hindlimb musculature, where a younger horse may not be able to do so through a circle. Body lean may also be increased when a lame forelimb is on the inside of the circle [10,29,64]. When circling at the trot, a two-beat diagonal gait, horses have decreased loading when the inside front leg and outside hind leg are in a stance, compared to a push-off pattern for the outer limbs [18,65].

Osteoarthritis (OA) and joint injuries have been reported as a leading cause of lost training days and horse wastage. There may be a connection between OA and circular exercise, but interactions between circular exercise and joint damage have not been explored. With up to 60% of lameness being related to OA, this gap in research greatly affects the equine community [46,47]. Within the United States, Quarter Horses and Thoroughbreds have been identified as making up half the population affected by OA [50]. Osteoarthritis can occur secondary to excessive loads on normal cartilage or normal loads on abnormal cartilage [66,67], with both mechanisms possibly exacerbated by circular exercise. It has been noted that horses are able to perform adaptations to limb position while exercising on a circle via abduction (pushing the limbs away from the midline of the body) and adduction (bringing the limbs towards the midline), but these adaptations performed over long periods of time and at faster gaits may lead to greater risk of injury to the distal limb [29,67].

Utilizing the Tekscan™ Hoof System (Tekscan, Inc., Boston, MA, USA), the aim of this study was to categorize the outputs (i.e., area, vertical force, and pressure) for the front hooves of horses during counterclockwise circular exercise, and to demonstrate that these outputs vary depending on circle diameter and gait. It was hypothesized that faster gaits and a decreased circle diameter would lead to greater disparity in the mean area, vertical force, and pressure of the front limbs, with the outer limbs having a smaller mean loaded area with greater mean vertical force and pressure.

## MATERIALS AND METHODS

This research was approved by the Michigan State University (MSU) Institutional Animal Care and Use Committee (PROTO201800148).

### *Horses*

A total of nine mature horses participated in this study (14 ± 2 years). Arabian horses were obtained from the MSU Horse Teaching and Research center (n = 4: Two mares and two geldings) and stock horses were obtained from a local training operation (n = 5: Two mares and three geldings). One week prior to beginning exercise, horses were evaluated by two board-certified veterinarians (large animal surgery and one also boarded in equine sports medicine and rehabilitation) with a Lameness Locator® and subjective lameness evaluation to be sound (American Association of Equine Practitioners lameness grade of <2 on each front leg). Horses were transported to the MSU Pavilion South Arena for one day for exercise analysis. During the day, while not being exercised, horses were given ad libitum access to water and hay and kept in individual stalls.

### *Hoof Preparation and Sensors*

Hoof and sensor preparations were performed in a method previously utilized when using the Tekscan Hoof System™ with a glue-on shoe [68]. Horses were trimmed by a certified farrier (Certified Journey Farrier, Advanced Skill Farrier, Associate of the Worshipful Company of Farriers) within a week before exercise. Horses were trimmed for medial-lateral balance according to the long axis of the limb observed with the hoof picked up. Excess hoof was removed as needed to achieve a flat plane to place sensors, and shoe placement was guided by the center of rotation (COR) [69]. Horse hooves were measured for width and length to determine an accurate glue-on shoe (SoundHorse Technology) size for each horse (Table 1). An exact fit for each front hoof and shoe was desired so that the loaded sensor area represented the loaded area of the hoof. Tekscan hoof sensors were trimmed to the size of the front hooves of each of the horses after initial hoof trimming. The trimmed sensors were sealed in two layers of liquid rubber (Flex Seal, Weston, Florida) to protect the sensors from moisture and sand exposure. The liquid rubber sealing was allowed to dry for 24–48 h before sensors were placed on horses.

The day before horses exercised, horses were weighed for calibration. Two scales of equal height were used. Horses stood with their hind legs on one scale and their front legs on another and were encouraged to stand square with their weight equally distributed. The weight of the front half of the horse was recorded on one scale for sensor calibration (Tru-Test Multipurpose MP600 Load Bars). This weight was divided in half, to represent the left limb and the right limb (Table 1).

On the day of exercise, the sealed and trimmed sensors were attached to the front hooves of each horse with a glue-on shoe and animal-safe epoxy. Horses were shod with a ratio of 60% in front of COR and 40% behind [69]. After the initial mixing, the two-part epoxy used to adhere the shoe to the hoof wall was dried for approximately 30 min to be cured for exercise. Before beginning exercise, the sensors were calibrated with the previously recorded weight of the forelimbs. Each horse walked the length of the indoor arena to pre-load the sensors. Afterwards, they were brought to a flat spot in the middle of the arena and encouraged to stand squarely with their weight evenly distributed. Using F-Scan research software (Tekscan™), the previously determined weight of the front limbs (Table 1) was inputted with the step calibration function, and a calibration file was saved for the left and right forelimbs for each horse.

Table 1: Horse details—forelimb calibration weights used for the Tekscan Hoof System and front hoof measurements used to accurately assign front hoof glue-on shoe size for each horse.

| Horse | Breed | Shoe Size | Forelimb Weight (kg) | Left Hoof Width (mm) | Right Hoof Width (mm) | Left Hoof Length (mm) | Right Hoof Length (mm) |
|---|---|---|---|---|---|---|---|
| 1 | Stock horse | 00 | 170 | 120 | 120 | 138 | 135 |
| 2 | Stock horse | 0.5 | 169 | 138 | 138 | 136 | 136 |
| 3 | Stock horse | 1.5 | 167 | 127 | 130 | 138 | 138 |
| 4 | Stock horse | 00 | 156 | 127 | 127 | 129 | 130 |
| 5 | Stock horse | 0 | 135 | 127 | 128 | 127 | 127 |
| 6 | Arabian | 1.5 | 139 | 135 | 130 | 145 | 140 |
| 7 | Arabian | 0.5 | 138 | 130 | 125 | 128 | 128 |
| 8 | Arabian | 0 | 131 | 122 | 135 | 130 | 125 |
| 9 | Arabian | 0 | 127 | 125 | 125 | 127 | 127 |

*Exercise*

Previous research with the Tekscan Hoof System™ has found that when used with a glue-on shoe on the front hooves, the sensors are reliable within a session of exercise [68]. Bearing this in mind, the current study was designed that each horse would complete all their exercise within one session, and each set of sensors would only be used once. Straight-line exercise was performed first for each horse, so that if a sensor was damaged during circular exercise, then all horses would have straight-line exercise recordings. The space in which straight-line exercise was recorded was 25 m in length. Multiple recordings have previously been suggested for exercise protocols utilizing sensors such as this to gain a mean of the desired outputs [70–73]. Each horse was recorded three times at both the walk and trot traveling in a straight line the length of the indoor arena. The canter was not recorded in a straight line, as the gait is not able to be safely and consistently attained when horses are led in-hand. Each recording included at least 10 steps of the horse performing the specified gait consistently with no break. All straight-line exercise was performed by the same handler.

After the straight-line exercise, each horse was led to the portable round pen in the middle of the indoor arena. Order of size for the circular exercise (small first or large first) was randomly assigned for each horse. The size of the small circle was 10 m in diameter, while the size of the large circle was 15 m. Circle diameter was adjusted by adding or removing portable round pen panels with the perimeter for each circle size marked in the sand of the arena so that both circles were set up the same each time. For each horse, three recordings of at least 10 strides at the walk, trot, and canter were taken for both the large circle and the small circle. All circular exercise was performed in a counterclockwise direction, with the left forelimb on the inside and the right forelimb on the outside. Only one direction was evaluated so that one limb could be consistently denoted as the outside limb and the other as the inside limb. Counterclockwise was chosen as this is the direction of travel for racing horses and is typically the first direction of travel for horses competing in judged classes on the rail. Horses were encouraged to maintain gait speed with a human handler either verbally or visually encouraging them to gain speed or slow down. The speed of the walk, trot, or canter was not controlled between animals, as each individual animal has a speed for each gait at which they are able to move comfortably and maintain their gait consistently through recordings. Other gait analysis studies have preferred to allow animals to travel at their natural speed within a gait during testing [6,18,47,71]. If there were errors such as an incorrect lead or break of gait, the recording in process was stopped and re-recorded. Following exercise, the glue-on shoes and sensors were removed from each horse.

*Data Analysis*

Sensor data were recorded at a sampling rate of 112 frames/second for all conditions. Recorded data were then analyzed with Tekscan F-Scan Software (version 6.85). F-Scan Software collected vertical force and area outputs, and calculated pressure from the coordinating vertical force and area for a frame. The first and last steps were removed from each recording dataset to ensure that no transitional steps between gaits were included in the dataset. Each recording dataset still had at least 10 steps with the first and last step removed. If individual sensor cells, sensels, were loaded during a suspension phase for a hoof, these erroneous sensels were manually removed. The mean vertical force, area, and pressure for each step were exported from F-Scan Software in an ASCII file.

*Statistical Analysis*

Data were exported from the F-Scan Software (version 6.85) and imported into SAS (version 9.4) for statistical analysis and were evaluated for normality via residual plots. To evaluate the impacts that gait and circle size have on kinematic outputs, two datasets were created. One dataset removed the gait "canter", so that at both the walk and trot outputs could be compared for the straight line and both circle sizes. A second dataset removed the exercise type "straight", so that at the walk, trot, and canter, the two circle sizes could be compared. These two separate datasets were necessary, as a canter on a straight line was not able to be safely included in the data collection for this study. The main effects of gait, exercise type, and leg were evaluated in PROC GLIMMIX with Tukey adjustment. Interactions of "gait and exercise type", "gait and leg", "exercise type and leg", and "gait, exercise type, and leg" were also evaluated. Horse, and all interactions including horse, was included as a random effect. Significance was set at $p < 0.05$. Means are reported at means ± standard error of the mean (SEM).

RESULTS

*Dataset without Canter*

Loaded Hoof Area

Gait ($p$ = 0.03) and exercise type ($p$ = 0.03) were significant main effects for area (Table 1), but leg was not ($p$ = 0.44). The walk had a greater mean area than the trot by 12%. Gait and exercise type constituted a significant interaction ($p$ = 0.005), but “gait and leg” as well as “exercise type and leg” were non-significant interactions ($p$ = 0.10 and 0.71, respectively). “Gait, exercise type, and leg” was not a significant interaction ($p$ = 0.48). At the walk and trot, there were no significant between-leg (left vs. right) differences in the mean area ($p$ = 0.33 and $p$ = 0.58, respectively)

At the walk, the area was different between exercise types ($p$ = 0.01, Table 2), but not at the trot. While trotting in a straight line, the mean area was lower than at the walk (Table 2). Within the small and large circles, gait was not different in terms of the area ($p$ = 0.09 and $p$ = 0.19, respectively).

Table 2: Mean area loaded on the front hooves for nine horses with each gait repeated three times by exercise type in the dataset excluding canter.

| Exercise Type | Walk Mean Area (Sensels Loaded) | Trot Mean Area (Sensels Loaded) |
|---|---|---|
| Straight* | $42^{a}$ | 35 |
| Small circle | $32^{ab}$ | 29 |
| Large circle | $29^{b}$ | 26 |
| Within gait SEM | 4 | 4 |
| Within gait $p$-Value | 0.01 | 0.11 |

[ab] Means with different superscripts are significantly different within a column ($p$ = 0.01).
* For an exercise type, mean area was different between gaits ($p$ < 0.01).

Vertical Force

Gait ($p$ = 0.007) and exercise type ($p$ = 0.007) were both significant main effects for vertical force (Table 3), but leg was not ($p$ = 0.71). The walk resulted in a greater mean vertical force than the trot by 14%. No two-way interactions were significant. The three-way interaction of "gait, exercise type, and leg" was significant ($p = 0.02$). At the walk and trot, there were no significant between-leg differences in the mean vertical force ($p = 0.75$ and 0.66, respectively).

At both the walk and trot, the exercise type resulted in different mean vertical force outputs, with straight-line exercise leading to a higher mean vertical force at both gaits, although only significantly higher than both circle sizes at the walk (Table 3). During straight-line exercise and small-circle exercise, the walk had a greater mean vertical force (Table 3).

Table 3: Mean front hoof vertical force in Newtons (N) for nine horses with each gait repeated three times by exercise type in the dataset excluding canter.

| Exercise Type | Walk Mean Vertical Force (N) | Trot mean vertical force (N) |
|---|---|---|
| Straight** | $1234^{a}$ | $1040^{a}$ |
| Small circle* | $810^{b}$ | $700^{ab}$ |
| Large circle | $736^{b}$ | $660^{b}$ |
| Within gait SEM | 356 | 134 |
| Within gait *p*-Value | 0.003 | 0.02 |

$^{ab}$ Means with different superscripts are significantly different within a column ($p < 0.05$).
* For an exercise type, mean vertical force was different between gaits ($p < 0.05$).
** For an exercise type, mean vertical force was different between gaits ($p < 0.001$).

Front Hoof Pressure

Gait was a significant main effect ($p = 0.0007$, Tables 4 and 5) for pressure, but exercise type and leg were non-significant effects ($p = 0.16$ and 0.14, respectively). The walk had a greater mean pressure than the trot by 23%. No two-way interactions were significant, but the three-way interaction of "gait, exercise type, and leg" was significant ($p = 0.0008$).

For the right and left legs, it was found that the mean walk pressure was greater than the mean trot pressure when all exercise conditions were averaged (Table 4). At the walk and trot, the exercise type did not lead to different mean pressures, but the walk had a greater mean pressure for all three exercise types compared to the trot (Table 5).

Table 4: Mean front hoof pressure by leg in kilopascals (kPa) for nine horses with each gait repeated three times per exercise type for the dataset excluding canter.

| Leg | Walk Mean Pressure (kPa) | Trot Mean Pressure (kPa) |
|---|---|---|
| Right leg** | 723 | 530 |
| Left leg** | 963 | 775 |
| Within gait SEM | 149 | 149 |
| Within gait *p*-Value | 0.15 | 0.14 |

** For one leg, mean pressure was different between gaits ($p < 0.001$).

Table 5: Mean front hoof pressure in kilopascals (kPa) for nine horses with each gait repeated three times by exercise type for the dataset excluding canter.

| Exercise Type | Walk Mean Pressure (kPa) | Trot Mean Pressure (kPa) |
|---|---|---|
| Straight** | 936 | 715 |
| Small circle** | 839 | 647 |
| Large circle* | 761 | 595 |
| Within gait SEM | 75 | 76 |
| Within gait *p*-Value | 0.09 | 0.31 |

* For an exercise type, mean pressure was different between gaits ($p < 0.01$).
** For an exercise type, mean pressure was different between gaits ($p < 0.0001$).

*Dataset without Straight-line Exercise*
<u>Loaded Hoof Area</u>

Gait was a significant main effect ($p = 0.03$, Table 6) for area. The canter had a 21% greater mean loaded area than the walk and a 29% greater mean loaded area than the trot. Exercise type and leg were non-significant effects ($p = 0.78$ and $p = 0.33$, respectively). The interactions of "gait and exercise type" ($p = 0.28$) as well as "exercise type and leg" ($p = 0.72$) were non-significant; however, the interaction of "gait and leg" was significant ($p = 0.02$). "Gait, exercise type, and leg" was a significant interaction ($p = 0.0035$). For the right front leg (outside leg), the canter had a greater mean loaded area than other gaits (Table 6), but this was not found in the left leg. For exercise in a large circle, the mean area loaded was different between gaits, with the canter being greater than the trot ($p = 0.01$, Table 6). While exercising in a small circle, the mean area was not different between gaits (Table 6).

Table 6: Mean area loaded on the front hooves for nine horses with each gait repeated three times by gait for the dataset excluding straight-line exercise.

| Gait | Right Hoof Mean Area (Sensels) | Left Hoof Mean Area (Sensels) | Large Circle Mean Area (Sensels) | Small Circle Mean Area (Sensels) |
|---|---|---|---|---|
| Walk | $34^{a}$ | 27 | $27^{ab}$ | 32 |
| Trot | $29^{a}$ | 26 | $26^{a}$ | 27 |
| Canter | $47^{b}$ | 29 | $39^{b}$ | 37 |
| Within leg or circle size SEM | 4 | 4 | 4 | 4 |
| Within leg or circle size *p*-value | 0.001 | 0.80 | 0.01 | 0.11 |

$^{ab}$ Means with different superscripts are significantly different within a column ($p < 0.05$).

*Vertical Force*

Gait, exercise type, and leg were non-significant main effects ($p = 0.33$, 0.88, and 0.87, respectively) for the vertical force. “Gait and exercise type” as well as “exercise type and leg” were non-significant interactions ($p = 0.17$ and 0.71, respectively), but “gait and leg” was a significant interaction ($p = 0.004$, Table 7). “Gait, exercise type, and leg” was a significant interaction ($p < 0.0001$).

At the walk ($p = 0.83$), trot ($p = 0.72$), and canter ($p = 0.31$), the right and left legs did not have different mean vertical forces between legs. The right leg did have a lower mean vertical force at the trot than the canter (Table 7). During the large- and small-circle exercise, the mean vertical force did not differ by gait. At the walk, trot, and canter, the circle size did not have a different mean vertical force (Table 7).

Table 7: Mean front hoof vertical force in Newtons (N) for nine horses with each gait repeated three times by gait for the dataset excluding straight-line exercise.

| Gait | Right Hoof Mean Vertical Force (N) | Left Hoof Mean Vertical Force (N) | Large Circle Mean Vertical Force (N) | Small Circle Mean Vertical Force (N) |
|---|---|---|---|---|
| Walk | 745[ab] | 804 | 736 | 813 |
| Trot | 629[a] | 729 | 660 | 698 |
| Canter | 938[b] | 647 | 817 | 768 |
| Within leg or circle size SEM | 94 | 94 | 84 | 86 |
| Within leg or circle size *p*-value | 0.01 | 0.27 | 0.20 | 0.40 |

[ab] Means with different superscripts are significantly different within a column ($p = 0.01$).

*Front Hoof Pressure*

Gait was a significant main effect ($p = 0.001$, Table 8) for pressure, while exercise type and leg were not ($p = 0.59$ and 0.11, respectively). The walk had 22% and 28% greater mean pressures than the trot and canter, respectively. No two-way interactions were significant. The three-way interaction of “gait, exercise type, and leg” was significant ($p = 0.005$).

For both the right and left limbs, the walk was found to have a greater mean pressure than other gaits (Table 8). In both the large and small circles, the walk was found to have a larger mean pressure than other gaits. Within each gait, there were no differences between the large and small circle sizes (Table 8).

Table 8: Mean front hoof pressure in kilopascal (kPa) for nine horses with each gait repeated three times by gait for the dataset excluding straight-line exercise.

| Gait | Right Hoof Mean Pressure (kPa) | Left Hoof Mean Pressure (kPa) | Large Circle Mean Pressure (kPa) | Small Circle Mean Pressure (kPa) |
|---|---|---|---|---|
| Walk | 679[a] | 924[a] | 762[a] | 813 |
| Trot | 484[b] | 759[b] | 596[b] | 698 |
| Canter | 467[b] | 693[b] | 586[b] | 768 |
| Within leg or circle size SEM | 61 | 61 | 55 | 86 |
| Within leg or circle size *p*-Value | 0.003 | 0.003 | 0.006 | 0.40 |

[ab] Means with different superscripts are significantly different within a column ($p < 0.01$).

DISCUSSION

The objectives of this study were to determine how changes in gait and circle diameter influence area, vertical force, and pressure of the front hooves. We hypothesized that a decrease in circle diameter and an increase in speed would lead to greater differences between inside and outside limb outputs. The results determined that changes to gait more frequently lead to differences in the mean vertical force, area, and pressure outputs than changes to the circle diameter size. Most of the differences noted in this study were driven by gait, with gait being a significant effect for all evaluated outputs except for vertical force in the dataset including canter.

When evaluating gait differences, the walk typically had greater mean area and vertical force in this study, but when canter was included, the canter had the greatest mean area loaded. The walk having the greatest pressure is driven by the inclusion of the area and vertical force in the calculation of pressure. Most studies utilizing the Tekscan Hoof System for gait analysis have done so at the walk [70–72,74] or trot [73,75,76]. The Tekscan sensors may not be able to record data as accurately when speed increases for gaits such as the canter or even a faster trot. It is also worth exploring that adaptation to circular exercise has been previously noted as gait-specific [29]. The differences in gait may be due to increased speed or different loading patterns, such as the presence of a lead while cantering, as horses are known to protract the lead limb of a canter by flexing the elbow, carpal, hip, and tarsal joints [33]. One study found that as speed within the walk or trot increases while exercising on a treadmill, vertical impulse to the forelimbs and hindlimbs decreases [77]. While the current study did not compare speed within gaits, we did find that as gait increased from walk to trot, and therefore speed increased, the mean area, vertical force, and pressure decreased for the forelimbs. Another study found peak stress of the metacarpus and radius to be lower at a slow trot than the walk and canter and attributed the lower values of the slow trot to the symmetrical, diagonal movement of the gait [78].

Due to its two-beat diagonal footfall, the trot is considered a symmetrical gait and is the preferred gait for a lameness evaluation. The lower outputs seen at the trot in this study may be due to the fact that horses are able to utilize both forelimbs and hindlimbs within a trot stride in a more-even manner than the walk and canter [78–80]. The trot and canter also have moments of suspension, where the walk does not. Given that the results in this study are reported as means of the area, vertical force, and pressure, the lack of suspension in the walk could contribute to longer data collection for the right and left forelimbs at the walk than the trot and canter. Using a pressure plate, the stance phase of the walk has been found to be longer than that of a trot when tracking over both a hard surface and a soft surface [81]. Horses may also use other parts of their body, such as the musculature of the hindquarters, more so in the trot and canter than the walk, potentially leading to a decrease in the forelimb outputs [12,29,82,83]. One study found that activity of the hindlimb biceps femoris is minimal during the walk, but highly active according to electromyography at the trot and canter [84]. Another study found that at the walk and canter, horses exercising on flat and banked curves have a shorter stride length of the inside leg compared to the outside leg [29]. As horses increase in gait speed from a walk, to a trot, to a canter, it has been found that trunk muscle engagement increases as well [85].

At the trot, the mean hoof area loaded was similar regardless of exercise type, once again suggesting the trot to be the more stable gait [79,80]. Vertical force was greatest on a straight line for both the walk and trot, while pressure was not found to be different between exercise types at the walk or trot. In humans, similar results have been found where peak vertical ground reaction forces are greater in a straight line than while running around a curve [12], similar to what was found in the current study. Considering the Tekscan™ sensors measure vertical forces normal (perpendicular) to the sensor, it is conceivable that shear vertical forces were higher during circular exercise. During straight-line exercise, when a horse is tracking upright, the resultant force would be vertically measured. However, when horses are tracking in a circle, as was performed in this study, lateral forces are also considered when calculating the resultant force [37]. As the sensors were worn on the front hooves and measured the force of the area that came into contact with the arena surface, only the vertical forces were included in this study. While the walk had greater vertical force in this study, other forces, such as lateral force, may be greater in the trot and canter, especially during circular exercise [37,86].

When the canter was retained in the dataset, at all gaits, the large circle did not have a different mean area, vertical force, or pressure than the small circle. When exercising in the large circle, the canter did have a greater mean area than the trot. When exercising at both small and large circles, the walk had greater pressure than both the trot and canter. With the use of a pressure plate, another study found the vertical impulse of the walk to be almost twice that of the trot on both hard and soft surfaces [81]. Comparisons between pressure plates and sensors such as the

Tekscan™ system should be made cautiously, as these two technologies have not been found to reliably produce the same outputs [76].

When the canter was removed from the dataset, the mean area and mean vertical force were not different between the right and left legs at the walk or trot. When the canter was maintained in the dataset, the mean loaded area of the right (outside) leg was greatest at the canter, and the mean vertical force for the right leg was greater at the canter than the trot. In this study, minimal differences were seen between limbs, but it was notable that the outside limb loaded area was greater at the canter. At racetracks with the smallest radii (>50–114 m), the outside front limb was found to have the highest number of fatal limb fractures [37]. A study evaluating body lean angle at the trot and canter lunged horses through a bitted bridle at a diameter of 10 m while wearing an inertial measurement unit on the sacrum [36]. The lean angle was reported to be greater at the canter (19°) than the trot (12°) when tracking both left and right. A greater lean-in angle at greater speeds could be cause for more push off with the outside leg [11,29], and therefore a greater mean area loaded in the outside limb at the canter, as was seen in this current study. Our findings are supported by another study, which found the third metacarpal of the outside limb endures greater strain than the inside limb when Thoroughbred horses are running around a turn [87]. While galloping around a turn, the stance phase for the inside front limb is greater, while the stance phase for the outside front limb is shorter, with larger centripetal, propulsive, and vertical forces [37]. The presence of greater peak ground reaction vertical forces in the outside leg compared to the inside leg on a curve has also been noted in humans [12]. This study did not evaluate horses tracking at a gallop, which many studies referenced in this study have evaluated. Instead, this study allowed for an exploration of gaits that are easily attainable and frequently used across the industry, including to exercise racing horses when they are not galloping. It is reasonable to expect when working a horse in a round pen or lunging a horse, especially in initial saddling and riding, that increased speed is needed to reach the optimal training state for the horse. However, given these results, the frequency of circular exercise via lunging or a round pen as a replacement to pasture turn-out or ridden exercise should be evaluated.

Circular exercise is frequently used to exercise and train animals, especially through lunging. A review of risk factors for lameness in dressage horses found lunging to be protective against lameness, while the use of walkers increased the risk of lameness [31]. Mechanical walkers are often used during recovery from lameness, so it may be difficult to separate horses that are being placed on a walker for recovery or for exercise. When on a mechanical walker, animals may be unsupervised, and are not controlled by a handler that would encourage them to travel upright and at consistent speeds. However, when lunging is utilized in dressage, often the use of a surcingle and bridle could encourage the horse to track in an upright and balanced manner, very similar to the way that a horse is "on the bit" while under saddle in dressage. In disciplines outside of dressage, lunging is typically performed with only the lunge line attached to a halter. This gives the handler less control of the horse, often resulting in lunging sessions where the horse is leaning into the circle and does not consistently engage the hindquarters and topline musculature to travel in an upright manner, making lunging in this manner less likely to be a protective factor against lameness. Circular exercise is also used under saddle for both training and competition in events such as dressage and reining. The presence of a rider is known to alter how horses utilize their back musculature at various gaits [85,88]. Further exploration into circular exercise with a rider present is needed to determine if differences between front limb outputs at the walk, trot, and canter are mitigated or exacerbated.

The current study evaluated straight line exercise versus circular exercise of a horse in a round-pen that was not attached to a lunge line. Further studies of similar design are needed to evaluate the impact of a lunge-line simply attached to a halter on forelimb disparity while an animal is in motion. When different head and neck positions were evaluated on a straight line on a treadmill, it was found that a high head position impacted limb functionality compared to an unrestrained horse [89]. Head and neck position has also been found to alter the center of motion of a horse while lunging [64]. The current study found frequent differences in gait, but limited differences in circle size for a horse moving freely in a round-pen. Differences in forelimb outputs between small and large circles may be detectable when a horse is exercised on a lunge line, as to make the circle smaller, greater tension could be applied to the lunge line attached to the horse's halter, potentially encouraging the horse to lean in and push off more with the outside leg.

A limitation in this study is that recordings of the canter on a straight line were not attainable, and thus two sets of data were evaluated to best compare gait and exercise types. Future studies could use a long aisle-way with an appropriate surface to have horses travel in a straight line without the need of a handler. This may help to better answer the question of whether circular exercise at a canter has differences in the outside limb because of the lead or just the increase in speed. Our current study only evaluated the front limbs, which permitted us to determine differences between the inside and outside limbs. It is recognized that the hindlimbs are important in the adaptation to motion, such as turning [37,82,86], and future studies to determine the impact of gait and the circle diameter to hind limb outputs should be explored. The Tekscan™ system used in the manner of this study measured vertical force and not shear force, which is certainly important for turning, especially for the hind limbs [37]. It should be noted that many technologies are utilized for gait analysis, such as vertical force plates, inertial measurement units, and sensors such as the Tekscan™ Hoof System. Between studies, the technology used for analysis, as well as attachment methods and locations, is not standardized. While our study protocol and recorded metrics have been shown to be reliable [68], comparisons between studies should be made recognizing the current limitation of no standardized protocol for quantitative gait analysis for horses in motion currently.

## CONCLUSIONS

While circular exercise is used frequently in the training, exercising, and competing of horses, little is known of its potential connection to joint and bone injury. This study explored the impact of gait as well as circle size to mean area, vertical force, and pressure of the front hooves. It was found that gait (walk, trot, canter) drives changes to outputs more than exercise type (straight, circular). The trot frequently had lower mean outputs than other gaits, suggesting that it is a more dynamically stable gait that could potentially allow horses to adapt to circular exercise easier than other gaits. Handlers looking to utilize circular exercise while maintaining the longevity of equine careers may consider doing so at slower gaits, as differences in outside limb output were noted at the canter, or minimizing the use of circular exercise. Future studies will help to determine if a round-pen allows the horse to adapt to changes in gait and diameter better than when exercised on a lunge line or under saddle. Lateral forces may be greater during circular exercise and should be evaluated and compared with the findings of vertical forces provided in this research.

## CHAPTER 4: Impact of circular exercise diameter on bone and joint health of juvenile animals

### SIMPLE SUMMARY

In many equestrian disciplines, circular exercise is utilized to train, exercise, and compete with horses and can vary in gait, as well as diameter. This study aimed to determine if circular exercise diameter impacts animal health. Calves have previously been used as a terminal skeletal model of juvenile horses, allowing for collection of musculoskeletal samples not acceptable from horses. Calves exercised on a small circle (12-m clockwise), large circle (18-m clockwise), treadmill, or served as non-exercised controls. Exercise was performed at a walking speed starting 5 minutes per day and increasing 5 minutes weekly until reaching 30 minutes per day during the 7-week study. The response to exercise was monitored in forelimb bones and joints. The small circular exercise group was found to have bone diameters which differed between the right and left fused third and fourth metacarpi, and between lateral and medial proximal phalanx bones. Cartilage glycosaminoglycan content was greater in the outside leg of the small circle exercise calves than the inside leg, with no differences noted within other treatments. These differences suggest that altering circular exercise diameter can impact bone and joint health, and that larger diameter circles may prevent asymmetric loading between inside and outside legs.

### ABSTRACT

Circular exercise is used in many equestrian disciplines, this study aimed to determine if circle diameter impacts juvenile animal forelimb bone and joint health. On d 0, 24 calves at 9 wks of age were assigned exercise treatments: small circle (12-m clockwise), large circle (18-m clockwise), treadmill, or non-exercised control. Exercise initiated at 1.1–1.5 m/s for 5 mins/d and increased 5 mins weekly until reaching 30 mins/d. On d 49 synovial fluid was collected from multiple joints, cartilage was collected from the proximal surface of fused third and fourth metacarpi (MC III & IV), and forelimbs underwent computed tomography scans. Statistical analysis (PROC mixed) was performed in SAS 9.4. The inside leg of the small circle treatment had larger MC III & IV dorsopalmar external diameter than the outside ($P = 0.05$). The medial proximal phalanx had greater mediolateral diameter than the lateral proximal phalanx of the small circle treatment ($P = 0.01$). Fetlock nitric oxide was greater in large circle and treadmill treatments ($P < 0.0001$). Cartilage glycosaminoglycan concentration was greater in the outside leg of the small circle exercise treatment than the inside leg ($P = 0.03$). Even at slow speeds, circular exercise diameter can impact joint and bone health, but faster speeds may have greater alterations.

## INTRODUCTION

The strain environment of bone can be influenced by exercise and consists of many factors, such as magnitude of strain, rate of strain, distribution of strain, and frequency of strain. Response of bone to exercise can be explained by the mechanostat theory. Under this theory, bone can adapt to its mechanical environment based on the strains it is subject to. When there is too little strain in the environment, the amount of bone needed is reduced, and bone resorption occurs. When there is an increase in strain, the amount of bone needed is increased, and bone formation occurs. The mechanostat theory also predicts bone can be maintained and repaired without net resorption or net formation when strain is high enough to prevent removal of unnecessary bone, but not too high to elicit an acquisition of more bone [90]. Dynamic exercise, such as sprinting, leads to an increase in bone strength in juvenile animals [60]. While straight-line sprint exercise has been found to benefit bone strength, little is known of the impacts that circular exercise has on bone or joint health of juvenile animals. Circular exercise is used frequently in training, exercise, and competition of horses across various disciplines. Common methods of circular exercise include riding under saddle, or working on the ground, such as in round pens and lunging. In the early training of a young horse, circular exercise via a round pen or lunging is used frequently. During circular exercise, the gait (walk, trot, canter, or gallop) and diameter can be altered by the handler or rider.

While exercising on a circle, horses will lean into the center of the circle by up to 20 degrees to maintain balance [9,10]. Speed and radius have been found to impact lean angle, with greater speed and smaller radii increasing lean-in towards the center of the circle [11]. Level of training can also impact lean angle, as a horse acclimated to traveling on a circle can travel more upright than a horse which is not acclimated to traveling in a circle or arc. This has especially been noted in dressage, where trained horses are able to travel “on the bit”, while inexperienced horses may not be able to engage the back and hindlimb musculature through a circle [8]. Quadrupeds, such as horses, may be at an advantage compared to bipedal runners during curve running, as they can redistribute weight to multiple stance legs within a stride. It has been found that while cantering in a 10-m circle, horses will have greater peak ground reaction force on the outside forelimb compared to the inside forelimb [17]. While traveling around a curve, Thoroughbred race horses experience greater strain to the outside forelimb, which increases as speed increases [19]. When both humans and horses sprint through a curve, the outside limb is known to generate more vertical and lateral force than the inside limb [11].

Sharpness of a turn is also found to impact horses and human runners. A sharply curved track (5-m radius) leads to greater torsion on the inside tibia of humans compared to running on a gently curved track (15-m) or straight line [16]. When running around a curve, the inside and outside limbs of human subjects are not biomechanically symmetrical. As circle radius decreases, peak resultant ground reaction forces to the inside leg decrease compared to straight line running

[12]. As speed increases, whole forelimb and third metacarpal angle in the horse increase, supporting that due to centripetal acceleration while traveling around a turn, horses lean into a turn at a greater angle as speed increases [11]. The area loaded by the outside front hoof is greater at the canter than the trot or walk during circular exercise, suggesting a push-off motion with the outside front leg at the canter [91]. Due to the reduced surface area of the inside hoof that is loaded, uneven forces may be placed on joints and bones of the fore and hind limbs during circular exercise. These forces exerted on a smaller surface area have the potential to lead to a higher risk for joint injury and osteoarthritis [9].

Circle radius can impact animal and rider safety, a retrospective study of risk factors for jockeys in Japanese Thoroughbred racing found smaller tracks to have a greater risk of injury [56]. Osteoarthritis and joint injuries have been reported as a leading cause of lost training days and horse wastage, yet little research has bridged the gap between circular exercise and joint damage. Osteoarthritis can be caused by excessive loads to normal cartilage, or normal loads to abnormal cartilage [66,67]. With up to 60% of lameness being related to osteoarthritis, this gap in research greatly affects the equine community [46,47].

Biomarkers can allow determination of bone and joint activity throughout an exercise trial. Osteocalcin (OC), a marker of bone formation, and c-telopeptide crosslaps of type I collagen (CTX-1), a marker of bone resorption, can be monitored simultaneously from serum samples to aid in determining bone activity in horses and bovines [60,92]. Procollagen II c-propeptide (CPII) is a marker of collagen synthesis [93]. Osteoarthritis affected horses have been found to have greater CPII serum and synovial fluid concentration during an exercise trial than horses without osteoarthritis [94]. Prostaglandin E2 ($PGE_2$) and nitric oxide (NO) are markers of inflammation and cartilage metabolism that are frequently evaluated in synovial fluid. Synovial fluid $PGE_2$ is found to be greater in joints affected by osteoarthritis than in joints that are not affected by osteoarthritis in exercising horses [94]. Exercise has been found to increase synovial fluid concentration of $PGE_2$ in healthy, sound horses as well [95]. Joints effected by osteoarthritis may experience both catabolic and protective functions of NO [96]. Glycosaminoglycans (GAG) are a hydrophilic proteoglycan in the extracellular matrix of articular cartilage, which provide shock absorption and resistance against forces [24]. Within articular cartilage, GAG concentration had been found to be heterogenous between joint locations [97,98]. Biomarkers can be combined with evaluation of bone morphology as well as tensile testing to determine the activity, shape, and strength of bone as well as joint health [60,61].

Many studies have evaluated gait characteristics of animals performing circular exercise, but few have evaluated bone and joint health. The objective of this study was to utilize a calf model to determine the impact of diameter during circular exercise at the walk to forelimb bone and joint health in juvenile animals. Calves have been used as a model for management and exercise of young horses successfully in previous studies [60–62,98]. It was hypothesized that exercise on a smaller diameter circle would lead to increased biological markers of joint inflammation and metabolism and greater asymmetry between inside and outside forelimbs.

## MATERIALS AND METHODS

### *Animals, housing, and exercise*

Holstein steer calves, previously dehorned, were obtained from a local farm at 8 weeks of age and moved into group-housing at the Michigan State University (MSU) Veterinary Research Farm. Group-housing consisted of a pen (112 $m^2$) including a partially enclosed three-sided shed (17 $m^2$) bedded in straw with a feeding trough and automatic water trough. Calves had *ad libitum* access to calf starter (Caledonia Farmers Elevator, Caledonia, MI) and water. Calves were given one week for acclimation to housing and halter training before exercise began at 9 weeks of age. Each calf underwent two days of halter training, in which they were introduced to the sensation of a rope halter and taught to move forward from halter pressure while being led for two laps in the group-housing pen. Calves were evaluated with daily health checks to assure the safety of exercise.

On d 0, calves were 9 weeks of age, and blood samples were taken along with height, weight, and length measurements. Calves were then randomly striated to treatment groups based on weight. Calves remained in their treatment groups for 7 weeks (49 d). Treatment groups consisted of non-exercise controls, treadmill exercise, small circle exercise, and large circle exercise. All treatment groups were maintained together in a group-housing pen. Calves randomized to the circle and treadmill exercise treatments exercised 5 d/wk starting at 5 min/d and increased by 5 min each week until reaching 30 min (Table 9). Exercise was performed at a speed of 1.1–1.5 m/s, allowing calves to maintain a walking gait. This exercise protocol allowed calves to acclimate to exercise throughout the study. Circular exercise was performed in a clockwise fashion, tracking to the right, on a mechanical walker (Q-line Horse Exercise) with both a small diameter (12 m) and large diameter (18 m) track. Treadmill exercise was performed on a Classic Treadmill equine treadmill (Classic Champion Model 940, Queensland, Australia).

Table 9: Exercise protocol for treadmill, small circle, and large circle treatment groups. *All exercise was performed 5 d/wk at a speed of 1.1–1.5 m/s*

| Week | Exercise duration |
|---|---|
| 1 | 5 min/d |
| 2 | 10 min/d |
| 3 | 15 min/d |
| 4 | 20 min/d |
| 5 | 25 min/d |
| 6 | 30 min/d |
| 7 | 30 min/d |

*Sample collection*

Starting on d 0 and continuing weekly, serum was collected via jugular venipuncture into non-heparinized vacutainers. Calf height, weight, and length were recorded weekly, starting on d 0. Height was measured from the floor to the top of the withers with an L-shaped measuring stick that was adjustable to the wither height of the calf. Weight was measured with a weight scale, (Tru-Test; Model 700; Mount Wellington, New Zealand) and length was from the point of shoulder to ischium, or pin bone. On d 49, all calves were humanely euthanized at the MSU Meat Laboratory, and the right and left forelimbs collected at the middle of the radius. From the right and left forelimb of each calf, synovial fluid was collected from the radiocarpal, middle carpal, lateral fetlock, and medial fetlock joints. Synovial fluid was kept on dry ice during sampling, after which it was placed in a -80° C freezer until analysis. Cartilage from the proximal surface of the fused third and fourth metacarpal (MC III & IV) was collected from the right and left forelimb of each calf. Cartilage was kept on dry ice during sampling, then stored in a -20° C freezer until analysis. Synovial fluid and cartilage samples were collected within 30 min of animal death. After cartilage and synovial fluid sample collection, the front legs of each calf were labeled and placed in a chiller (4.8° C) overnight until computed tomography (CT) scanning.

*Computed tomography scans*

Computed tomography scans were performed at the MSU College of Veterinary Medicine in a GE Revolution Evo Scanner (Boston, Massachusetts). Both the right and left forelimb of each calf were CT scanned within 36 hours of euthanasia. Position was set to "lumbar spine", slice thickness was set at 0.625-mm, with settings of 120kV and 320 mAmp. Field of view was 180 mm with a 512 x 512 matrix size, leading to a pixel size of 0.35 mm x 0.35 mm. Voxel volume was 0.077 $mm^3$ (0.35 mm x 0.35 mm x 0.625 mm). Calcium hydroxyapatite phantoms (Image Analysis, Inc; Colombia, KY) were included in each scan, with rows representing 0, 75, and 100 mg mineral/$cm^3$ for BMD comparison. All CT scans were analyzed with Mimics 24.0 software (Materialise, Leuven, Belgium). For each MC III & IV and lateral and medial proximal phalanx bone, the proximal and distal end were recorded to calculate the midpoint of the bone. Measurements of BMD, area, and cortical widths were performed at the midpoint of each MC III & IV and lateral and medial proximal phalanx. Bone mineral density, cross sectional area, cortical widths, and cortical areas were determined with a mask threshold value of 400 Hounsfield Units (HU). Moment of inertia (MOI) was calculated from MC III & IV diameters measured in Mimics with the calculation for a hollow ellipse described in the American Society of Agricultural and Biological Engineers (ASABE) standards [60,61,99]. Values for average BMD are reported as HU. Average HU values were recorded at each of the 3 concentrations of calcium along the length of the phantom at 10 locations for each scan. Average HU values for each concentration were then compared in a scatter plot to the known concentrations of the phantom. An equation from the regression line converted HU to mg mineral/$cm^3$. This method of determining BMD from CT scans has been previously utilized [60,100].

*Biomechanical testing*

Before biomechanical testing, front legs were removed from the freezer (-20° C) to thaw for 5 days at 4.8° C in order to clean the MC III & IV. Skin, soft tissue, and remaining bones were removed with a scalpel so the fused MC III & IV to allow tensile testing of only the MC III and IV. Cleaned MC III & IV were then stored in the freezer until tensile testing (-20° C). Three days before tensile testing, MC III & IV were removed from the freezer to thaw at 4.8° C. Fracture force of the MC III & IV was determined by four-point bending at room temperature with an electromechanical testing system (MTS Criterion, Model 43) equipped with a 60 kN load cell. Left and right MC III & IV for each calf were placed individually with the palmar aspect of the bone facing upwards toward the force applicators [60]. The bottom supports were 82-mm apart, and upper supports, which applied the vertical force from the load cell, were 52-mm apart. All samples were loaded to failure at a rate of 10mm/min. Fracture force was recorded as the maximum force (ultimate load) before failure. Data acquisition rate was set to 100 Hz.

*Osteocalcin*

Calf serum samples were analyzed for OC concentration, a marker of bone formation reflecting osteoblastic activity, with the commercially available MicroVue Osteocalcin Enzyme Immunoassay (Quidel, San Diego, CA). Calf serum samples utilized for OC analysis were diluted at a 1:15 ratio with wash buffer for samples taken through week 4. At week 4, depending on individual calf samples, sera were diluted at 1:20 or 1:25 with wash buffer, without this dilution samples were outside of the threshold of sensitivity. Analysis was performed according to kit instructions. Coefficients of variation below 10% were accepted.

*C-telopeptide crosslaps of type I collagen*

Calf serum samples were analyzed for CTX-1 concentration, a marker of bone resorption, with the commercially available Serum CrossLaps kit made by Immunodiagnostics Systems (Gaithersburg, MD). Samples were run neat and analysis was performed according to kit instructions. Coefficients of variation below 10% were accepted.

*Procollagen II c-propeptide*

An ELISA kit from IBEX Pharmaceuticals (Montreal, Quebec, Canada) was obtained for analysis of CPII concentration in serum, a marker of collagen synthesis. This competitive assay measures CPII which is released from type-2 collagen during collagen synthesis. Serum samples were run in triplicate and with a 1:4 dilution, except d 0 which was run with a 1:2 dilution. Assay procedure was performed according to instructions accompanying the kit. Coefficients of variation below 10% were accepted.

*Prostaglandin $E_2$ & Nitric oxide*

Calf synovial fluid samples were analyzed for concentration of $PGE_2$ and NO, markers of inflammation. Fetlock joint synovial fluid samples were analyzed with the commercially available Thermo Fisher (Waltham, Massachusetts) $PGE_2$ ELISA kit. Due to supply chain issues, sufficient kits from Thermo Fisher for carpal synovial fluid were not able to be procured. Carpal synovial fluid samples were analyzed with a $PGE_2$ ELISA kit made by Enzo (Farmingdale, New York). Synovial fluid samples were digested with 50 µg/mL of hyaluronidase from bovine testes. Digested samples were diluted at a 1:2 ratio with reagent diluent, analysis was performed according to kit instructions. Coefficients of variation below 10% were accepted.

Nitric oxide was measured by quantifying nitrite (an end product of nitric oxide metabolism) using a Greiss reaction and sodium nitrite standard [101,102]. Samples were not digested with hyaluronidase or diluted, as this caused sample readings to be too low and outside of the threshold of sensitivity. Nitric oxide results are expressed in micromoles per well. Coefficients of variation below 30%, larger than with other assays, were accepted due to the variability of undigested and undiluted synovial fluid.

*Glycosaminoglycan*

Cartilage slices from the proximal surface of the MC III & IV were digested with papain to determine the GAG concentration with a dimethylmethylene blue assay [103]. This assay is based on the binding of anionic GAGs to cationic 1,9-dimethylmethylene blue. Sulfated GAG content was measured against a chondroitin sulfate standard, and sample concentration determined with a linear curve [101]. Papain digested samples were diluted 1:25 with a sodium acetate and tween dilution buffer. Coefficients of variation below 10% were accepted.

*Statistical analysis*

All statistical analyses were performed in SAS 9.4. Residuals were plotted against predicted means and observed for normality. Nitric oxide data were found to be abnormally distributed and were log transformed to achieve normal distribution. All other data were found to be normally distributed. Height, weight, length, OC, CTX-1, and CPII were evaluated with the repeated effect of day, with calf as subject, fixed effects of day and treatment, as well as the interaction between day and treatment. Repeated measurements of OC, CTX-1, and CPII were run with d 0 as a covariate. Fracture force and glycosaminoglycan content were evaluated with fixed effects of leg (right or left) and treatment as well as the interaction between leg and treatment, with calf as a random effect. Fetlock NO and $PGE_2$ concentration were evaluated with the fixed effects of treatment and joint (lateral or medial fetlock) and the interaction between treatment and joint. Carpal NO and $PGE_2$ concentration were evaluated with fixed effects of treatment and joint (radiocarpal or middle carpal) and the interaction between treatment and joint. Calf was included as a random effect for both fetlock and carpal NO and $PGE_2$. Fused MC III & IV CT data were evaluated with the fixed effects of treatment and leg, as well as the interaction between treatment and leg, calf was included as a random effect. Proximal phalanx CT data were evaluated with the fixed effects of treatment, leg, and bone (lateral or medial phalanx) and interactions, calf was included as a random effect. Means are reported as averages ± standard error of the mean (SEM). Error bars in graphs represent SEM. Significance was set at $P \leq 0.05$ and trends at $P \leq 0.10$.

## RESULTS

Study day was a significant effect for calf height ($P < 0.001$), weight ($P < 0.001$), and length ($P < 0.001$), supporting animal growth throughout the study. The interaction between day and treatment was not significant for calf height, weight, or length ($P = 0.99$, $P = 0.99$, and $P = 0.68$, respectively). Average daily gain (ADG) was not different among treatments (Table 2). On both d 0 and d 48, calf height, weight, and length were not different among treatments (Table 10).

Table 10: Initial (d 0) and final (d 48) calf height, weight, and length by treatment as well as average daily gain (ADG)

| Treatment | Height (d 0) cm | Height (d 48) cm | Weight (d 0) kg | Weight (d 48) kg | Length (d 0) cm | Length (d 48) cm | ADG, kg |
|---|---|---|---|---|---|---|---|
| Control | 89 | 100 | 75 | 146 | 90 | 113 | 1.5 |
| Large | 88 | 100 | 81 | 145 | 90 | 112 | 1.3 |
| Small | 89 | 102 | 81 | 156 | 89 | 113 | 1.6 |
| Treadmill | 87 | 100 | 80 | 145 | 87 | 114 | 1.4 |
| SEM | 1.2 | 1.1 | 4.0 | 6.0 | 2.02 | 1.2 | 0.10 |
| n | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| P – Value | 0.52 | 0.51 | 0.71 | 0.44 | 0.71 | 0.61 | 0.17 |

*Fused MC III & IV CT scan results*

There were no differences in MC III & IV internal or external diameters at the midpoint of the bone between left and right leg or treatments. There was no interaction between treatment and leg for dorsopalmar internal diameter (P = 0.54), mediolateral internal diameter (P = 0.42), or mediolateral external diameter (P = 0.54). There was a trend for an interaction between treatment and leg for dorsopalmar external diameter (P = 0.09). This interaction was driven by the right leg of the small circle treatment group having larger dorsopalmar external diameter than the left leg (P = 0.04). Moment of inertia was not different among treatments (P = 0.50), but there was a trend for an effect of leg (P = 0.10), with the left leg having higher moment of inertia. There was no interaction between treatment and leg (P = 0.15).

The bone length of MC III & IV was different among treatments (Table 11, P = 0.04), with large circle treatment group having shorter MC III & IV compared to all other treatments. There was no difference between left and right leg length (0.57) and no interaction between treatment and leg (P = 0.96). The cortical area was not different between leg (P = 0.15), or treatment (P = 0.057), but there was a trend for an interaction between treatment and leg (P = 0.07). This trend was driven by the left leg MC III & IV of the treadmill group having larger cortical area then the right leg (P = 0.01). The cross-sectional area was not different between left and right legs (P = 0.42), treatment (P = 0.026), and there was no interaction between treatment and leg (P = 0.36).

Table 11: Bone length of the right and left metacarpal III & IV (MC III & IV) by treatment

| Treatment | MC III & IV bone length, mm |
|---|---|
| Control | 199[a] |
| Large | 192[b] |
| Small | 195[a] |
| Treadmill | 195[a] |
| n | 12 |
| SEM | 1.6 |
| P – Value | 0.04 |

[a,b] Values lacking common superscripts within a column differ (P = 0.04)

For the dorsal, lateral, medial, and palmar cortices, as well as the entire slice of the midpoint, BMD was not different between left and right legs (P = 0.73, 0.64, 0.17, 0.82, and 0.12, respectively) nor treatment (0.95, 0.73, 0.76, 0.97, and 0.96, respectively). No interactions between leg and treatment were significant.

Dorsal, lateral, medial, and palmar cortical widths of the MC III & IV were not different among treatments (0.38, 0.86, 0.63, and 0.70, respectively). The medial cortex width of the MC III & IV tended to be larger for the left leg compared to the right leg (P = 0.063). No interactions between leg and treatment were significant.

*Medial and lateral proximal phalanx CT scan results*

Dorsopalmar internal and external diameter were not different among treatments (P = 0.68, 0.70), leg (P = 0.85, 0.64), or bone (P = 0.36, 0.53). Mediolateral internal and external diameter were not different among treatments (P = 0.90, 0.56) or leg (P = 0.84, 0.61). There was a difference between proximal phalanx bones with the medial proximal phalanx having greater mediolateral internal (P = 0.0003) and mediolateral external diameter (P = 0.01). The interaction between treatment and bone was significant for the mediolateral external diameter (Table 12, P = 0.01).

Table 12: Internal (int) and external (ext) cortical diameters of the lateral and medial proximal phalanx of both front legs

| Treatment | Proximal phalanx bone | Dorsopalmar int diameter, mm | Dorsopalmar ext diameter, mm | Mediolateral int dimeter, mm | Mediolateral ext diameter, mm |
|---|---|---|---|---|---|
| Control | Lateral | 20.7 | 25.5 | 17.2 | 23.6* |
| | Medial | 20.4 | 25.4 | 18.0 | 24.5* |
| Large | Lateral | 19.8 | 25.0 | 17.2 | 23.6 |
| | Medial | 20.1 | 25.2 | 17.4 | 23.8 |
| Small | Lateral | 20.1 | 25.4 | 17.1 | 23.7* |
| | Medial | 20.6 | 25.7 | 18.0 | 24.8* |
| Treadmill | Lateral | 19.9 | 24.9 | 17.2 | 23.5 |
| | Medial | 20.0 | 24.9 | 17.4 | 23.6 |
| n | | 12 | 12 | 12 | 12 |
| SEM | | 0.45 | 0.42 | 0.42 | 0.41 |
| P – Value | | 0.44 | 0.63 | 0.26 | 0.01 |

* Denotes lateral and medial proximal phalanx are different within a treatment (P = 0.01)

Proximal phalanx bone length was not different among treatments (P = 0.40), lateral or medial bones (P = 0.76), or the left and right legs (P = 0.72). No interactions were significant. Cortical area was not different among treatments (P = 0.65) or legs (P = 0.17) but was different between the lateral and medial proximal phalanx bones (P = 0.01), with the medial phalanx having greater cortical area. No interactions were significant. Cross sectional area was not different among treatments (P = 0.71), leg (P = 0.83) or proximal phalanx bones (P = 0.18). There was a tendency for an interaction between leg and bone (P = 0.07). This trend was driven by the right medial proximal phalanx having more area than the right lateral proximal phalanx (P = 0.03).

For the dorsal cortex, BMD was not different among treatments ($P = 0.59$) or leg ($P = 0.79$), but there was a trend for an interaction between leg and bone (Table 13, $P = 0.07$). Lateral cortical BMD was not different among treatments ($P = 0.69$) or leg ($P = 0.75$) but was different between proximal phalanx bones ($P < 0.0001$), with the lateral proximal phalanx having greater lateral cortical BMD than the medial proximal phalanx. Medial cortical BMD was not different among treatments ($P = 0.30$) or leg ($P = 0.66$) but was different between proximal phalanx bones ($P < 0.0001$), with the medial proximal phalanx having greater cortical BMD. There was a trend for an interaction between leg and bone (Table 13, $P = 0.06$). For the palmar cortex, BMD was not different among treatments, legs, or proximal phalanx bones ($P = 0.75$, $P = 0.34$ and $P = 0.20$, respectively). There was a significant interaction between leg and bone (Table 13, $P = 0.03$) and a trend for an interaction between treatment and proximal phalanx bone ($P = 0.07$). This trend was driven by the palmar cortex in the medial proximal phalanxes of the treadmill group having greater density than the palmar cortex of the lateral proximal phalanxes ($P = 0.005$). For midpoint slice BMD, treatments and leg were not different ($P = 0.67$ and $P = 0.40$, respectively). There was a tendency for difference between the proximal phalanx bones ($P = 0.08$), with the medial proximal phalanx trending towards greater BMD. There was a significant interaction between leg and bone (Table 13, $P = 0.001$).

Table 13: Cortical bone mineral density of the lateral and medial proximal phalanx of left and right legs

| Leg | Proximal phalanx bone | Dorsal cortex, mg mineral/cm$^3$ | Lateral cortex, mg mineral/cm$^3$ | Medial cortex, mg mineral/cm$^3$ | Palmar cortex, mg mineral/cm$^3$ | Midpoint slice, mg mineral/cm$^3$ |
|---|---|---|---|---|---|---|
| Left | Lateral | 845 | 959 | 728* | 799* | 737* |
| | Medial | 855 | 776 | 974* | 837* | 764* |
| Right | Lateral | 853# | 973 | 769* | 812 | 759 |
| | Medial | 831# | 755 | 949* | 803 | 751 |
| n | | 24 | 24 | 24 | 24 | 24 |
| SEM | | 16 | 21 | 23 | 17 | 12 |
| P–Value | | 0.07 | 0.19 | 0.06 | 0.03 | 0.001 |

* Denotes lateral and medial proximal phalanx are different within the left or right leg (P < 0.05)
# Denotes lateral and medial proximal phalanx tend to differ within the left or right leg (P < 0.10)

Dorsal, lateral, medial, and palmar cortical widths all had significant effects of phalanx bone (Table 14). There were no effects for treatments or legs for cortical widths of the dorsal, lateral, medial, or palmar cortices.

Table 14: Cortical widths of the lateral and medial proximal phalanx of left and right front limbs

| Proximal phalanx bone | Dorsal cortex, mm | Lateral cortex, mm | Medial cortex, mm | Palmar cortex, mm |
|---|---|---|---|---|
| Lateral | 2.6$^a$ | 3.7$^a$ | 2.6$^b$ | 2.6$^a$ |
| Medial | 2.5$^b$ | 2.6$^b$ | 3.8$^a$ | 2.7$^b$ |
| n | 48 | 48 | 48 | 48 |
| SEM | 0.07 | 0.12 | 0.11 | 0.06 |
| P – Value | 0.04 | <0.0001 | <0.0001 | 0.02 |

$^{a,b}$ Values lacking common superscripts within a column differ (P < 0.05)

*Fracture force of MC III & IV*

There was no significant effect of treatment (P = 0.70) or leg (P = 0.88), but there was an interaction between treatment and leg (Table 15, P = 0.05), with the right fused MC III & IV of the treadmill treatment having lower fracture force than the left.

Table 15: Fracture force of calf MC III & IV by treatment and leg

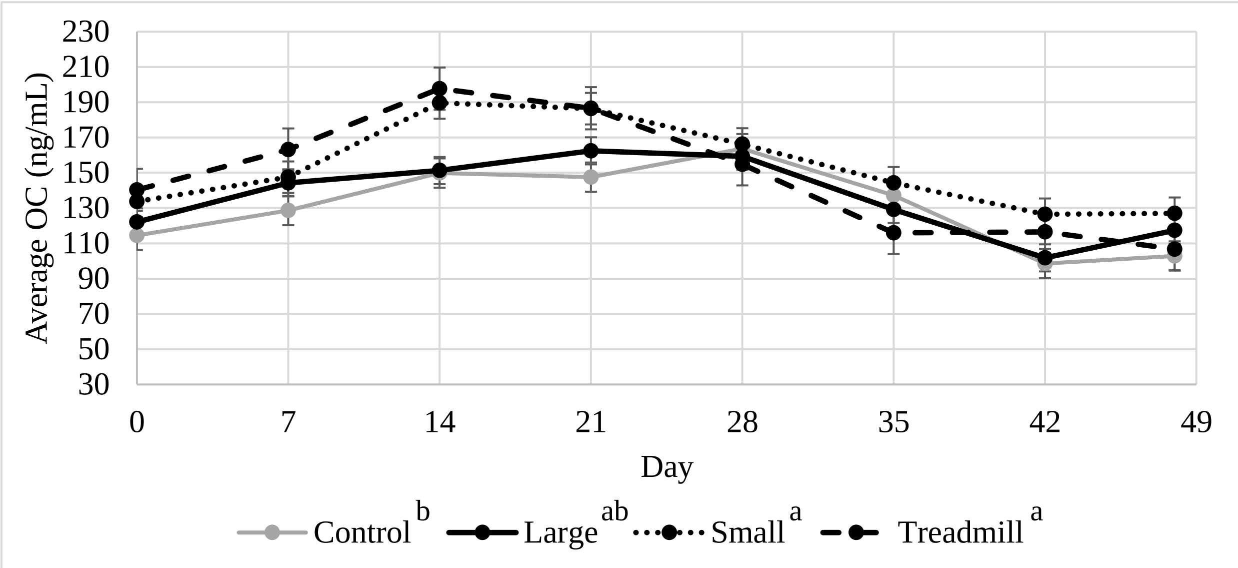
| Treatment | Leg | Force (N) |
|---|---|---|
| Control | Left | 8,700 |
| | Right | 9,300 |
| Large | Left | 8,800 |
| | Right | 9,200 |
| Small | Left | 10,100 |
| | Right | 10,400 |
| Treadmill | Left | 9,400* |
| | Right | 8,300* |
| n | | 6 |
| SEM | | 960 |
| P – Value | | 0.05 |

* Denotes lateral and medial proximal phalanx are different within the left or right leg ($P < 0.05$)

*Average serum OC*

There was a significant effect of day and treatment on average OC concentration (Figure 3, $P < 0.001$ and $P = 0.049$, respectively), with the small circle and treadmill exercise groups having greater osteocalcin concentration than the control group. Overall osteocalcin concentrations were higher on d 14 and 21. The interaction between day and treatment was not significant ($P = 0.97$).

Figure 3: Calf mean serum osteocalcin (OC) by treatment throughout the 7-week study period

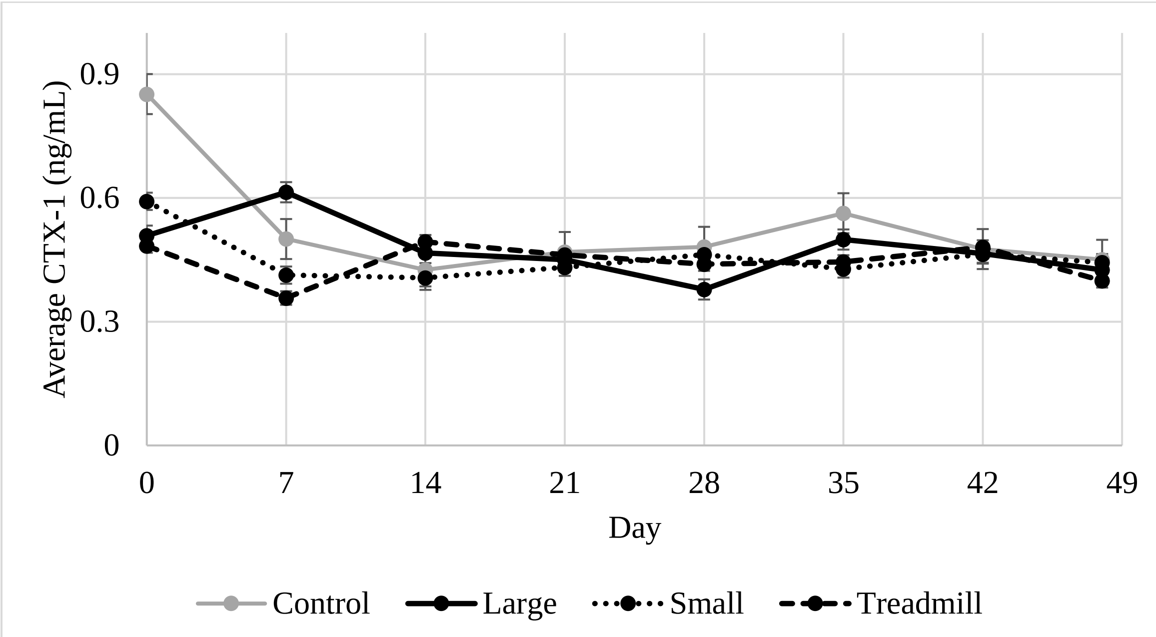



[a,b] Treatments lacking common superscripts differ (P = 0.05)

*Average serum CTX-1*

There was a significant effect of day on serum CTX-1 concentration (Figure 4, P = 0.02) but not treatment (P = 0.15). Day 0 had greater average concentration of CTX-1 than other dates. The interaction between day and treatment was not significant (P = 0.42).

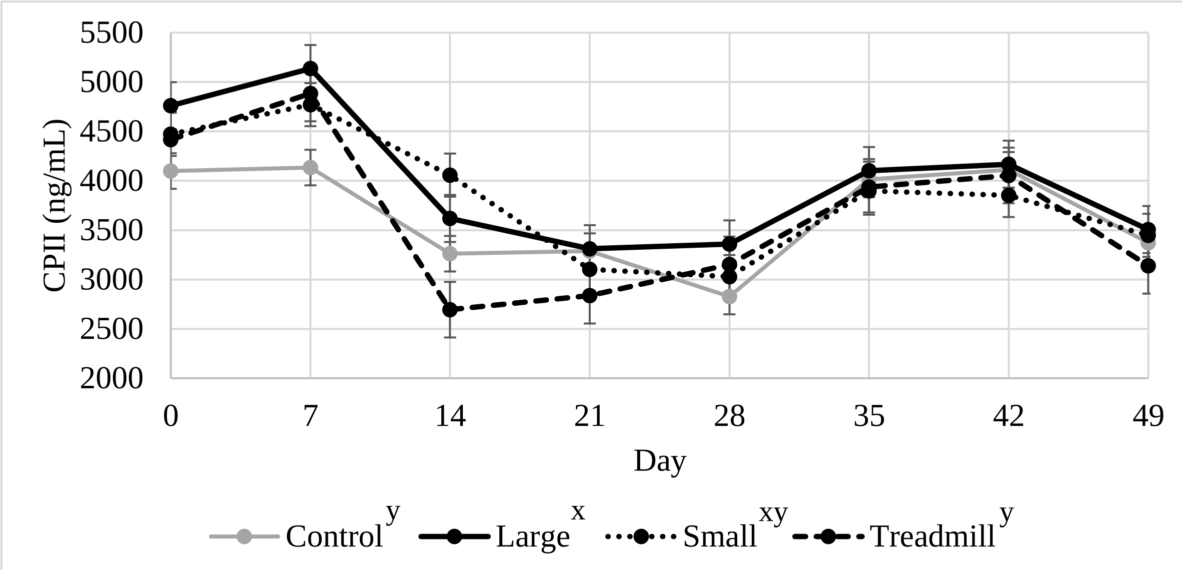
Figure 4: Calf mean serum C-telopeptide crosslaps of type I collagen (CTX-1) by treatment throughout the 7-week study period




*Average serum CPII*

There was a trend for a treatment effect on serum CPII concentation (P = 0.08) and a day effect (P < 0.0001), with day 14, 21, 28, and 49 having lower concentrations than other days (Figure 5). The interaction between day and treatment was not significant (P = 0.73).

Figure 5: Calf mean procollagen II c-propeptide (CPII) by treatment throughout the 7-week study period

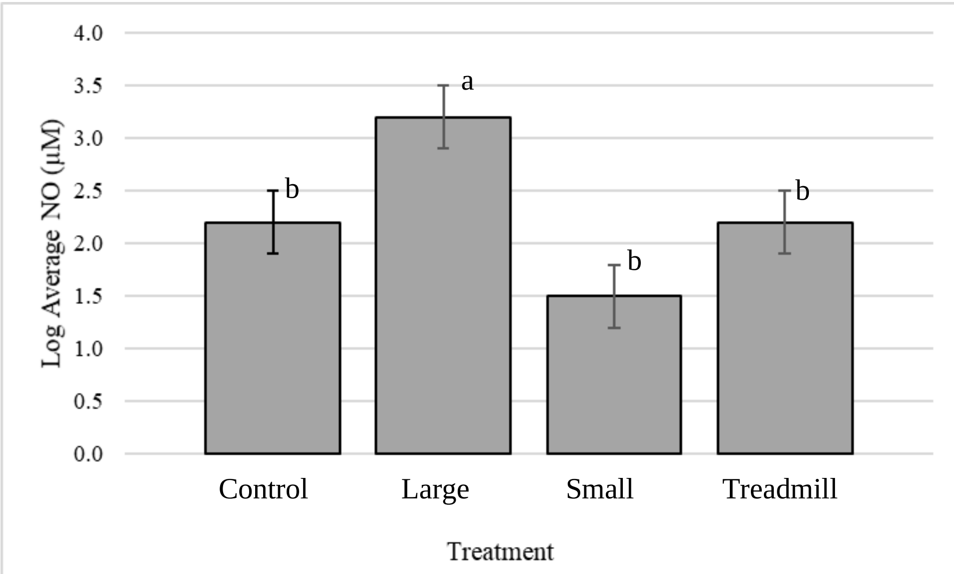



[x,y] Treatments lacking common superscripts have a tendency to differ ($P = 0.08$)

*Fetlock synovial fluid NO*

Treatment was a significant effect on synovial fluid NO concentration (Figure 6, $P = 0.0005$) with the large circle treatment having the highest concentration of NO. Joint was not a significant effect ($P = 0.80$) nor was the interaction of treatment and joint ($P = 0.51$).

Figure 6: Log transformed average nitric oxide (NO) concentration in the lateral and medial fetlock joints by treatment

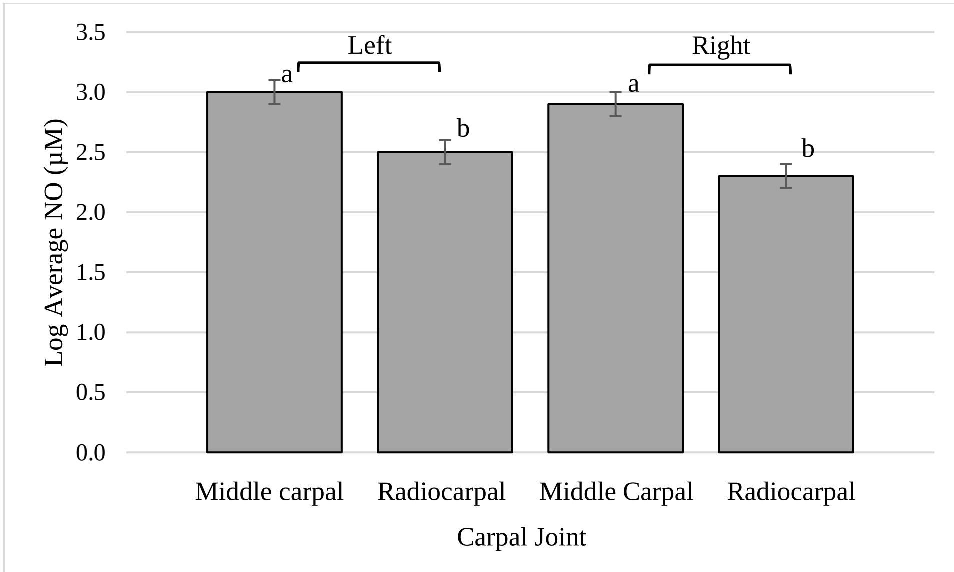



[a,b] Treatments lacking common superscripts differ ($P = 0.0005$)

*Carpal synovial fluid NO*

Treatment was not a significant effect on synovial fluid NO concentration ($P = 0.27$), but joint was (Figure 7, $P < 0.0001$), with middle carpal joints having greater average NO concentration than radiocarpal joints. The interaction of treatment and joint was not significant ($P = 0.91$).

Figure 7: Log transformed average nitric oxide (NO) concentration in the middle carpal and radiocarpal joints

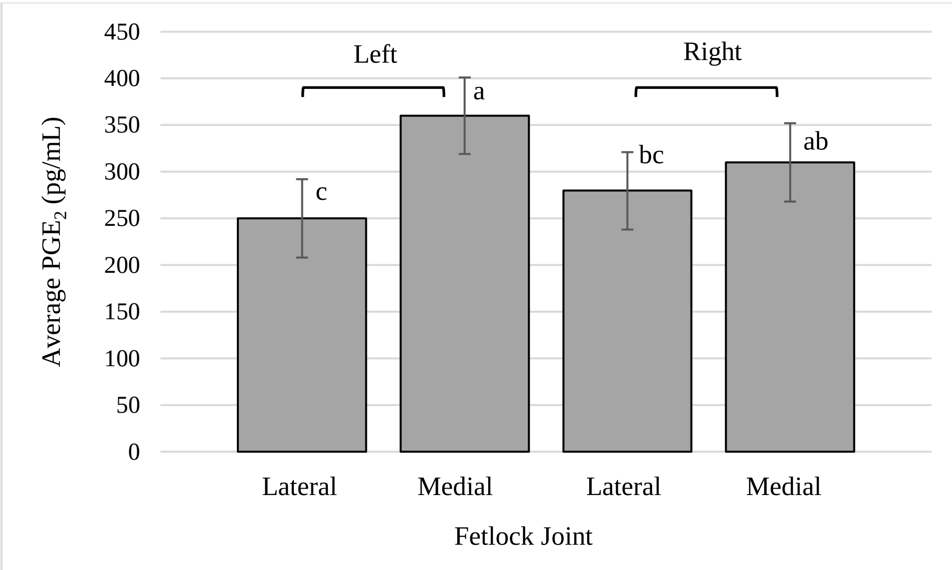



[a,b] Values lacking common superscripts differ ($P < 0.0001$)

*Fetlock synovial fluid $PGE_2$*

Treatment was not a significant effect on synovial fluid PGE2 ($P = 0.99$) but joint was (Figure 8, $P = 0.0005$), with both medial fetlock joints having greater average PGE2 concentration than the left lateral fetlock joint. The interaction of treatment and joint was not significant ($P = 0.16$).

Figure 8: Average prostaglandin E2 (PGE2) concentration in lateral and medial fetlock joints

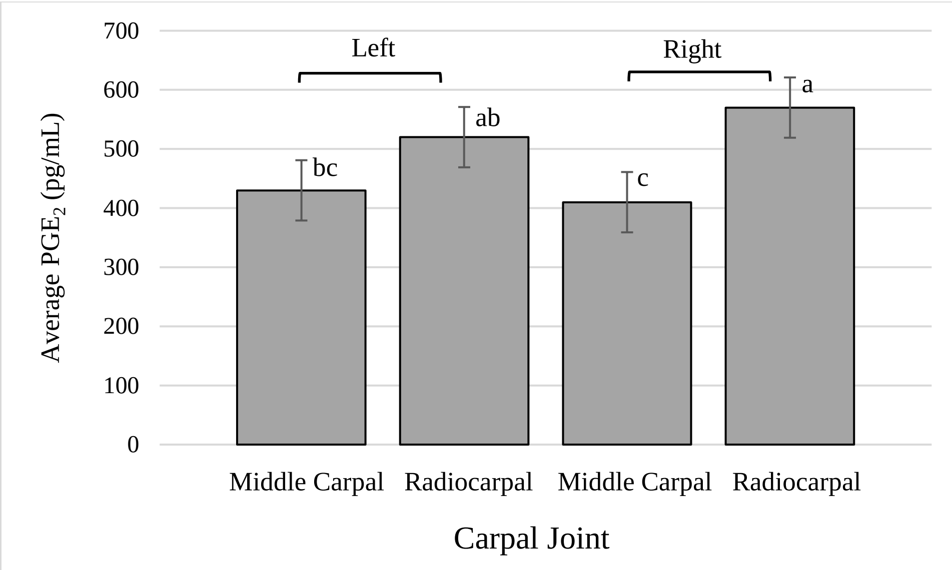



[a,b,c] Values lacking common superscripts differ (P = 0.0004)

*Carpal synovial fluid $PGE_2$*

Treatment was not a significant effect on synovial fluid PGE2 (P = 0.78) but joint was (Figure 9, P = 0.007), with the right radiocarpal joint having higher average concentration than both left and right middle carpal joints. The interaction of treatment and joint was not significant (P = 0.60).

Figure 9: Average prostaglandin $E_2$ ($PGE_2$) concentration in middle carpal and radiocarpal joints




[a,b,c] Values lacking common superscripts differ (P = 0.007)

*Cartilage glycosaminoglycan content*

There was no significant effect of treatment (P = 0.73) or leg (P = 0.35), nor was the interaction between treatment and leg significant (P = 0.14). Within the control, large circle, and treadmill treatments, the left and right legs did not have different GAG concentration at the proximal surface of the MC III & IV (Table 16). However, it was determined that the left leg of the small circle exercise treatment had greater GAG concentration at the proximal surface of the MC III & IV than the right leg (Table 16, P = 0.03).

Table 16: Average Glycosaminoglycan concentration in cartilage slices from the proximal surface of the MC III & IV by treatment and leg

| Treatment | Leg | GAG (mg/g) |
|---|---|---|
| Control | Left | 88 |
| | Right | 90 |
| Large | Left | 84 |
| | Right | 71 |
| Small | Left | 113[a] |
| | Right | 68[b] |
| Treadmill | Left | 79 |
| | Right | 99 |
| n | | 6 |
| SEM | | 14 |
| P - Value | | 0.14 |

[a,b] Values lacking common superscripts differ ($P < 0.05$)

## DISCUSSION

This study utilized calves as a model for young horses to determine the impact circle diameter can have to joint and bone health of the front limbs. It was hypothesized that a smaller diameter circle would lead to asymmetry between inside and outside forelimbs and increased markers of joint inflammation and cartilage metabolism. This hypothesis is partially accepted, as bone morphology and cartilage GAG content differences were noted within the small circle exercise group, but differences in markers of joint inflammation were not observed within the small circular exercise group.

Computed tomography scans in this study found the right (inside) leg of the small circle treatment group to have larger dorsopalmar external diameter than the left (outside) leg. In racing Thoroughbreds, the inside forelimb is the most common location for injuries (56% of catastrophic musculoskeletal injuries) [53]. While galloping around a turn the outside front limb has a pushing function, and the weight of the horse will be pushed forward and laterally onto the lead forelimb [34]. While there is great speed difference between the walking gait of calves in this study and

Thoroughbreds galloping around a turn, the location of bone adaptation in the small circle exercise calves seems to be a result of exercising on a turn.

Computed tomography analysis also identified treatment differences in the more-distally located proximal phalanxes. The small circle exercise group was found to have greater external mediolateral diameter in the medial phalanxes compared to the lateral phalanxes. This was also noted in the control treatment group. Both treadmill and large circle exercised animals had similar-sized lateral and medial phalanx external mediolateral diameter. The straight-line treadmill exercise and large circle exercise diameters being similar between the two phalanxes may suggest that the large circle exercise is more similar to straight-line exercise than the small circle exercise at such low speeds. Calves in the control treatment only had access to movement in the group housing pen. These movements may have included tight turns similar to the small circle exercise group.

Cortical density and widths of the proximal phalanxes were not impacted by treatment, but were however, different between the lateral and medial proximal phalanx bones. Very few studies have evaluated cortical bone characteristics of the proximal phalanxes in exercising horses or bovines. Horses have a single proximal phalanx unlike calves. This current study provides a necessary categorization of the differences in bovine lateral and medial proximal phalanxes which is important in the continued use of cloven-hoof species, such as calves, to serve as a skeletal model for equines. Lack of treatment differences between cortical widths and BMD from computed tomography are not surprising. It is known that dynamic exercise, such as sprinting, leads to bone adaptations [60–62,104]. As has been previously determined, gait is an important factor in the response of loading during circular exercise [91]. In this study, exercise only occurred at slow speeds of 1.1–1.5 m/s, leading to a walking gait for the calves, and no treatment differences between cortical widths or BMD. Based on the mechanostat theorem, localized strain within 1500-2500 microstrain will lead to bone maintenance, but greater strain will lead to a formation response in bone in order to reduce strain [90,105]. A limitation of this current study is that strain was not evaluated or calculated. Stride rate or other gait characteristics were not recorded in this study to allow for strain calculation. Strain evaluation among the cortexes of the MC III & IV would aid in the determination of calf bone response to circular exercise, treadmill exercise, as well as the free exercise in group housing.

The fracture force of the MC III & IV was found to be lower for the right leg of the treadmill exercise treatment group compared to the left. Similarly, the left MC III & IV of the treadmill group was found to have larger cortical area than the right leg. During treadmill exercise, calves wore rope halters which were tied with a quick release knot to either the left or the right side of the treadmill depending on which side of the treadmill the calves were on. Depending on calf size, between 2 and 4 calves could fit on the treadmill at a time. During the last week of the study, calves were large enough that only two calves could fit on the treadmill at once, both tied to the left side of the treadmill as handlers were only able to stand on the left side of the treadmill. While calves were tied with their rope halter loose enough so that they were encouraged to travel straight, there is a potential that having their rope halter tied to their left side could have caused asymmetric loading between the right and left forelimb. Kinematics between treadmill and over ground exercise are not identical. Exercise on a treadmill exposes animals to footing that is different than that of normal ground surfaces. Treadmill exercise can impact gait factors, such as a longer stance duration of the forelimbs at the trot, less lumbar motion at the trot, and longer stride length at the canter [106]. The difference between the right and left MC III & IV fracture force may have simply been a result of a small number of animals on each treatment, and not method of exercise, but both possibilities should be noted. The presence of a shorter MC III & IV for the large circular exercise group is most likely due to very little variation of bone length within and among treatments, causing for a relatively small SEM that could allow any minor difference, whether related to exercise treatment or not, to be found significant.

Similar to bone morphology, some treatment differences, as well as multiple joint differences, were found in markers of joint inflammation and metabolism, NO and $PGE_2$. Average fetlock NO concentration was found to be greatest in the large circular exercise treatment. During the first two weeks of exercise the large circle calves were reluctant to exercise, and while they maintained an average speed within the range of this study, they often paused during exercise and needed verbal encouragement to begin walking again. Once they began walking again they needed to take a few steps at a faster stride to avoid getting shocked by the panels in the electric walker. The small circle exercise and treadmill groups exercised continually with no pauses in a walking session unlike the large circle group. This behavioral difference may contribute to the greater concentration of NO in the large circle exercise groups. It is also worth noting in this study, there was large variation of NO concentration in synovial fluid samples due to the viscous nature of un-diluted and un-digested synovial fluid. Future studies may have more success evaluating NO concentration in serum.

In this study we specifically chose not to compare results of the fetlock synovial fluid to the carpal synovial fluid, understanding that the range of motion and relative loading of these joints are vastly different due to their locations. However, differences within fetlock and carpal joints were found. The carpal NO concentration was different between locations, with middle carpal joints having higher NO concentration than radiocarpal joints, providing further categorization of joint characteristics of cloven-hooved animals. Nitric oxide is considered to be important in the initiation of repair and attracts bone cells to the site of injury. NO may also be involved in the regulation of osteoclasts, as *in vitro* osteoclast cell death has been found after high doses of NO [107]. NO can lead to dysregulation of osteoblast and osteoclast balance, which can result in cartilage destruction through chondrocyte apoptosis [108]. Fetlock $PGE_2$ was greater in medial fetlock joints than lateral fetlock joints of both legs. Carpal $PGE_2$ was lower for the right middle carpal compared to left and right radiocarpal joints regardless of treatment. Synovial fluid $PGE_2$ in two-year-old horses and dogs has been found to increase after surgically-induced osteoarthritis [94,109]. Middle carpal NO concentration was greater than radiocarpal, but middle carpal $PGE_2$ concentration was lower than the radiocarpal. Injuries to carpal joints have been found to vary based on animal function. Middle carpal joint injuries are found frequently in racing horses due to repeated trauma, but pleasure and sport horses are found with osteoarthritis most commonly in the radiocarpal joint [110]. Exercise performed in this study did not impact fetlock or carpal $PGE_2$ concentration. In this study, synovial fluid concentrations of NO and $PGE_2$ were mostly influenced by joint location, similar to the proximal phalanx bone location. These results provide important information on joint-based differences of biomarkers in calves, which have not been previously analyzed as a result of exercise.

Determination of GAG content in the proximal surface of MC III & IV was not different among treatments. It has been previously speculated that in 15-wk-old calves, the joints may still be homogeneous and GAG content was not influenced by short-duration exercise [98]. In this study within the small circle exercise group the left (outside) leg had greater GAG content in the MC III & IV proximal surface compared to the right (inside) leg. Another study found, after a 5-month period, horses confined to box stalls had elevated cartilage GAG content compared to horses afforded pasture access [111]. As has been previously discussed, lean angle is increased as a result of smaller radii circles and has the potential for uneven loading between the inside and outside limbs [8,29,67]. With only 6 animals per treatment, and exercise at a slow rate of speed in this study, treatment differences may not have been detectable. However, a future experiment with a combination of more animals and higher speeds may find GAG content to be different between cartilage surfaces of the front limbs.

Average OC, a marker of osteoblastic activity and thus bone formation [112], was greater for the small circle and treadmill exercise treatments compared to the control treatment. However, the large circle group was not greater than the control exercise group. During the first two weeks of exercise the large circular exercise calves were reluctant to exercise and needed encouragement to achieve the basic requirements of exercise at 1.1–1.5 m/s. However, the treadmill and small circle exercise treatment groups were amenable to exercise and needed less auditory coercion to continue to exercise at the speed required for the study. In this study, behavior was not evaluated or analyzed, but the described behavioral differences may have been a contributing factor to the treatment differences in average OC concentration. A limitation of this study is the lack of internal load indicators evaluated during exercise. Future studies including an analysis of internal load of exercising calves, such as heart rate response, can aid in characterizing more of the psychophysiological response of calves to exercise [113].

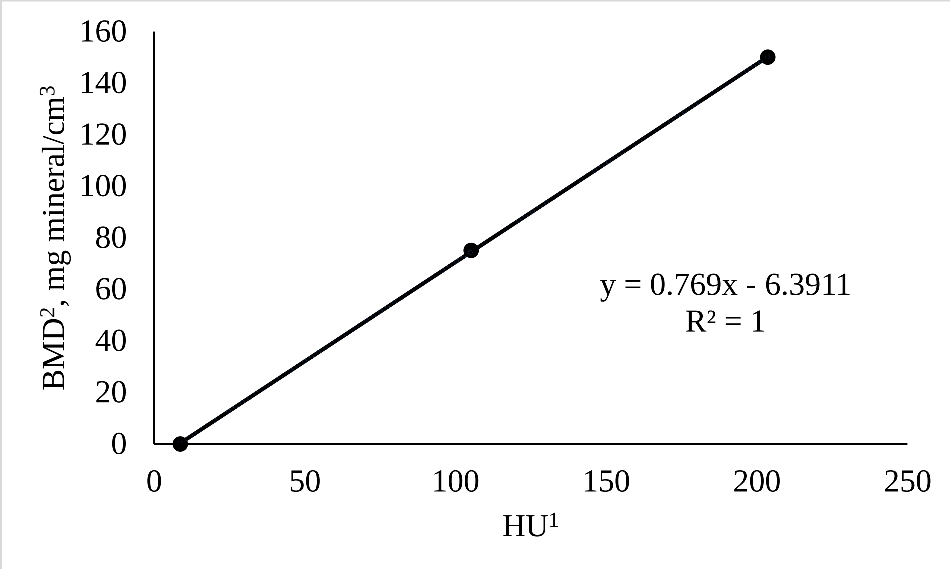
Average OC, CTX-1, and CPII were different among treatments on d 0, thus, d 0 was a significant covariate for all serum markers. All calves were randomly assigned to treatments and striated based on weight at d 0, assuring that each treatment had calves of equal sizes at the start of the study. Calves were transported to the farm one week before d 0. In future studies utilizing calves for exercise, animals may need more than one week for housing acclimation before beginning exercise. It is interesting to note, that in the middle of the study, on days 14, 21, and 28, CPII concentration appears to decrease, then increase after day 28. At this time, OC was also elevated. By d 14 all treatment groups were acclimated to their exercise treatments and successfully exercising with little coercion. These day differences observed may be a result of calves settling into their exercise and housing after acclimation. The lack of day effects in CTX-1 after d 0 is not surprising, as calf serum CTX-1 has not previously exhibited a day effect during sprint exercise or confinement [60].

## CONCLUSIONS

Circular exercise is used frequently, and by varying methods across the equine industry. Circle size, speed of travel, and training of the animal are all factors which should be considered when utilizing circular exercise. Based on the results of this study, altering circle size can impact joint and bone health, with a smaller circle size leading to differences in bone diameters as well as cartilage glycosaminoglycan content. This study provides initial characterization of physiological responses to circular exercise performed by calves on a walker at slow speeds. Results from this study, coupled with other studies available in the literature suggest that circular exercise, even at a slow speed, can impact joint and bone health of young animals. The circular exercise in this study was performed at slow speeds, and for a duration of 7 weeks, a very short time span in relation to horse training. Alteration of speed or duration of exercise could eventually lead to greater changes to bone morphology and biomarkers. Further information needs to be explored on circular exercise, such as the impact of different styles of riding, presence of a rider, and effect to the hind limb function. Handlers and riders utilizing circular exercise should recognize the manner in which they exercise animals can impact overall health, and should consider the circle size at which animals exercise to be a factor contributing to bone and joint health.

APPENDIX

Figure S1: Equation utilized to calculate bone mineral density (BMD) of values measured in computed tomography (CT) scans




[1]Hounsfield Units (HU): Values along the x-axis are average HU values obtained from CT scans

[2]Bone Mineral Density (BMD): Values along the y-axis are known concentrations of rows in the hydroxyapatite phantom (0, 75, and 150 mg mineral/cm$^3$)

Table S1: Calf height, weight, and length expressed throughout the weekly measurements

| Day | Height (cm) | Weight (kg) | Length (cm) |
|---|---|---|---|
| 0 | $88^{g}$ | $78^{g}$ | $89^{e}$ |
| 7 | $90^{f}$ | $90^{f}$ | $89^{e}$ |
| 14 | $92^{e}$ | $87^{f}$ | $95^{d}$ |
| 21 | $96^{d}$ | $108^{e}$ | $95^{d}$ |
| 28 | $96^{d}$ | $119^{d}$ | $103^{c}$ |
| 35 | $98^{c}$ | $130^{c}$ | $104^{c}$ |
| 42 | $99^{b}$ | $140^{b}$ | $109^{b}$ |
| 48 | $100^{a}$ | $148^{a}$ | $113^{a}$ |
| SEM | 1 | 3 | 1 |
| P – Value | $P < 0.001$ | $P < 0.001$ | $P < 0.001$ |

$^{a,b,c,d,e,f,g}$ Values lacking common superscripts within a column differ ($P < 0.001$)

Table S2: Internal (int) and external (ext) dorsopalmar and mediolateral diameters as well as moment of inertia (MOI) from cross-sectional views at the midpoint of fused metacarpal III & IV of left and right front legs

| Treatment | Dorsopalmar int, mm | Dorsopalmar ext, mm | Mediolateral int, mm | Mediolateral ext, mm | MOI, $mm^4$ |
|---|---|---|---|---|---|
| Control | 12 | 21 | 17 | 29 | 11,600 |
| Large | 12 | 21 | 19 | 29 | 11,200 |
| Small | 12 | 22 | 18 | 30 | 12,900 |
| Treadmill | 12 | 21 | 17 | 28 | 11,500 |
| SEM | 0.40 | 0.39 | 0.52 | 0.61 | 820 |
| P – Value | 0.60 | 0.27 | 0.16 | 0.29 | 0.50 |

Table S3: Cortical and midpoint slice bone density of metacarpal III & IV (MC III & IV) of left and right front legs

| Treatment | Dorsal cortex, mg mineral/$cm^3$ | Lateral cortex, mg mineral/$cm^3$ | Medial cortex, mg mineral/$cm^3$ | Palmar cortex, mg mineral/$cm^3$ | Midpoint slice, mg mineral/$cm^3$ |
|---|---|---|---|---|---|
| Control | 1,240 | 1,220 | 1,220 | 1,060 | 1,030 |
| Large | 1,240 | 1,240 | 1,230 | 1,070 | 1,030 |
| Small | 1,250 | 1,230 | 1,240 | 1,070 | 1,040 |
| Treadmill | 1,250 | 1,230 | 1,240 | 1,060 | 1,030 |
| SEM | 21 | 12 | 14 | 17 | 11 |
| P – Value | 0.95 | 0.73 | 0.76 | 0.97 | 0.96 |

Table S4: Cortical widths at midpoint of the metacarpal III & IV (MC III & IV) of left and right front legs

| Treatment | Dorsal cortex, mm | Lateral cortex, mm | Medial cortex, mm | Palmar cortex, mm |
|---|---|---|---|---|
| Control | 5.0 | 5.6 | 5.7 | 4.1 |
| Large | 5.0 | 5.2 | 5.2 | 3.9 |
| Small | 5.4 | 5.4 | 5.7 | 4.0 |
| Treadmill | 5.2 | 5.4 | 5.5 | 4.2 |
| SEM | 0.20 | 0.31 | 0.28 | 0.21 |
| P – Value | 0.38 | 0.86 | 0.63 | 0.70 |

Table S5: Dorsal, lateral, medial, and palmar cortical widths of the metacarpal III & IV separated by left and right leg

| Leg | Dorsal cortex, mm | Lateral cortex, mm | Medial cortex, mm | Palmar cortex, mm |
|---|---|---|---|---|
| Left | 5.15 | 5.44 | $5.62^{x}$ | 4.12 |
| Right | 5.11 | 5.35 | $5.40^{y}$ | 4.01 |
| n | 24 | 24 | 24 | 24 |
| SEM | 0.11 | 0.16 | 0.15 | 0.11 |
| P – Value | 0.48 | 0.40 | 0.06 | 0.21 |

$^{x,y}$ Values lacking common superscripts within a column tend to differ (P < 0.06)

# CHAPTER 5: Overall discussion and conclusions

Compared to straight-line exercise, it is considerably more difficult to determine the overall effects of circular exercise in horses. Radius and gait speed can both be altered independently, but also simultaneously, making for infinite combinations of radius and gait speed. When studying quadrupeds, the impact of radius, gait, as well as radius and gait has the potential to be unique to all four limbs, as well as the possible combinations of limbs (forelimbs, hindlimbs, inside limbs, outside limbs, or diagonal pairs), and the rest of the musculoskeletal system. Outside of alterations to radius and gait, there are multiple methods to circular exercise: round pen, ridden, lunged, mechanical walker. All these methods, combined with varying radii and gaits, provide exponentially more unique situations of circular exercise to evaluate. This concluding chapter is prefaced with these limitations to recognize that the research performed in this dissertation, and the previously published research that exists on the topic, truly have only been able to evaluate a small sector of the impacts of circular exercise to equine health.

Specifically, this dissertation found that in horses exercising on a 10-m or 15-m circle, the area loaded by the outside front hoof is greater at the canter than the walk or trot. In this study, we expected to find that circle size would have an impact for forelimb average solar area, vertical force, and pressure outputs. This was not the case, only the gait (walk, trot, or canter) impacted the outputs. In this study, horses were exercised in a round pen with no physical restraint, and only auditory or visual encouragement to travel at a specific gait. Of particular interest is how utilization of a lunge line and halter to restrain a horse instead of a round pen would impact forelimb outputs. The use of lunging with simply a halter and lunge line is incredibly common as a method of exercise and training and is utilized by handlers of varying skill levels. Combinations of circle size, gait, and handler experience would be interesting to evaluate. Of course, a rider can also impact how a horse travels; unpublished data in our lab found that a professional rider on a horse leads to lower peak force when traveling straight or in a circle. This is a very small slice of data that has not been evaluated statistically nor with numerous horse/rider combinations. This early relationship found in the preliminary data and supported with other studies is an exciting area to consider for future research.

During the above-mentioned study, the Tekscan™ Hoof System was used to determine loaded area and force, and subsequently calculated pressure. This system needs further development for use in circular exercise, especially at speeds above a walk and moderate trot. The material which the sensors are embedded in is not strong enough on its own to withstand loading from horses, and environmental exposure to dirt and moisture. Further experimentation is needed to evaluate a sealing method which aids the durability of the sensors, but also allows for collection of data without hindering natural movement.

When using calves as a model for juvenile horses, it was found that even at a slow, walking gait, circular exercise for 7 weeks led to potentially negative impacts in bone and joint health at a small circle (12-m). Ideally, the calves would have travelled at a trotting gait, but unfortunately they were not willing to travel at a trot for a long duration on a mechanical walker or treadmill. While faster speed would have been preferred, horses are frequently exercised at a walk on mechanical walkers, allowing connections between these results and the equine industry to be made. While calves in this study were reluctant to exercise faster than a walk, other studies in the Spartan Equine Research Lab have found that sheep will exercise at faster speeds willingly and safely. Future studies are in development to utilize a sheep model which can tolerate mechanical walker and treadmill exercise at faster speeds for longer durations. Using older, exercise trained bovines may also be an option, but these animals may need housing and management needs that are more expensive than using sheep as an equine model.

A researcher could spend the lifetime of their career and beyond evaluating the many combinations of circular exercise radius, gait of travel, method of exercise, and their infinite combinations. However, before losing oneself to the depths of possibility in research, one should remember the reason for the research: the horse. Beneficial application of this topic of research is to find the combination which is most beneficial to the horse, and could prevent injuries, while providing an effective, and safe method of exercise for both human and horse. This priority of horse health and safety should not only be recognized by researchers, but also riders, trainers, breeders, governing associations, and spectators alike. Instead of determining which circle size, speed, and method elicits to the least amount of damage in the horse, we may need to incur a change in how horses are trained and evaluated, and put less priority on small, repetitive maneuvers, that are not beneficial to the overall health, purpose, of affective state of the horse.

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LITERATURE CITED

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