

Calves, as a model for juvenile horses, need only one sprint per week to experience increased bone strength

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ABSTRACT: Previous research has determined that maintaining young animals in stalls is detrimental to their bone health, while the addition of 50 to 82-m sprints 5 d/week aids in counteracting the reduction of bone strength from confinement. The current research aims to determine if 1 or 3 d/week of sprinting affords the same benefits to bone as 5 d/week of sprinting compared to animals confined with no sprinting. Twenty-four Holstein bull calves were obtained from the Michigan State University Dairy Cattle Teaching and Research Center. At 9 wk of age, calves were randomly assigned to treatments of 1, 3, or 5 d/week of sprint exercise, or to the confined control group sprinted 0 d/week. Each treatment had 6 calves. Individual sprinting bouts included a single sprint down a 71-m concrete aisle. For the duration of the 6-wk study, calves were housed at the MSU Beef Cattle Teaching and Research Center in stalls which afforded calves room to stand, lay down, and turn around. Serum was collected weekly via jugular venipuncture to obtain concentrations of osteocalcin (OC) and C-telopeptide crosslaps of type I collagen (CTX-1)—markers of bone formation and degradation, respectively. Sprints were videotaped weekly to determine stride frequency and sprint

velocity. On day 42, calves were humanely euthanized at the Michigan State University Meat Lab and both front limbs were immediately harvested. Computed tomography scans and mechanical testing were performed on the left fused third and fourth metacarpal bones. Serum OC concentration was greatest for calves sprinted 5 d/week ($P < 0.001$). Calves sprinted 5 d/week had both greater stride frequency ($P < 0.05$) and lower sprint velocity ($P < 0.05$). All exercise treatments experienced greater dorsal cortical widths compared to control animals ($P < 0.01$). Through mechanical testing, fracture forces of all sprinting treatments were determined to be greater than the control treatment ($P < 0.02$). Results from this study support that sprinting 1, 3, or 5 d/week during growth can increase bone health and cause favorable alterations in bone markers. While all exercise treatments had over a 20% increase to fracture force, calves sprinted 1 d/week sprinted only 426 m over the 6-wk study and still experienced over a 20% increase in bone strength compared to confined calves. This study demonstrates the remarkably few strides at speed needed to enhance bone strength and emphasizes the danger to skeletal strength if sprinting opportunities are not afforded.

Key words: bone, confinement, exercise, horse, juvenile, strength

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INTRODUCTION

Horse racing has experienced increases to starters and purses in Quarter Horse and Thoroughbred racing over the past few decades, despite a reduction in the U.S. horse population (American Quarter Horse Association, 2010, 2017; American Horse Council Foundation, 2018; The Jockey Club, 2019a,b). The rise of interest and participation in racing comes with inherent risks due to the fast-paced, high-intensity nature of the sport, and resulting prevalence of injuries. Injuries to racehorses can lead to career-ending retirement, death of horses, injury to or death of jockeys, adverse perceptions of horse racing by the public, and detrimental effects to the horse industry's economics (Stover, 2003).

Catastrophic musculoskeletal injuries occur at a rate of 1.33 injuries per 1,000 race starts during Quarter Horse races (Beisser et al., 2014) and fatal injuries occur at a rate of 1.61 per 1,000 starts in Thoroughbred races (The Jockey Club, 2018). Beyond affecting horses while racing, musculoskeletal injuries are the main cause of missed training days and wastage of racing horses and horses of all other disciplines (Rogers, 2012). One such musculoskeletal injury is dorsal metacarpal disease, which is commonly seen in 2-yr-old racing Quarter Horses as well as racing Thoroughbreds. Dorsal metacarpal disease is characterized by stress fractures in the dorsal region of the metacarpal, caused by the lag time between bone formation during remodeling, as rebuilding of bone happens much slower than the resorption of bone. Young animals are often afflicted with dorsal metacarpal disease likely because they have not been accustomed to the strains of racing as they have been removed from pasture, kept in stalls, and not afforded voluntary exercise with strides at speed (Ross and Dyson, 2011).

Past research has depicted that bone mineral loss in the third metacarpal occurs as a result of horses being removed from pasture and kept in stalls at the onset of race training and that the loss of bone mineral content leads to increased incidence of injuries as training progresses (Nielsen et al., 1997).

Bone responds to strains placed on it, as sprints between 50 and 82-m performed 5 d/week for 6 wk increased bone mineral content of the third metacarpal in Quarter Horse weanlings and bone mineral density (BMD) of the fused third and fourth metacarpal (MC III & IV) in Holstein bull calves. Bone morphology was also positively impacted in sprinted animals of both species, compared to confined counterparts. Sprinted Quarter Horse weanlings and Holstein bull calves both tended to have greater cortical bone. The sprinted bull calves were determined to have greater fracture force of the MC III & IV compared to confined animals (Hiney et al., 2004a,b). In the United States, euthanizing young horses solely for the purpose of research would not be well received by the general public. Young bull calves fit as a model for juvenile horses as they can be euthanized for research and have similar skeletal structure. Previously, Hiney et al. determined that short sprints 5 d/week lead to greater skeletal health and that calves were an applicable skeletal model for juvenile horses (2004a,b).

The bones of young horses are most responsive to stimulus from the yearling stage up to 2 yr of age. If loading occurs between these ages, there is opportunity for the animal to be able to resist damage to and failure of bone in adulthood (Rogers et al., 2012). Furthermore, low impact exercise, such as that from endurance training over long distances, does not alter bone density compared to young horses kept on pasture with no additional exercise (Spooner et al., 2008). The intensity of exercise is important to consider when evaluating the effects of exercise on bone health. The knowledge that 5 d/week of short-duration high-intensity exercise increases bone health in young animals leads to the inquiry of how many days of sprint exercise are truly needed to experience benefits to bone health which counteract bone loss from stalled confinement.

In this current study, Holstein bull calves were sprinted 0, 1, 3, or 5 d/week to evaluate the effects to bone morphology, BMD, and bone strength as well as biomarkers of bone formation and resorption in an effort to determine the frequency of

weekly sprints needed to achieve beneficial skeletal changes. Calves were used as a model for juvenile horses allowing for postmortem mechanical testing of bone strength. It was hypothesized that sprinting 1 or 3 d/week would have the same beneficial impacts to bone health of juvenile animals as sprinting 5 d/week, when compared to confined animals.

MATERIALS AND METHODS

Animals and Management

This project was approved by the Michigan State University Institutional Animal Care and Use Committee (Protocol 10/16-183-00). Twenty-four Holstein bull calves were obtained from the Michigan State University Dairy Cattle Teaching and Research Center over a span of 6 mo. Calves were age-matched before entering the study, to have a span of no more than 10 d between the oldest and youngest calf in each group. Groups contained between 3 and 6 calves (Table 1). At 7 and 8 wk of age, all calves underwent 2 halter training sessions in which they were walked 4 laps on a 10-m long concrete barn aisle. Calves were weaned from milk replacer at 8 wk of age, after which their diet consisted of 3.6 kg of a commercially available pelletized calf starter daily (Ampli-Calf STR 20P R50 DBZ9.1 Medicated, Purina Animal Nutrition LLC) and ad libitum access to water. This pelletized calf starter meets the National Research Council's requirement of Ca and P for young calves (NRC, 2001). At day 0, each calf was presented with 3.6 kg of calf starter. Fresh calf starter was given every 24 h. If a calf left less than 0.5 kg of calf starter uneaten, then the presented amount of calf starter was increased by 0.5 kg.

Table 1. Treatment assignment of calves based on age-matched groups

Group ¹	Treatment ² 0 d/week	Treatment 1 d/week	Treatment 3 d/week	Treatment 5 d/week	Total calves
1	2	1	1	1	5
2	1	1	1	1	4
3	0	1	1	1	3
4	1	2	2	1	6
5	1	1	0	1	3
6	1	0	1	1	3
Total calves	6	6	6	6	24

¹Calves assigned to a group had no more than a 10-d span between birthdates and groups had between 3 and 6 calves.

²Treatments are based on the number of sprints a calf performed per week: 0 sprints/wk, 1 sprint/wk, 3 sprints/wk, or 5 sprints/wk. A sprint was 71 m in length, and no calf performed more than 1 sprint in a day.

Study Design

Each group of calves entered the study when their average age was 9 wk, at which time the group was transported to the Michigan State University Beef Cattle Teaching and Research Center where they were housed for the duration of the 42-d study. Individuals from each group were randomly assigned to 1 of 4 treatments, leading to 6 calves per treatment (Table 1). Treatments were based on how many days of sprinting an animal would perform each week: 0, 1, 3, or 5 d/week. Calves sprinted 0 d/week served as a control group and spent the duration of the 6-wk study confined. For a sprinting session, animals were individually walked out of their stalls, down a 71-m concrete aisle away from their stalls, released, then verbally encouraged to sprint the 71-m back down the aisle. At the end of the aisle, the calf was collected and walked back to their stall. Regardless of treatment, all animals were housed in stalls that were 93 cm in width and 175 cm in length. The size of these stalls ensured that calves could stand up, lie down, and turn around with ease, but could not get any extraneous exercise beyond controlled sprints determined by treatment assignment. Height, weight, and length of each calf were taken on day -1 and day 41. Hoof angles of the calves were taken by an equine farrier on day -1 and day 41 as well. On day 42, calves were transported from the MSU Beef Cattle Teaching and Research Center to the MSU Meat Lab, where the animals were humanely euthanized by a captive bolt pistol, with a United States Department of Agriculture inspector present.

Sprint Videos

On day 7 and continuing weekly until day 42, calves were videotaped while sprinting. Calves were not videotaped while sprinting until day 7 to permit calves an acclimation period to the exercise treatments. The video camera was positioned so that calves were running towards it during their entire sprint. The entire 71-m sprint was videotaped, but only the middle 21 m of the sprint was analyzed to calculate stride frequency and sprint velocity. Calves were recorded in the middle 21 m of their 71-m sprint to avoid confounding sprint analysis with acceleration and deceleration of calves. The middle 21-m of the 71-m barn aisle was marked with bright tape on a wall along the aisle so that the middle 21-m portion was identical and visible for all sprints. Stride frequency was calculated by counting from the recorded video the amount of

impacts the left front limb made during the middle 21-m portion of the sprint. Sprint velocity was determined from the recorded video by calculating the time it took a calf to complete the 21-m middle portion of the sprint and dividing 21 m by that span of time. Videos needed to meet the criterion that all impacts of the left front limb during a calf's sprint could be counted during the middle 21 m. Videos that did not meet this criterion due to video quality, field of view, or calf behavior were excluded from the dataset. Four calf sprints were removed from the dataset due to not meeting the video quality criterion. Two of the calves that were removed sprinted 1 d/week, 1 calf sprinted 3 d/week, and 1 calf sprinted 5 d/week.

Sample Collection

Starting on day 0 and continuing weekly until day 42, 8.5 mL blood was collected from each calf via jugular venipuncture into nonheparinized serum-separator vacutainers between 0730 and 0830 h. Blood was allowed to coagulate for 1 h, then centrifuged at $1,000 \times g$ for 15 min to attain serum separation. Serum samples were pipetted into microcentrifuge tubes, then frozen at -20°C for later analysis. Serum was analyzed for concentrations of osteocalcin (OC) and C-telopeptide crosslaps of type I collagen (CTX-1)—respective markers of bone formation and degradation.

After euthanasia on day 42, the left front limb from each calf were removed above the carpal bones. The left limb was immediately placed into a -20°C freezer for later computed tomography (CT) analysis and mechanical testing.

Sample Analysis

Calf serum samples were analyzed for OC utilizing the commercially available MicroVue Osteocalcin EIA (Quidel, San Diego, CA). Serum OC reflects osteoblastic activity and is a marker of bone formation (Lee et al., 2000; Hiney et al., 2004a,b). Serum samples utilized for osteocalcin analysis were diluted with deionized water at a 1:15 ratio. Serum samples were analyzed for CTX-1 concentration using the commercially available Serum CrossLaps (CTX-1) ELISA (Immunodiagnosics Systems Gaithersburg, MD). Concentration of CTX-1 in serum is a marker of bone resorption used in both equine and bovine research (Donabedian et al., 2008; Matsuo et al., 2014). Serum samples utilized for CTX-1 analysis were not diluted.

Computed Tomography

The left limb remained in a -20°C freezer until CT scans were performed. Before CT scans were performed, limbs were removed from the freezer and placed in a chiller to thaw for 2 to 3 d at 4.8°C . Scans were performed by a technician at the Michigan State University College of Veterinary Medicine. Computed tomography scans were performed with the following settings: 120 kV, 320 mAmp, 0.625 mm slice thickness, 2,000 slices per scan, and lumbar spine position using a GE Revolution Evo scanner (General Electric Healthcare, Princeton, NJ). All limbs were positioned perpendicular to the gantry as straight as possible. Solid calcium hydroxyapatite phantoms (Image Analysis, Inc; Columbia, KY) with rows representing 0, 75, and 150 mg/cm^3 Ca were scanned along with limbs for BMD comparison. Analysis of CT scans was completed using Mimics 21.0 (Materialise, Leuven, Belgium). The midpoint of the left MC III & IV was calculated for cross-sectional measurements. The distal and proximal ends of the fused MC III & IV were found, and the average CT slice between the 2 ends was calculated and denoted as the midpoint of the whole-bone (MID). Internal and external diameters of cortical bone, and the dorsal, palmar, lateral, and medial cortical widths (CW) were measured at MID for the left fused MC III & IV for each calf. Internal diameters represented the diameter of the medullary cavity. Diameters and CW were calculated with a mask at threshold of 400 Hounsfield Units (HU); this mask permitted that only bone, and not soft tissue, was included in the reported value. Using an angle measurement tool in Mimics, perpendicular dorsopalmar, and mediolateral lines were drawn to measure the internal and external diameters, as well as to identify the locations to measure CW.

Bone mineral density was measured in a cross-sectional view at MID. Values for average BMD were collected from the dorsal, lateral, medial, and palmar cortices and whole slice at MID. A mask with a threshold value of 400 HU was used to collect BMD. Squares measuring 10 mm^2 were used to determine the average BMD in the 4 cortices. For whole-slice BMD, the entire slice was highlighted with the mask, and BMD of the entire cortical bone at MID was recorded. Values collected from CT scans for average BMD were reported in HU. Average HU were recorded at each of the 3 concentrations of calcium along the length of the phantom at 10 locations for each individual scan. The HU values were then averaged

for each scan and compared in a scatter plot to the known concentrations of the phantom (Fig. 1). The equation produced from the regression line converted density in HU to mg Ca hydroxyapatite/cm³. This method of determining BMD is supported by Robison and Karcher (2019).

Cross-sectional and cortical areas were determined with a mask at threshold of 350 HU. This mask threshold was selected so that medullary contents were included in the cross-sectional area. Cross-sectional area was obtained by using a tool which reports the area of an entire ellipse, including the medullary cavity. Cortical area was calculated by determining the area of the inner medullary cavity and subtracting that from the cross-sectional area, to yield only the area of cortical bone selected at MID. After CT scanning, limbs were placed back into the -20 °C freezer until mechanical testing was performed.

Mechanical Testing and Calculations

Before mechanical testing, the left limbs were removed from the -20 °C freezer and thawed for 4 d at 4.8 °C. Once thawed, the limbs were skinned and remaining tissues were removed from the fused MC III & IV. The bones were wrapped in paper towel, covered in phosphate buffer saline, and kept in an upright refrigerator in individual plastic bags overnight until mechanical testing. Mechanical testing was performed via 4-point bending on an Instron machine (Model number: 4202, Serial number: 537) at room temperature. Fused MC III & IV were placed with the palmar aspect of the bone facing upwards toward the force applicators,

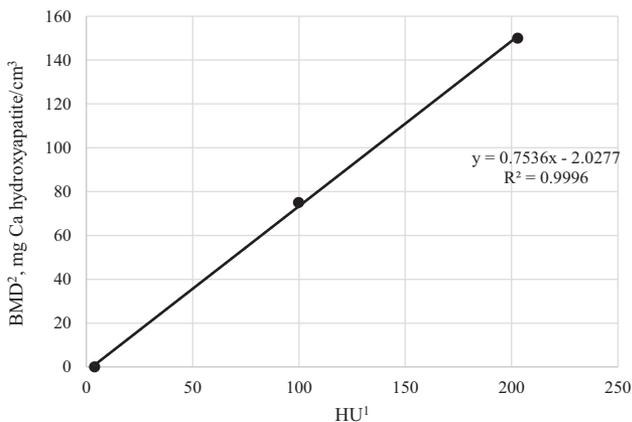


Figure 1. Graph used to calculate bone mineral density (BMD) of values obtained from computed tomography (CT) scans analyzed with Mimics. ¹Values along the X-axis are average Hounsfield Units (HU) obtained from CT scans. ²Values along the Y-axis are known concentrations of rows in the hydroxyapatite phantom (0, 75, and 150 mg/cm³ Ca). The equation obtained from this regression outputs HU values as mg Ca hydroxyapatite/cm³.

and the dorsal portion facing the bottom supports. For all bones tested, loading speed was 10 mm/minute, span (L) between the bottom supports was 100 mm, and the load cell used was 10 kN (Fig. 2).

Moment of inertia (I, mm⁴) was determined with the calculation for a hollow ellipse described in ASABE standards (2017, Fig. 3): $I = 0.049[(B * D^3) - (b * d^3)]$ where B = exterior lateromedial diameter, D = exterior dorsopalmar diameter, b = interior lateromedial diameter, d = interior dorsopalmar diameter.

Flexural rigidity (EI, N mm²) and Young's modulus of elasticity (E, N/mm²) were determined based on respective calculations appropriate for 4-point bending with an Instron: $EI = (F/V)(a^2/12)(3L - 4a)$ and $E = EI/I$. While calculating EI, the slope of the force deformation curve (F/V) was calculated from 4 to 5.5 mm of deformation. For all calves, 4 to 5.5 mm of deformation was part of the linear portion of the curve, with an R² of 0.99. The value of a was calculated individually for all calves. At the time of bone breaking, the distance between the 2 downward force applicators was measured for each calf. This value was subtracted from L and then divided by 2 to determine a. On average, a had a value of 38.6 ± 0.4 mm.

Statistical Analysis

Results are reported as means \pm SEM. All reported data were analyzed using the MIXED procedure of SAS 9.4 (SAS Inst. Inc., Cary, NC). Bone marker and video data were evaluated with a model containing fixed effects of treatment and week, interaction of treatment and week, as well as repeated measures of week with calf as subject. Calf size parameters, CT data, fracture force, and data obtained from mechanical property calculations were analyzed with a model containing only the fixed effect of treatment. All data, except for stride frequency, were deemed to be normally distributed. Stride frequency was transformed as 1/y and subsequently followed a normal distribution after transformation. This transformation was selected as a box-cox 95% confidence interval performed with the TRANSREG procedure included a lambda of -1, which yields a transformation of 1/y. Significance was set at $P \leq 0.05$ and trends were observed at $P \leq 0.10$.

RESULTS

The average age of calves when entering the study was not different among treatments (Table 2). Calves started and finished the project with initial

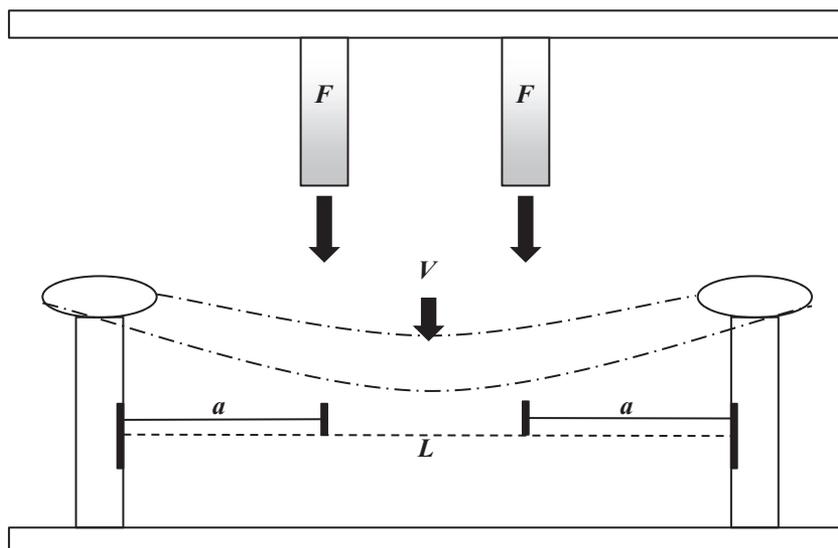


Figure 2. Diagram of a bovine fused third and fourth metacarpal subjected to 4-point bending on an Instron. Load exerted (F) on the bone is shown, as the Instron measures the bone's displacement (V). Measurements: L is span length, set at 100 mm; a is the distance between F , and the support on either end of the bone, this was measured individually for each calf and averaged 38.6 mm. Adapted from [Pruyn et al. \(2000\)](#).

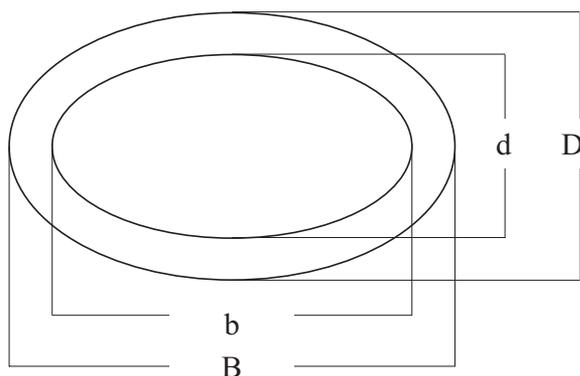


Figure 3. Cross-section of bovine fused third and fourth metacarpal illustration. Cortical diameters included: B = exterior lateromedial diameter, b = interior lateromedial diameter, D = exterior dorsopalmar diameter, d = interior dorsopalmar diameter. Adapted from [Hiney et al. \(2004a\)](#).

and final heights, weights, and lengths that were not different among exercise treatments. Average daily gain was not different among treatments either ([Table 2](#)).

Video Data

There was no treatment \times week interaction in terms of stride frequency ([Table 3](#)). There were differences in stride frequency among treatments ($P < 0.05$), with calves sprinting 5 d/week having lower inverse stride frequency, indicative of greater stride frequency. There were differences in stride frequency among weeks ($P < 0.05$), with weeks 1 and 2 having lower inverse stride frequency, indicative of greater stride frequency. There was no treatment \times week interaction in terms of mean sprinting

velocities ([Table 3](#)). However, there were differences in sprinting velocities among treatments ($P < 0.05$), with calves sprinting 5 d/week having the lowest velocity. There were differences in sprinting velocity among weeks ($P < 0.05$), with week 1 having lower velocity than weeks 3, 4, and 5, and week 2 having lower velocity than weeks 4 and 5.

Bone and Biological Markers

There was a difference among treatments in mean OC concentrations ($P < 0.001$, [Fig. 4](#)). The 5 d/week exercise group had the greatest mean OC concentration compared to all other treatments ($P < 0.05$). There were no differences among weeks or interactions between treatment \times week. For CTX-1, there were no differences among treatments or weeks, nor was there an interaction between treatment \times week ([Fig. 5](#)).

Computed Tomography and Mechanical Testing

Calves sprinted 1 d/week tended to have increased internal ML diameter at MID ([Table 4](#); $P < 0.10$). Dorsal CW at MID was greater for all exercise treatments compared to nonexercised calves ([Table 5](#); $P < 0.01$). There were no differences in cross-sectional areas or cortical areas at MID among treatments ([Table 5](#)). Likewise, there were no differences among treatments in cortical bone densities or whole-slice bone densities at MID ([Table 6](#)).

In terms of mechanical properties, no differences by treatment were found in moment of

Table 2. Calf size parameters measured at the beginning and end of the study

Treatment	Calf age at day 0	Initial ¹ height, cm	Final ² height, cm	Initial weight, kg	Final weight, kg	Initial length, cm	Final length, cm	ADG ³ , kg/d
0	62 ± 1	90 ± 1	100 ± 1	89 ± 3	115 ± 7	67 ± 1	75 ± 2	0.6 ± 0.2
1	62 ± 1	92 ± 1	101 ± 1	85 ± 3	117 ± 7	67 ± 1	75 ± 2	0.8 ± 0.2
3	63 ± 1	93 ± 1	101 ± 1	85 ± 3	119 ± 7	68 ± 1	74 ± 2	0.8 ± 0.2
5	63 ± 1	91 ± 1	101 ± 1	79 ± 3	116 ± 7	65 ± 1	75 ± 2	0.9 ± 0.2
<i>P</i> values	0.99	0.49	0.90	0.16	0.97	0.49	0.99	0.60

¹All initial measurements were taken on day -1.

²All final measurements were taken on day 41.

³ADG = Average daily gain = $\frac{\text{Final weight} - \text{initial weight}}{42d}$

Table 3. Inverse mean calf stride frequency and velocity by sprinting treatment over the 6-wk study period

Treatment	Inverse stride frequency, 1·strides ⁻¹ ·sprint ⁻¹			Velocity, m/s			
	1 d/week ^a	3 d/week ^a	5 d/week ^b	Treatment	1 d/week ^a	3 d/week ^a	5 d/week ^b
Week 1 ^a	0.07	0.05	0.07	Week 1 ^a	2.8	2.1	2.9
Week 2 ^a	0.07	0.08	0.05	Week 2 ^{ab}	3.1	3.6	1.9
Week 3 ^b	0.09	0.10	0.08	Week 3 ^{bc}	3.8	4.1	4.1
Week 4 ^b	0.12	0.10	0.08	Week 4 ^c	5.4	5.2	3.0
Week 5 ^b	0.10	0.10	0.06	Week 5 ^c	5.4	5.0	2.5
Week 6 ^{ab}	0.07	0.09	0.08	Week 6 ^{abc}	3.1	4.0	3.6
SEM	0.01	0.01	0.01	SEM	1	1	1

^{a-c}Within a row or column, main effect means differ ($P < 0.05$).

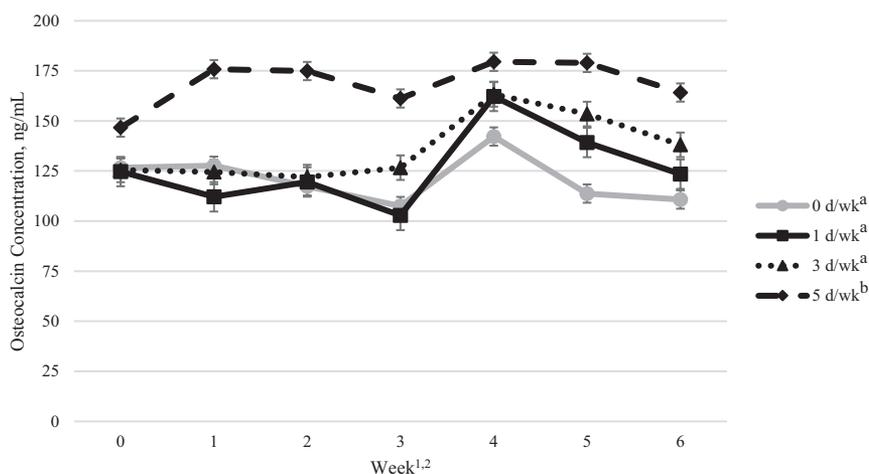


Figure 4. Mean calf serum osteocalcin concentration by treatment throughout the 6-wk study period. ¹Difference among weeks not significant ($P = 0.11$). ²Interaction between treatment and week not significant ($P = 0.99$). ^{a,b}Treatments lacking common superscripts in legend differ ($P = 0.0004$).

inertia, flexural rigidity, or Young's modulus. However, fracture force did exhibit a difference among treatments ($P = 0.01$). Calves sprinting 1, 3, or 5 d/week had increased fracture force compared to control calves as determined by 4-point bending (Table 7). All exercise treatments had similar fracture force, even just 1 d/week of sprinting had a greater fracture force of 7,940 N compared to the 6,300 N fracture force of calves sprinted 0 d/week ($P = 0.004$).

DISCUSSION

Disuse by way of exercise reduction, immobilization, or confinement has deleterious effects on bone (Snow et al., 2001; van Harreveld et al., 2002). In the absence of loading, the skeleton reverts to its genetic minimum, a reduced bone mass that can support basic function without failure (Skerry, 2008). Immobilization for 6 wk has been shown to lead to decreased BMD, ultimate load, and stiffness

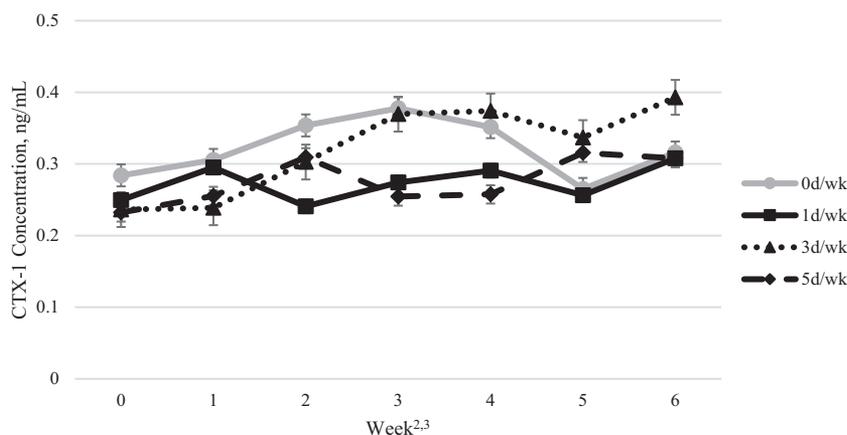


Figure 5. Mean C-terminal telopeptides of type 1 collagen (CTX-1) concentration in calf serum by treatment throughout the 6-wk study period. ¹Difference among treatments not significant ($P = 0.16$). ²Difference among weeks not significant ($P = 0.32$). ³Interaction between treatment and week not significant ($P = 0.94$).

Table 4. Internal (Int) and external (Ext) dorsopalmar (DP) and mediolateral (ML) diameters from cross-sectional views at midpoints calculated from the proximal end of the fused third and fourth metacarpal to the distal end (MID)

Treatment	MID ML Int, mm	MID ML Ext, mm	MID DP Int, mm	MID DP Ext, mm
0	17.4 ^x	26.8	11.9	20.3
1	18.9 ^y	28.2	11.6	20.5
3	17.0 ^x	27.3	11.0	20.7
5	17.3 ^x	27.3	11.2	20.3
SEM	0.5	0.7	0.5	0.4
<i>P</i> -values	0.06	0.48	0.53	0.89

^{x,y}Values lacking common superscripts tend to differ ($P < 0.10$).

Table 5. Cross-sectional cortical widths (CW), cross-sectional cortical areas, and cortical areas measured at midpoints calculated from the proximal end of the fused third and fourth metacarpal to the distal end (MID)

Treatment	Dorsal CW, mm	Palmar CW, mm	Lateral CW, mm	Medial CW, mm	MID cross-sectional area, mm ²	MID cortical area, mm ²
0	4.3 ^a	4.1	4.5	4.9	437	288
1	4.9 ^b	3.9	4.9	4.7	465	309
3	5.5 ^b	4.2	4.9	5.3	451	320
5	4.9 ^b	4.1	5.0	5.1	453	318
SEM	0.2	0.2	0.2	0.2	20	15
<i>P</i> -value	0.006	0.67	0.16	0.24	0.80	0.43

^{a,b}Values lacking common superscripts differ ($P < 0.01$).

in rats (Inman et al., 1999). Calves subjected to stall confinement for 6 wk in this study did not experience alterations to BMD, but ultimate load (fracture force) determined through 4-point bending was negatively impacted as a result of confinement. All 3 exercise treatments experienced a 23% increase in fracture force compared to the nonsprinted calves, with just 1 d/week of sprinting leading to a 26% increase in fracture force compared to calves sprinted 0 d/week. When calves were sprinted 50 m 5 d/week, fracture force tended to be greater in sprinted animals compared to confined animals (Hiney et al.,

2004a). Differences between studies, such as mechanical testing method used and length of sprints, may be the cause of differing results in terms of fracture force. In the current study, mechanical testing was performed through 4-point bending, while 3-point bending was utilized by Hiney et al. (2004a). It has been noted in polymer testing that differences are present in mechanical properties between 3-point and 4-point bending of the same specimen (Mujika, 2006). This phenomenon, coupled with the fact that sprint lengths were 50-m in the initial study and 71-m in the current study may explain

Table 6. Cortical and whole-slice bone densities from a cross-sectional view at a midpoint calculated from the proximal end of the fused third and fourth metacarpal and the distal end (MID)

Treatment	Whole-slice, mg mineral/cm ³	Dorsal, mg mineral/cm ³	Lateral, mg mineral/cm ³	Medial, mg mineral/cm ³	Palmar, mg mineral/cm ³
0	996	1,210	1,220	1,230	1,060
1	997	1,130	1,250	1,240	1,040
3	987	1,210	1,220	1,210	1,070
5	982	1,220	1,220	1,210	1,050
SEM	13	28	20	17	18
<i>P</i> -value	0.82	0.13	0.57	0.55	0.78

Table 7. Fracture force and calculated mechanical properties of the fused third and fourth metacarpal of calves separated by treatments

Treatment	Moment of inertia, mm ⁴	Flexural rigidity, × 10 ⁷ Nmm ²	Young's modulus, N·mm ⁻²	Fracture force, <i>N</i>
0	9,660	2.3	2,530	6,300 ^a
1	10,400	2.6	2,490	7,940 ^b
3	10,800	2.4	2,210	7,850 ^b
5	10,200	3.0	3,220	7,550 ^b
SEM	813	0.5	543	358
<i>P</i> -value	0.81	0.78	0.61	0.01

^{a,b}Values lacking common superscripts differ ($P = 0.01$).

why only a trend was noted in the initial study, but a treatment difference was present in fracture force in the present study.

Similar to Hiney et al. (2004a), the exercised calves in this study had greater terminal dorsal CW compared to the confined group. As mentioned in the results, confined calves had similar BMD as exercised calves. However, the calves exercised 1, 3, and 5 d/week all had increased dorsal CW, suggesting that they had more physical bone of similar BMD than the confined calves. Greater dorsal CW in exercised calves may have contributed to the greater fracture force of exercised calves; force during 4-point bending was applied to the palmar cortex with the dorsal cortex facing the bottom supports of the Instron.

Lack of differences in external and internal lateromedial and dorsopalmar diameters lead to a lack of differences in moment of inertia. As Young's modulus of elasticity is calculated based on moment of inertia and flexural rigidity, no differences among treatments could be expected. Absence of differences in flexural rigidity may be caused by the lack of differences in area of cortical bone between treatments. While dorsal CW at MID was greater for sprinted treatments, cross-sectional and cortical area at MID were not different. Values for cross-sectional and cortical area were far greater than values for dorsal CW, subsequently, increased dorsal CW in exercised calves was not great enough to lead to changes in area. In future studies, a change of bone morphology from baseline could

better detect responses to exercise. Dynamic strains to bone, such as those from sprinting, are known to lead to bone formation (Allen and Burr, 2014). Unfortunately, formation of bone by osteoblasts can take months, while resorption of bone by osteoclasts can occur in the time span of a few days to 2 wk (Stover, 2003). For this reason, differences in area and diameter of the cortical bone may not have yet been detectable at the end of this 6-wk study but may have been detectable if the study period spanned a few months.

Bone morphology was found to be affected when calves were sprinted 50 m 5 d/week, leading to the overall effect of sprinted calves having a smaller medullary cavity and larger cortical bone area than confined counterparts (Hiney et al., 2004a). In this original calf exercise study, confined calves were kept in tie-stalls in which they could stand up and lie down only. In the current study, calves were maintained in stalls which afforded room to stand up and lie down as well as turn-around 360°. Additional loading opportunities afforded to the calves in the current study may explain why minimal differences in bone morphology were detected. In retrospect, video analysis of calf behavior or use of pedometers could depict how often calves moved in their stalls. The additional bone strength and dorsal cortical width attained in sprinted calves in this study should not be discounted, as all calves had equal opportunities for movement and loading in their stalls.

Removing animals from pasture and confining them to stalls does have an influence on markers of bone formation and bone degradation (Hoekstra et al., 1999). A difference among treatments was evident in OC concentration—calves exercised 5 d/week had greater OC concentration compared to all other treatments. In calves sprinted 5 d/week, greater OC concentration suggests greater osteoblastic activity and therefore greater bone formation (Lee et al., 2000). Greater OC in calves sprinted 5 d/week may be a result of the greater distance covered during the study by calves exercised 5 d/week. Calves exercised 5 d/week sprinted 2,130 m over the 6-wk study while calves exercised 1 d/week sprinted 426 m during the same period. Osteocalcin reflects systematic bone formation (Lee et al., 2000). There is a potential that bone formation occurred in other locations beyond the MC III & IV and differed among treatments. The number of sprints per week did not differ in influence on dorsal CW or fracture force of the MC III & IV, suggesting that OC could be affected by loading cycles while bone size and strength are affected by presence of sprints. This difference may also be an artifact of comparison between treatments as, while there were no treatment differences at day 0, the 5 d/week treatment also did not increase in OC over the course of the study. Calves sprinted 50 m 5 d/week also experienced greater OC concentration compared to confined animals (Hiney et al., 2004a). Concentration of OC has been shown to increase in horses in response to simulated race training on a treadmill composed of 2 min of trotting, 2 min of galloping, then 2 more minutes of trotting 5 d/week (Frisbie et al., 2008).

Lack of differences in overall CTX-1 concentrations between treatments are not surprising, as calves were housed individually in small stalls and not afforded exercise before the study began, beyond the 2 short walking sessions for halter training. Calves sprinted 0 d/week did not experience changes to their normal activity once the study started, as the stimuli their skeleton was accustomed to had not changed as a result of study initiation. The lack of a difference in CTX-1 concentration among weeks suggests that initiation of exercise did not lead to increased bone resorption, nor did maintenance in stalls. In a similar study, serum deoxypyridinoline, another marker of bone resorption, did not differ among treatments (Hiney et al., 2004a). If the calves in this study were maintained in pastures or in large group-housed pens before being housed in confinement during the study, then there may have been evident changes

to CTX-1 concentration in the calves sprinted 0 d/week. The minimal differences in CTX-1 concentration among treatments are reasonable given the lack of changes between prestudy housing and confinement during the study. The calves in this study were all at a juvenile age of 9 wk, at which point skeletal growth was occurring and would continue to occur if calves were maintained in calf-hutches of similar size to the stalls used in this study, as is normal industry practice. Any potential differences among treatments or weeks were most likely masked by the skeletal growth endured by all calves on this study. Concentration of CTX-1 was not expected to differ among treatments as a result of sprint exercise, or lack thereof.

While calves were sprinted on a hard-concrete surface for this study, young horses in training are typically exercised on a dirt or turf track. Calves sprint at slower speeds than horses do, with Quarter Horses peaking at 24 m/s, Thoroughbreds at 18 m/s, and calves sprinting an average of 3.6 m/s in this study. Strides at speed are relative to the species of interest. Swiss heifers have been found to walk normally on a treadmill at an average speed of 1.33–1.40 m/s (Meyer et al., 2007). While traveling at 3.6 m/s may not be a sprint for horses, a velocity of 3.6 m/s is fast in terms of the normal traveling speed of a calf and results in dynamic loading which yields a skeletal response. The slower sprinting speed of calves compared to horses, and short distance of the sprints, justifies the use of a concrete surface to obtain dynamic strains similar to those experienced by a racehorse. It has been demonstrated by Hiney et al. (2004a,b) that calves sprinted on concrete and weanling horses sprinted on grass yield similar changes to cortical bone morphology and bone markers of resorption and formation. Video analysis confirmed that calves sprinted 1 and 3 d/week sprinted at similar speeds. Response of bone in terms of CW and fracture force was the same for all sprinted treatments, even though calves sprinted 5 d/week did not sprint as fast as calves sprinted 1 and 3 d/week. Calves sprinted 5 d/week also took more strides during each sprint, as a result of the lower sprint velocity. As mentioned above, distance covered may have had an impact on concentration of OC, but presence of strides at speed, not quantity of strides at speed, seems to have a notable influence on bone health via increased cortical bone width and ultimate strength.

It is important to note that calves may have underwent a gradual increase to speed at the beginning of the study, as sprint exercise was not incorporated into their management until day 0. At the

beginning of the study, calves were not oriented to the performance of sprints nor to the direction of travel. Faster velocities towards the end of the 6-wk study period suggest that calves had acclimated to sprint exercise by week 3. Stride frequency was greater during the first 2 wk of the study, suggesting that calves took more strides at the beginning of the study while they were traveling at slower velocities. After week 3, calves took fewer strides to complete a sprint and completed their sprints at faster velocities. Calves increased in height and length during the 6-wk study, potentially attributing to the increase in sprint velocity and decrease in stride frequency. Familiarity to the sprint exercise as the study progressed likely attributed to the changes in sprint velocity and stride frequency also. Few cycles of dynamic loading are needed to produce a stimulus which leads to the achievement and maintenance of bone mass. In roosters, as few as 4 and 36 cycles of loading were needed to maintain or increase long bone mineral content, respectively (Rubin and Lanyon, 1984).

The value of dynamic loading has been recognized in other industries beyond the horse industry. Egg-laying hens are known to have increased bone resorption during periods of egg laying. Normally, medullary bone is mobilized during egg laying as a labile source of calcium for shell formation, but resorption of cortical bone during egg laying puts birds at high risk for fracture. This is especially prevalent in domesticated egg-laying hens as they lay year-round, unlike wild hens (Whitehead, 2004). Intensive production coupled with minimal opportunities for load-bearing exercise in conventional cages leads to a high incidence of osteoporosis and fractures. Studies evaluating management styles have determined that housing which allows hens and pullets opportunities for load-bearing and wing loading lead to better bone health compared to birds housed in conventional cages (Jendral et al., 2008; Regmi et al., 2015).

It is common practice in the horse industry that young horses are removed from pasture and kept in stalls during race training. While young horses are in the initial stages of race training, they undergo walking, trotting, and cantering before any speed is added to their exercise regimen. This schedule of slow exercise, coupled with the loss of free-exercise from pasture leads to decreased bone mineral content, and presumably strength (Nielsen et al., 1997; Hoekstra et al., 1999). The addition of dynamic loads to confinement is crucial in counteracting the loss of bone strength (Hiney et al., 2004a,b). Sprinting young horses short distances 1, 3, or 5 d/week, as done with

the calves in this study, should lead to a subsequent increase in strength of the MC III & IV, potentially reducing the risk of catastrophic injury during their racing career.

CONCLUSION

Collectively, the results of this study support that animals sprinted the short distance of 71-m 1, 3, or 5 d/week attained heightened dorsal CW and fracture force needed to bend bone to failure. Sprint exercise also influences bone formation evidenced by the fact that calves sprinted 5 d/week had greater OC concentration. Calves sprinted 1 d/week exhibited a 26% increase to fracture force compared to calves confined without sprinting. Over the 6-wk study, calves assigned to sprinting 1 d/week only sprinted 426 m. This demonstrates the very few strides at speed needed to increase bone health, and that lack of dynamic loading for just 6-wk leads to deleterious effects on skeletal strength. From an implementation stand-point, sprint-exercising young animals 1 d/week for 6 wk while young requires little extraneous time and funds of the owner while increasing the physical welfare of the young animal and potentially reducing the risk of a musculoskeletal injury during training and racing. Further research in this topic is needed to determine if sprinting animals at least 1 d/week at a young age can maintain heightened bone strength into maturity.

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