Effects of stacked wedge pads and chains applied to the forefeet of Tennessee Walking Horses for a five-day period on behavioral and biochemical indicators of pain, stress, and inflammation

James B. Everett DVM
Jim Schumacher DVM, MS
Thomas J. Doherty MVB, MSc
Randi A. Black PhD
Lisa L. Amelse MS
Peter Krawzel PhD
Johann F. Coetzee BVSc, PhD
Brian K. Whitlock DVM, PhD

Received March 7, 2017.
Accepted July 26, 2017.

From the Departments of Large Animal Clinical Sciences (Everett, Schumacher, Doherty, Amelse, Whitlock) and Animal Science (Black, Krawzel), College of Veterinary Medicine, University of Tennessee, Knoxville, TN 37996; and the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University, Ames, IA 50011 (Coetzee). Dr. Coetzee's present address is Department of Anatomy and Physiology, College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506.

Address correspondence to Dr. Schumacher (jschumac@utk.edu).

OBJECTIVE
To determine the effects of stacked wedge pads and chains applied to the forefeet of Tennessee Walking Horses on behavioral and biochemical indicators of pain, stress, and inflamation.

ANIMALS
20 Tennessee Walking Horses.

PROCEDURES
Horses were randomly assigned to 2 treatment groups: keg shoes (control; n = 10) or stacked wedge pads and exercise with chains (10). Ten days before treatment application, an accelerometer was attached at the left metatarsus of each horse to record daily activity. Horses were exercised for 20 minutes daily, beginning on day –7. On day 0, exercise ceased, the forefeet were trimmed, and the assigned treatment was applied. From days 1 through 5, horses were exercised as before. Blood samples for measurement of plasma cortisol, substance P, and fibrinogen concentrations were collected on days –5, 1, and 5 before and after exercise and every 30 minutes thereafter for 6 hours.

RESULTS
No significant differences in plasma concentrations of cortisol, substance P, and fibrinogen were detected between groups. Although lying behaviors changed after shoes were applied, these behaviors did not differ significantly between groups. Shoeing appeared to have altered behavior to a greater extent than did the type of treatment applied.

CONCLUSIONS AND CLINICAL RELEVANCE
Application of stacked wedge pads and chains to the forefeet of horses for a 5-day period as performed in this study evoked no acute or subacute stress or nociceptive response as measured. Although these findings should not be extrapolated to the long-term use of such devices in Tennessee Walking Horses performing the running walk, the data should be considered when making evidence-based decisions relating to animal welfare and the use of stacked wedge pads and chains. (*Am J Vet Res* 2018;79:21–32)

Tennessee Walking Horses have 3 distinct natural gaits: the flat walk, running walk, and canter.[1] The flat walk is a brisk, long-reaching walk at a pace of 4 to 8 miles/h (1.8 to 3.6 m/s). The running walk is an exaggerated and faster version of the flat walk at a pace of 10 to 20 miles/h (4.5 to 9 m/s). The flat and running walks are 4-beat gaits in which each of the horse's feet strike the ground separately, at regular intervals. Overstride, an important component of the gait of a Tennessee Walking Horse, is characterized by the horse gliding over the track left by a forefoot with the ipsilateral hind foot. The overstride and 4-beat gait provide a smooth, comfortable ride.

Tennessee Walking Horses are trained and perform in shows with shoes composed of stacked wedge pads (so-called performance packages) attached to the soles of the forefeet and maintained in place with a metal band encircling the hoof.[1] This type of shoe is applied to accentuate the horse's natural gait by encouraging exaggerated elevation of the forelimbs and overstriding of the hind limbs.[2,3] Chains, a type of so-called action device, are commonly applied to the pastern region of the forefeet of these horses to enhance the exaggerated movement of the forelimbs brought about by the stacked wedge pads. The weight of the chains applied to the pastern region has been accredited with contributing to the exaggerated movement of the forelimbs of the Tennessee Walking Horse, but attachment of a light-weight bracelet to the pastern region of the forelimbs and hind limbs can induce a higher flight arc of the hoof by providing tactile stimulation to the area.[4] The change in flight arc

of the forefeet induced by chains may, therefore, be the result of tactile stimulation rather than the weight provided by the chains.

In horses, wedges are commonly used to elevate the heels for therapeutic reasons, such as treatment for navicular disease, but not often to the degree in which the heels are elevated in Tennessee Walking Horses.[5] Severe alterations in joint angles and landing, bearing, and breakover periods induced by elevating the heels with stacked wedge pads could induce inflammation of altered structures if horses are exercised while shod with the pads.

Use of stacked wedge pads and chains in the training Tennessee Walking Horses used for show is legal and within the limits set by the USDA through the Horse Protection Act of 1970.[6] However, use of these training devices to accentuate gait incites criticism from some members of the public, who presume that the use of stacked wedge pads and chains causes stress and pain in horses.[7] In addition, the US Equestrian Federation, which is the national organization governing all equestrian sports in the United States, disallows the use of stacked wedge pads and chains in the show ring for all recognized national breed affiliates.[8] This ban on the use of stacked wedge pads and chains is supported by the AVMA and the American Association of Equine Practitioners.[2,3] To our knowledge, no scientific evidence exists to suggest that the legal use of these instruments induces pain or stress in horses.

Stress can be defined as "the state of homeostatic imbalance induced by any physical or psychological stressor, involving physiological (neural and endocrine) and behavioral responses that tend to re-establish the homeostasis."[9,10] Pain can be defined as "an aversive feeling or sensation associated with actual or potential tissue damage and resulting in physiologic, neuroendocrine, and behavioral changes that indicate a 'stress' response."[11–13]

Pain and stress are unpleasant sensory and emotional experiences that evoke physiologic and behavioral changes to maintain homeostasis.[14,15] To measure pain and stress objectively in veterinary species, physiologic and behavioral changes must be assessed. Adrenal glucocorticoids, such as cortisol, defend against threats to homeostasis.[16] In horses, plasma cortisol concentration is correlated with pain and stress[1–20] and increases during transport,[21] surgery,[22] application of a nose twitch,[23] or isolation.[24] Substance P, a neurokinin, is released in the spinal cord in proportion to the intensity and frequency of noxious or aversive stimulation.[25] This peptide lowers the threshold for activation of neural C fibers and may directly activate them. Evidence suggests that substance P is also an integral part of pathways in the CNS involved in psychological stress.[26] Plasma concentrations of acute phase proteins, such as fibrinogen, increase in response to inflammation of infectious and noninfectious sources, surgical trauma, or stress.[27] Fibrinogen is involved in homeostasis, providing a substrate for formation of fibrin, and in tissue repair, providing a matrix for the migration of cells involved in inflammation.[28]

The physiologic changes induced by pain or stress, however, do not always directly correlate with the degree of pain or stress, and this lack of a direct relationship has prompted a shift among investigators of animal welfare toward reliance on behavioral changes, such as alterations in stance, posture, gait, and movement, for detection of pain or stress in veterinary species.[29–31] Such detection in horses through observation of behavioral changes is particularly difficult because horses generally avoid obvious displays of pain, presumably to decrease the likelihood of predation.[32]

The lying behavior of horses has been used to establish the welfare implications of housing horses in groups on wood shavings or rubber mats with shavings, in a shelter with sand, or in a sand paddock.[33] Lying time has been used to assess stress induced by housing horses in tie stalls, box stalls, or groups.[34] Results of these studies[33,34] indicate that behavioral changes can be used to assess the stress or pain induced in horses by various managerial strategies.

The purpose of the study reported here was to examine the effects of stacked wedge pads and chains used to train Tennessee Walking Horses on horse behavior and biochemical indicators of stress, pain, and inflammation. The overall objective was to help determine whether these training devices, when used in a legal manner,[6] could cause stress or pain to horses. Specific objectives were to compare behaviors (ie, daily number of steps taken, number and duration of lying bouts, and lying time) and plasma concentrations of chemical indicators of stress, pain, and inflammation (ie, cortisol, substance P, and fibrinogen) before and after horses were shod and exercised with stacked wedge pads and chains and to compare these behaviors and plasma biochemical concentrations between horses shod and exercised with these training devices and horses shod and exercised with keg shoes.

Materials and Methods

Animals

Twenty Tennessee Walking Horse geldings with an age range of 3 to 18 years (mean ± SD, 9.25 ± 4.40 years) were selected for this study. All horses were privately owned, and each owner consented to the use of his or her horse in the study. Six of the 20 horses had been maintained at pasture for at least the preceding year, but during that time had been intermittently allowed access to a stall. The other 14 had been kept entirely at pasture for at least the preceding year. Before the study began, all horses had been judged as sound (ie, without clinically apparent lameness) by a member of the research team (JBE) through observation of the horses at a flat walk on firm ground.

On the first day of the study (day –10), horses were randomly assigned by drawing of cards to 2

groups: control (shod with keg shoes; n = 10; mean ± SD age, 9.50 ± 3.87 years) or treatment (shod with stacked wedge pads and exercised with chains; 10; mean age, 9.00 ± 5.08 years). All 20 horses were introduced into the study unshod or shod with keg shoes. Thirteen had never been trained with stacked wedge pads and chains, and the other 7 had neither been shod with stacked wedge pads nor ridden with chains for at least 1 year. The 7 horses that had been previously trained were distributed as evenly as possible between the treatment and control groups (ie, 3 in the treatment group and 4 in the control group).

All horses were housed in the same barn, in individual stalls and on wood shavings. Eighteen horses were housed in 3.2 × 3.8-m stalls (12.2 m^2), and because of insufficient stalls of this size, 2 horses (1 assigned to the control group and 1 assigned to the treatment group) were housed individually in a 3.6 × 7.3-m stall (26.3 m^2). Each horse could see horses in adjacent stalls between widely spaced vertical metal bars atop horizontal wooden planks used to partition the stalls. Horses were provided free access to grass, hay, and water. No grain was provided during the study. The study protocol was approved by the University of Tennessee's Institutional Animal Care and Use Committee (protocol No. 2268-0514).

Study protocol

On day –10, an accelerometer[a] was attached to the distal aspect of the left metatarsus of each horse by means of a flexible plastic strap provided by the manufacturer **(Figure 1)**. This device recorded on a daily basis the lying time (h/d), standing time (h/d), number of lying bouts, duration of lying bouts (min/bout), and number of steps taken. Between days –10 and –6, horses were allowed to acclimate to the study conditions. Rectal temperature, heart and respiratory rates, and digital pulses were evaluated once daily throughout the study,

Beginning on day –7 and, except for day 0, continuing until study completion on day 5, horses were exercised daily for 20 minutes at a flat walk (1.21 m/s) on firm ground by use of a horse walker apparatus. Horses were separated into 5 exercise groups for this purpose, each composed of 4 horses (2 treated horses and 2 control horses) because the walker apparatus was able to accommodate only 4 horses at a time. The groups were exercised daily in the same order and at the same time of day. Horses were evaluated for lameness throughout the study, and before, during, and after exercise, by 2 members of the research team (JBE and JS).

On day –6, the area over a jugular vein in each horse was desensitized by SC administration of 2% mepivacaine hydrochloride solution, and a 14-gauge, 9-cm catheter[b] was inserted into the vein. The catheter was attached to a short extension set,[c] which was attached to the horse's neck with a suture. The catheters and extension sets were then flushed with heparinized saline (0.9% NaCl) solution (20 U of heparin/mL).

Pretreatment period—On day –5, a blood sample (20 mL) for measurement of plasma cortisol, substance P, and fibrinogen concentrations was collected from each horse via the jugular catheter into a syringe immediately before and after the horses were exercised and then every 30 minutes for 6 hours (referred to as the 6-hour intense sample collection period). Before each sample collection, 10 mL of fluid was removed from the catheter and extension, and after each collection, the catheter and extension set were flushed with 5 mL of heparinized isotonic saline solution. Collected blood samples for measurement of plasma cortisol and fibrinogen concentration were injected into tubes containing lithium heparin, and those for measurement of plasma substance P concentration were placed into tubes containing protease inhibitors to prevent degradation of substance P during collection and processing. All plasma was harvested by centrifugation at 1,500 × *g* for 10 minutes and stored at –80°C pending analyses. Catheters were removed on day –5 after the last blood sample was collected.

Acute period—On day 0, the forefeet of each horse assigned to the control group were shod with keg shoes[d] (ie, traditional steel shoes) that weighed between 227 and 369 g (weight depended on the size of the foot to which they were applied), were 0.8-cm thick, and had 1.9-cm-wide branches and heel caulks. The forefeet of each horse assigned to the treatment group were trimmed and shod with stacked wedge

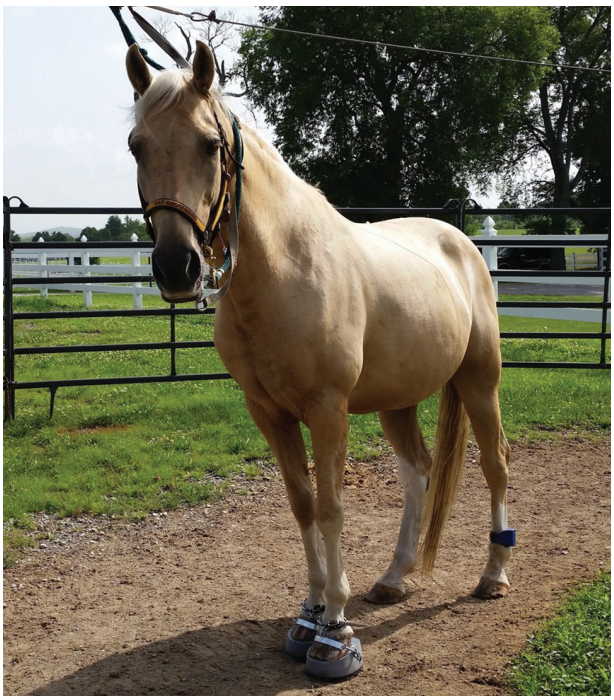

Figure 1—Photograph of a Tennessee Walking Horse tethered to a horse walker apparatus and equipped with stacked wedge shoes on the forefeet, chains on the pastern region of the forelimbs, and an accelerometer attached to the distal aspect of the left metatarsus.

pads in accordance with regulations established in the Horse Protection Act of 1970.[6] The stacked wedge pads were custom-made by a farrier for each horse and fitted according to the size and shape of the hoof. The wedges were composed of plastic, and the bearing surface of the shoe was composed of a 2.5-cm-thick rubber pad.[e] The total weight of the wedge constructs (approx 1.9 kg) varied slightly according to the size of the foot, and the heel elevation achieved (12°) was within the typical elevation used for Tennessee Walking Horses in training. Stacked wedge pads were anchored loosely to the foot by a metal band encircling the hoof.[6] A catheter was inserted into the jugular vein contralateral to the one catheterized on day −6 and connected to an extension set, as described for day −6. Horses were not exercised on this day.

The next day (day 1), the exercise regimen was reinstituted and continued until day 5. For horses in the treatment group only, the hoof bands were tightened immediately before exercise and loosened immediately after exercise. In addition, a 6-oz, flat-linked chain was attached around the pastern region of each forelimb with a plastic strap (Figure 1) so that the chain encircled the region in a manner that allowed it to rotate around or up and down the region. Blood samples were collected via a jugular catheter and processed as described for day −5, and catheters were subsequently removed. No blood samples were collected on days 2 through 4.

Subacute period—On day 4 after exercise, a jugular catheter was inserted and connected to an extension set as previously described, and horses were confined to their stalls. On day 5, blood samples were collected via an IV catheter and processed as previously described. Jugular catheters and accelerometers were removed after the last blood sample was collected.

On day 6, stacked wedge pads were removed from horses in the treatment group. Horses shod with keg shoes retained those shoes.

Biochemical responses

Cortisol—Cortisol concentration was measured in each plasma sample as described elsewhere.[35] To validate the immunoassay, cortisol was extracted from pooled equine plasma by use of diethyl ether. Blank plasma was fortified with 5 concentrations of cortisol (obtained from a stock solution of 0.5 g/dL) that spanned the expected analytic range of the assay. Each spiked sample was then analyzed in triplicate. The coefficient of variation for triplicate samples at each spiked concentration was < 15%. The linear regression line for the 3 points at each of the 5 concentrations had an R^2 value of 0.99.

Substance P—For measurement of plasma substance P concentration, 7 mL of blood was collected into K_3-EDTA tubes containing the protease inhibitor benzamidine hydrochloride.[f] To prepare these tubes, 20mM solution of benzamidine was prepared in water, and 300 µL was added to each tube for a final blood benzamidine concentration of 1mM to serve as a protease inhibitor. Tubes were stored on ice for no more than 30 minutes after blood samples were collected before centrifugation at 1,500 × *g* for 10 minutes at 4°C. Plasma was harvested by use of 3-mL transfer pipettes, stored in 2-mL cryogenic vials, and frozen with dry ice until transported to a laboratory, where the vials were stored at −80°C until analyzed as described elswhere.[12]

Fibrinogen—Fibrinogen concentration was measured in 1-mL portions of the obtained plasma samples by use of the heat precipitation method and a refractometer, as described elswhere.[36]

Behavioral responses

Behavioral data were extracted from the accelerometer by use of software[g] and a desktop reader[h] provided by the manufacturer. Data concerning lying bouts < 2 minutes in duration were removed from the final data set.[37] Data obtained on the days the accelerometers were applied (day −10) and removed (day 6) were not analyzed.

Statistical analysis

The observational and experimental unit of the study for plasma biochemical concentrations was the horse. Cortisol data were analyzed by means of mixed-effects linear regression,[i] in which treatment, day, time, and their interactions were considered explanatory variables. Exercise group, horse within treatment by group, and day by horse within treatment by group were considered random variables. Cortisol data were repeated by time, with day by horse within treatment by group as the subject and an autoregressive covariate structure. Fibrinogen and substance P data were analyzed by means of generalized linear mixed modeling,[j] in which treatment, day, time within day, and their interactions were considered explanatory variables. Exercise group, horse within treatment by group, and day by horse within treatment by group were considered random variables. Fibrinogen and substance P data were repeated by time, with day by horse within treatment by group as the subject and an autoregressive covariate structure.

The observational and experimental unit of the study for behavioral data was also the horse. Mixed linear regression[i] was used to determine the effects of the explanatory variables treatment, day, and treatment by day on daily lying time, number of lying bouts, duration of lying bouts, and number of steps taken. Group and horse within group by treatment were considered random variables, and behavior data were repeated by day, with horse within group by treatment as the subject and an autoregressive covariate structure. For all analyses, normality of data distribution was assessed.[k] Results of all analyses were considered significant at $P < 0.05$. Values are reported as mean ± SE.

Results

Animals

All horses remained free of lameless throughout the study, and no horse had signs of discomfort after shoes were applied, as determined by subjective evaluation. Rectal temperature, heart and respiratory rates, and digital pulses were within reference ranges before and after shoe application. No loss of hair or signs of dermatitis were detected over the pastern region in any horse, nor were any changes in appetite noted for the duration of the study. Samples were successfully collected from all horses at all points.

Biochemical responses

Cortisol—Mean ± SE plasma cortisol concentration for all 3 measurement days combined (–5, 1, and 5) was 37.6 ± 2.7 ng/mL for all control horses (shod with keg shoes) and 40.7 ± 3.2 ng/mL for all treated horses (shod with stacked wedge pads and exercised with chains). No effect on plasma cortisol concentration was identified for treatment ($P = 0.43$), treatment by day interaction ($P = 0.25$), treatment by time interaction ($P = 0.14$), day by time interaction ($P = 0.14$), or day by treatment by time interaction ($P = 0.81$), but there was an effect of day ($P < 0.001$) and time ($P < 0.001$). Mean values for samples obtained during the 6-hour intense collection periods on day –5 (pretreatment period), day 1 (acute period), and day 5 (subacute period) were 46.2 ± 2.2 ng/mL, 40.8 ± 2.2 ng/mL, and 30.6 ± 2.2 ng/mL, respectively. These concentrations differed significantly ($P < 0.01$ for all comparisons) among days. Mean plasma cortisol concentration for all horses (treatment and control) for all measurement days combined was greatest ($P < 0.05$) 330 minutes after exercise **(Figure 2)**.

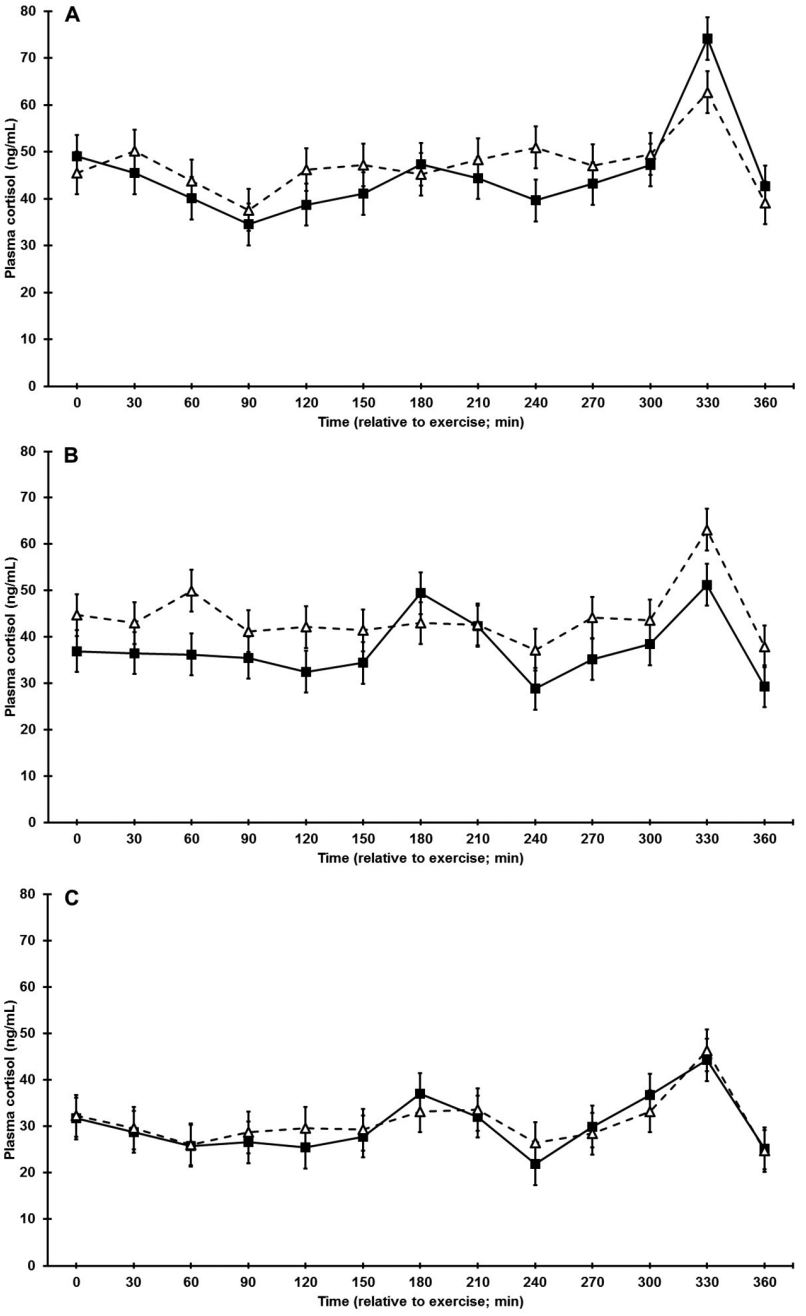

Figure 2—Mean cortisol concentrations in plasma samples obtained on days –5 (A), 1 (B), and 5 (C) from 10 horses shod on the forelimbs with keg shoes (control group; n = 130 samples; squares) or shod on the forelimbs with stacked wedge pads and exercised with chains (treated group; 130 samples; triangles) on day 0. Time immediately before exercise was designated as 0 minutes. Exercise sessions (20 min of flat walking/d) began on day –7 and, except for day 0, continued until study completion on day 5. Error bars represent pooled SEM. No effect (ie, $P \geq 0.05$) on plasma cortisol concentration was identified for treatment, treatment by day interaction, treatment by time interaction, day by time interaction, or day by treatment by time interaction, but there was a significant effect of day ($P < 0.001$) and time ($P < 0.001$). Mean plasma cortisol concentrations during the 6-hour intense sample collection period differed significantly ($P < 0.01$ for all comparisons) among days. Concentrations were greatest ($P < 0.05$ for all comparisons) 330 minutes after exercise, compared with other measurement points.

Substance P—Mean plasma substance P concentration for all 3 measurement days combined was 91.8 ± 6.9 pg/mL for all control horses and 74.4 ± 6.9 pg/mL for all treated horses. No effect on plasma substance P concentration was identified for treatment ($P = 0.11$), day ($P = 0.34$), treatment by day interaction ($P = 0.97$), time within day ($P = 0.24$), or treatment by time within day ($P = 0.68$; **Figure 3**).

Fibrinogen—Mean plasma fibrinogen concentration for all 3 measurement days combined was 281.9 mg/dL for all control horses and 216.0 ± 29.1 mg/dL

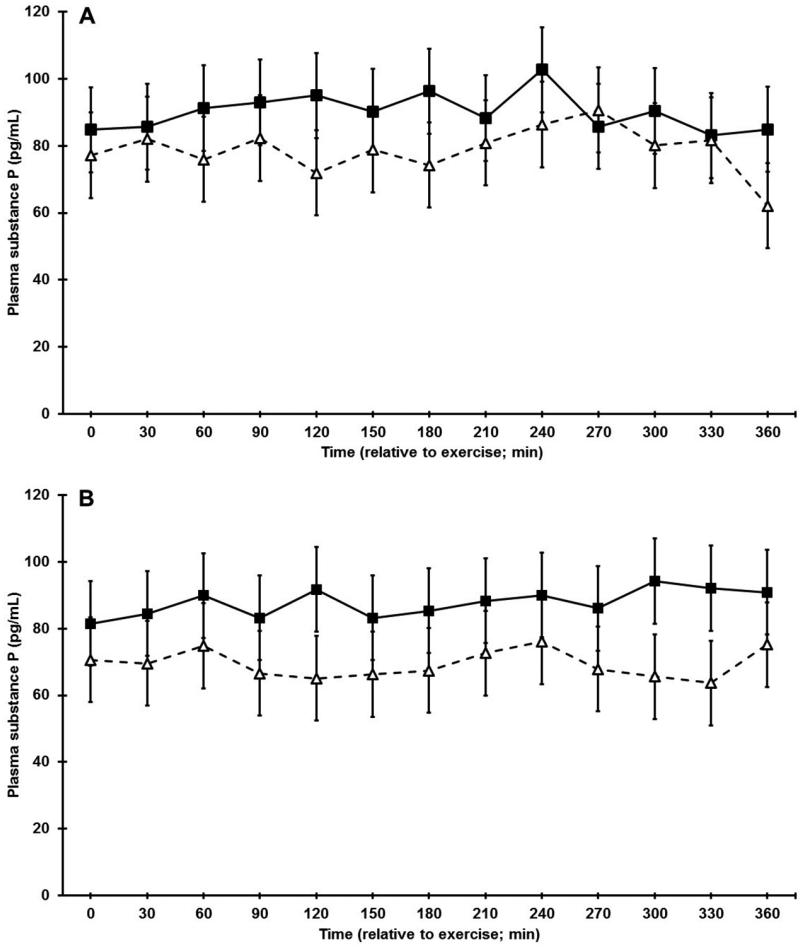

Figure 3—Mean substance P concentrations in plasma samples obtained on days 1 (A; n = 130 samples) and 5 (B; 130 samples) from the horses in Figure 2. No effect on plasma substance P concentration was identified for treatment, day, treatment by day interaction, time within day, or treatment by time within day. ***See*** Figure 2 for remainder of key.

for all treated horses. No effect on plasma fibrinogen concentration was identified for treatment (P = 0.15) or treatment by day interaction (P = 0.31), but there was an effect of day (P = 0.009), time within day (P = 0.003), and treatment by time within day (P = 0.05). Mean values for samples obtained during the 6-hour intense collection period on all 3 days combined were 286.1 ± 34.0 mg/dL at 0 hours (immediately) after exercise), 211.1 ± 34.0 mg/dL at 0.5 hours after exercise, and 207.2 ± 34.0 mg/dL at 6 hours after exercise. Mean plasma fibrinogen concentration in both treated and control horses was greatest (P < 0.05 for all comparisons) on day –5. No difference in plasma fibrinogen concentration was identified between treated and control horses on day –5, but on day 1, the least squares mean at 0.5 hours after exercise was greater (P = 0.03) for treated horses (340.0 ± 48.5 mg/dL) than for control horses (186.7 ± 48.5 mg/dL); conversely, on day 5, the least squares mean at 0 hours after exercise was greater (P = 0.04) for control horses (236.7 ± 48.5 mg/dL) than for treated horses (93.3 ± 48.5 mg/dL; **Figure 4**).

Behavioral responses

Mean ± SE daily number of steps taken was 7,218 ± 697 for control horses and 8,126 ± 697 for treated horses. No effect on daily number of steps taken was identified for treatment (P = 0.37), exercise group (P = 0.12), or treatment by day interaction (P = 0.30), but there was a significant (P < 0.001) effect of day **(Figure 5)**. These mean values were greatest for both treated and control horses on day –7 (first day of exercise; 10,469 ± 593 steps taken; P < 0.05 for all comparisons) and least on day 0 (ie, the day the horses were shod and not exercised; 3,566 ± 593 steps taken; P < 0.05 for all comparisons).

Mean daily lying time was 2.26 ± 0.26 hours for control horses and 2.03 ± 0.26 hours for treated horses. No effect on daily lying time was identified for treatment (P = 0.53), exercise group (P = 0.12), or treatment by day interaction (P = 0.61), but there was a significant (P < 0.001) effect of day on daily lying time. Daily lying time was less for both control and treated horses on days 1 through 4 (after being shod) than on all other days (P < 0.05 for all comparisons).

Mean daily number of lying bouts was 12.4 ± 2.7 for control horses and 8.7 ± 2.7 for treated horses. No effect on daily number of lying bouts was identified for treatment (P = 0.33), day (P = 0.40), exercise group (P = 0.09), or treatment by day interaction (P = 0.16).

Mean daily lying time was 19.1 ± 4.0 minutes for control horses and 25.9 ± 4.0 minutes for treated horses. Daily lying time was generally (but not significantly) greater for treated horses than control horses on day –3 (45.2 ± 6.5 minutes vs 19.1 ± 6.5 minutes, respectively) and day 0 (36.4 ± 6.5 minutes vs 16.0 ± 6.5 minutes, respectively). No effect on number of daily lying bouts was identified for treatment (P = 0.25), exercise group (P = 0.61), or treatment by day interaction (P = 0.06), but there was a significant (P < 0.001) effect of day.

Discussion

Stacked wedge pads and chains have been used illegally to create an exaggerated gait in horses by eliciting a painful response[2,3,7]; however, we could find no scientific evidence that the legal use of stacked wedge pads and chains, as indicated by the Horse Protection Act,[6] is detrimental to the health and welfare of horses. The objective of the present study was, therefore, to examine the acute and subacute physiologic and behavioral responses of horses to application of stacked wedge pads and chains to infer whether these training devices may cause stress or pain. No significant changes were identi-

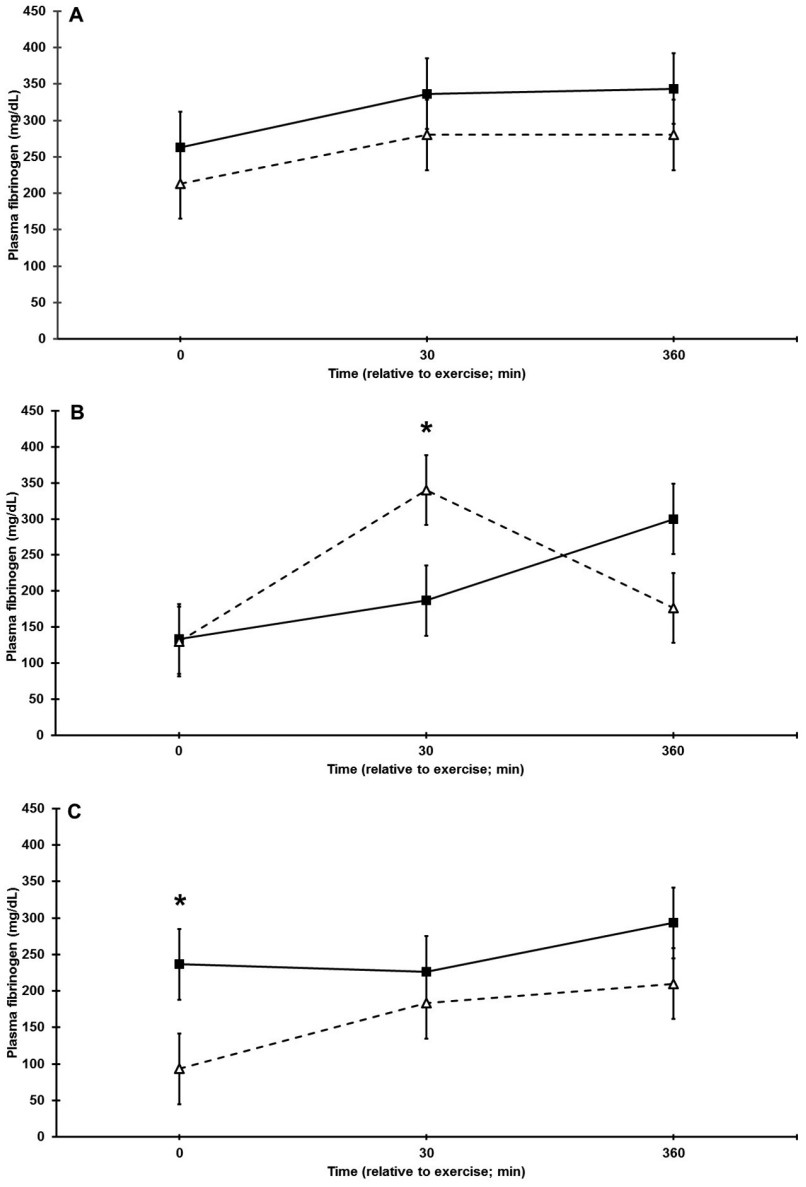

Figure 4—Mean fibrinogen concentrations in plasma samples obtained on days –5 (A; n = 60 samples), 1 (B; 60), and 5 (C; 60) from the horses in Figure 2. No effect on plasma fibrinogen concentration was identified for treatment, treatment by day interaction, or treatment by time within day, but there was a significant effect of day ($P = 0.009$) and time within day ($P = 0.003$). *Value differs significantly ($P < 0.05$) between groups at the indicated measurement point. ***See*** Figure 2 for remainder of key.

fied in plasma concentrations of biochemical indicators of stress, pain, and inflammation after stacked wedge pads and chains were applied, nor were any significant differences in measured biochemical and behavioral responses identified between horses that wore these devices versus horses that did not. No signs of lameness were observed during the study, although bilateral lameness of the forelimbs or hind limbs can be challenging to identify.[38] In addition, the chains caused no loss of hair or signs of dermatitis in the pastern region.

Cortisol concentration in biological fluids is widely used to indicate whether an animal is stressed.[39] Almost any kind of threat to homeostasis or stress causes the amount of glucocorticoids in circulation to rise.[40] In a study[23] involving horses, serum cortisol concentrations took almost 20 minutes to reach peak values after induction of stress through application of a lip twitch and stayed high for 60 minutes. The widespread use of cortisol as a stress biomarker stems from cortisol being the primary glucocorticoid released by the hypothalamic-pituitary-adrenal axis, which is activated during stressful or painful events.[41,42]

Mean cortisol concentrations in all plasma samples pertaining to the 6-hour periods of intense sample collection in the pretreatment period (day –5), posttreatment acute period (day 1), and posttreatment subacute period (day 5) in the present study differed significantly among days but not between control horses and treated horses. Concentrations were highest in the pretreatment period and decreased during the posttreatment acute and subacute periods, indicating that the horses had become acclimated to their new environment and handlers unfamiliar to them. Although the mean plasma cortisol concentration for both groups of horses was highest at 330 minutes after exercise, values at that time, or at any other time, did not differ between control horses and treated horses.

Plasma cortisol concentration in horses fluctuates by circadian rhythm, with a peak early in the morning and a trough in the late afternoon or early evening, but this rhythm can be disrupted by even the minor perturbation of removing a horse from an environment to which it is accustomed,[43] thereby making clinical interpretation of changes in cortisol concentration difficult. However, blood sample collection from treatment and control horses in the present study was performed at the same time of day, and both groups of horses had been allowed to acclimate to their surroundings and to exercise for at least 5 days before the study began, minimizing the impact of circadian rhythm and environmental changes on the results.

Lack of changes in plasma cortisol concentration does not suggest horses have no stress. We interpret the lack of significant differences in effects of treatment and treatment by time by day between treated and control horses and before and after application of training devices as indicating that wearing stacked wedge pads and chains, under the study conditions,

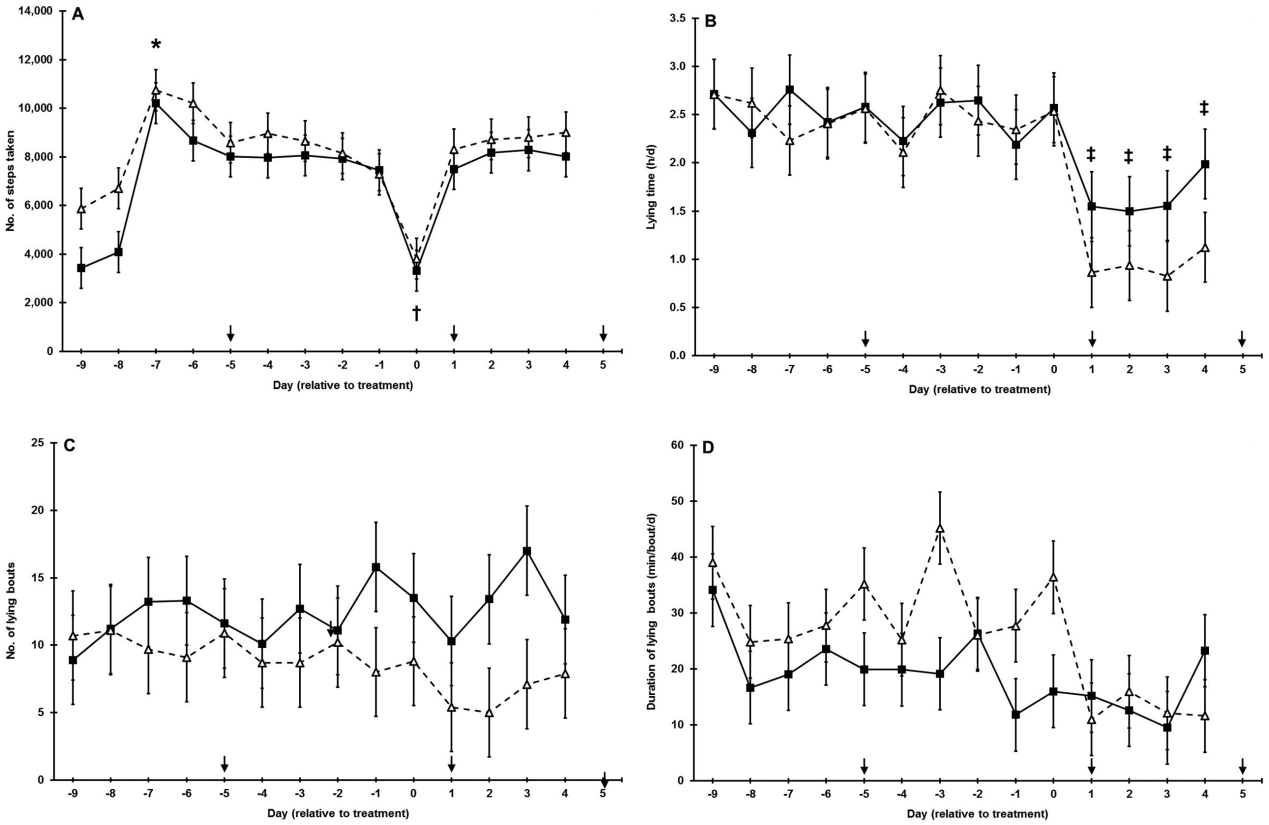

Figure 5—Mean number of steps taken (A), lying time (B), number of lying bouts (C), and duration of lying bouts (D) on days –9 through 4 for the horses in Figure 2 as measured by accelerometer. Arrows along the x-axis indicate the days on which blood samples were collected. A—No effect on the daily number of steps taken was identified for treatment, exercise group, or treatment by day interaction, but there was a significant ($P < 0.001$) effect of day. *Values were greatest in both control and treated horses on day –7 (first day of exercise; $P < 0.05$). †Values were least on day 0 (ie, the day the horses were shod and not exercised; $P < 0.05$). B—No effect on the daily lying time was identified for treatment, exercise group, or treatment by day interaction, but there was a significant ($P < 0.001$) effect of day. ‡Values were significantly ($P < 0.05$) less for control and treated horses on days 1 through 4 (after being shod) than on all other days. C—No effect on the daily number of lying bouts was identified for treatment, day, exercise group, or treatment by day interaction. D—No effect on the daily duration of lying bouts was identified for treatment, exercise group, or treatment by day interaction, but there was a significant ($P < 0.001$) effect of day. ***See*** Figure 2 for remainder of key.

yielded no detectable physiologic response indicative of stress on the basis of plasma cortisol concentration. Indeed, post hoc power analyses ($\alpha = 0.05$; n = 10 horses/group) revealed that the study had 94% power to detect differences between the groups in plasma cortisol concentration (SD, 6 ng/mL; effect size, 10 ng/mL), plasma substance P concentration (SD, 40 pg/mL; effect size, 66 pg/mL), and plasma fibrinogen concentration (SD, 150 mg/dL; effect size, 250 pg/mL). Consequently, sufficient power existed to detect subtle differences in plasma cortisol concentration as the horses became acclimated to the housing and handling as well as an unsuspected difference in this concentration at approximately 330 minutes after exercise. Therefore, we propose that if the application of stacked wedge pads and chains, compared with application of standard horse shoes, was stressful to horses, then it was no more stressful than the introduction of horses to a novel environment. Indeed, if it had been more stressful, then plasma cortisol concentrations in both groups would not have declined progressively as the study progressed.

Substance P is a more specific indicator of nociception than cortisol, and assessment of plasma substance P concentration has been used to discriminate between stressful events that cause a transient increase in plasma cortisol concentration and nociceptive events.[12,44,45] In the present study, no significant increase in plasma substance P concentration was identified in horses after stacked wedge pads and chains were applied to their forefeet, either before or after exercise, nor was any difference identified between horses, maintained under identical conditions, shod with stacked wedge pads and exercised with chains and horses shod with keg shoes and exercised without chains.

Whereas an increase in plasma substance P concentration indicates nociception, no change in this variable does not necessarily indicate a complete absence of nociception. However, we interpret the lack of significant differences in plasma substance P concentrations between treatment and control horses in the present study as indicating that wearing stacked wedge pads and chains, under the conditions out-

lined in this study, yielded no detectable or discernable nociceptive response.

Heel wedges increase maximal flexion of the proximal and distal interphalangeal joints and maximal extension of the metacarpophalangeal joint.[46] In a 3-D kinematic study[47] performed while the horses were walking, investigators found that elevating the heels of the forelimbs 6° increased and delayed maximal flexion of the metacarpophalangeal joint and increased maximal flexion and decreased maximal extension of the proximal and distal interphalangeal joints. This elevation also influenced the gait by extending the landing and bearing periods and shortening the breakover period. In the present study, the horses' heels were elevated 12°, and we presume that this degree of elevation altered the angles of joints and the landing, bearing, and breakover periods more than it would have by elevating the heels 6°.

Fibrinogen concentration was measured in the study reported here to determine whether elevating horses' heels with stacked wedge pads would induce inflammation of the altered structures, because local inflammation such as synovitis induced in ≥ 1 joint can, if strong enough, elicit a measureable systemic acute phase inflammatory response.[48] Plasma concentration of fibrinogen, a protein involved in blood clotting, rises in response to injury and to a degree that may be related the degree of injury.[49] In a study[48] involving horses, chemical induction of synovitis of the carpal joint resulted in an increase in serum fibrinogen concentration 24 hours later, and concentrations remained greater than before induction for 2 weeks. In that study, the relative maximal increase in concentration was 87%, and maximal concentrations were observed between 36 and 72 hours after synovitis induction. Mean fibrinogen concentrations in all plasma samples from both treatment and control horses were significantly greater on day –5 (before shoe application) than on days 1 and 5 (after shoe application), indicating that wearing shoes, whether keg or wedged, incited no inflammatory response or a response too little to be measured.

Although no increase in plasma fibrinogen concentration was identified after stacked wedge pad application or between treatment and control horses, we acknowledge that inflammation of the joints of the distal portion of the limb induced by changes in angles of the joints and changes in the landing, bearing, and breakover periods may have been insufficient to elicit a systemically observable increase in fibrinogen concentration. Other unmeasured acute phase proteins that may rise in plasma concentration after induction of noninfectious arthritis in horses include haptoglobin and serum amyloid A.

In the study reported here, a 20-minute exercise period was chosen because 20 minutes is the typical duration of a training session for Tennessee Walking Horses.[1] We were unable to exercise the horses at a running walk, which is the gait at which Tennessee Walking Horses are typically trained for competition (4.5 to 9 m/s), and this represents a study limitation. We had neither the riders nor the facilities to exercise 20 horses at speeds at which Tennessee Walking Horses are typically exercised, and consequently, the horses were exercised for 20 minutes at the fastest speed possible with the walker apparatus used (1.21 m/s). Exercising the horses in this manner, although not fully mimicking a typical training session, kept the duration and intensity of the exercise equal among horses. In addition, the height and weight of the stacked wedge pads worn by treated horses were typical of those worn by Tennessee Walking Horses in full training, which are usually applied only after gradually increasing (ie, over a period of weeks or months) the height and weight of the stacked wedge pads. That is, the weight and height of the stacked wedge pads used in the present study were those typically used on a horse that has been in training for 8 months to a year.

Another limitation of the present study was the brief period during which horses were evaluated. Tennessee Walking Horses that wear stacked wedge pads do so for extended periods, often years, but evaluation of the long-term effect of stacked wedge pads on biochemical and behavioral signs of pain and stress was beyond the scope of the study.

The accelerometer used in the present study is an electronic instrument that, when used with a software program, measures the activity of an animal in 3 dimensions. It has been applied to hind limbs of cattle to estimate, with reasonably high accuracy, the number of steps taken per unit of time and to estimate the frequency and duration of walking and standing.[50] The accelerometer can be used to collect and record data regarding acceleration at a rate of 8 readings/s and can store up to 60 days of data. It provides information for each second about posture (standing vs lying), whether the limb to which the sensor is attached is moving, and the number of steps taken.[50] Proprietary algorithms are used to convert the data into a summarized form, with raw data reported at 1-minute intervals for the duration of data collection.

Use of this specific accelerometer[a] for behavioral data collection has been validated in dairy cows[50] but not in horses; however, the general use of triaxial accelerometers has been validated in horses.[51,52] Furthermore, placement of the accelerometer on a hind limb, as was done in the present study, reportedly yields the most accurate data.[51] In a study[53] involving calves, use of an accelerometer to examine the effect of castration revealed that castrated calves spent a significantly larger amount of time standing during the subsequent 24-hour period (or longer) than uncastrated calves, but studies[54–56] of the effect of lameness on cattle behavior have shown that lame cattle spend more time lying down than do nonlame cattle. We hypothesized that horses with pain in ≥ 1 limb would behave similarly to lame cattle. Use of the accelerometer revealed that time spent walking and

standing increased on the days after shoe application, regardless of the type of shoe applied. The authors are unaware of any studies examining the effect of shoe application on the behavior of horses, and our finding that daily lying time decreased after keg shoes were applied was unexpected.

In the days leading up to shoe application in the present study, horses spent approximately 2.5 h/d lying. This time decreased to 1 to 1.5 h/d after shoe application, regardless of whether the horse received stacked wedge pads or keg shoes. In a study[34] of housing systems for horses, horses kept for 17 h/d in individual box stalls and 7 h/d in a communal paddock spent approximately 0.75 hours lying in the box stall. Lying times were not quantified while these horses were within the paddock, which may explain the differences observed in lying time between that study[34] and the present study. In a study[33] examining the effect of housing type on lying bouts and lying time, maximum lying time was approximately 90 min/d for group-housed horses, which is similar to the posttreatment lying times in the present study. We believe that these results collectively indicate that the lying behaviors of the control and treated horses did not reflect diminished welfare. The main change that coincided with shoe application may have been driven by the hoof trimming and shoeing process, regardless of the type of shoe applied. We were unable to find any reports of studies of the effect of shoe application on lying time in horses; therefore, we recommend further investigation of the effects of podiatric procedures, such as shoeing, on horse behavior.

For horses in the present study, the number of lying bouts was approximately 10/d before shoe application. Findings of other studies[33,52,57] regarding the number and duration of lying bouts of horses greatly differ. Horses housed in groups on different types of bedding reportedly have between 2 and 3 lying bouts/d, with 32% of these horses spending a full 24-hour period without any lying bouts.[33] Similarly, in a study[52] designed to validate the use of an accelerometer for evaluation of lying behaviors, horses had 3 to 5 lying bouts/d. A primary difference between these studies and the study reported here was that the horses in the present study were housed in individual box stalls, except when exercised, whereas the horses in the other studies[33,52] spent a portion of the day in a paddock. This difference in study conditions, however, likely fails to explain the difference in daily number of lying bouts because horses housed totally within a stall reportedly have 2.5 lying bouts/d (range, 0 to 6 bouts/d),[57] which is similar to the number of lying bouts associated with horses spending only a portion of the day in a stall.[33,52] Despite these differences in the total number of lying bouts, the mean duration of lying bouts in the present study was consistent with that in other studies (18 to 32 minutes[57] or 30 minutes[52]).

Patterns of lying down and stepping activity may change when horses have pain, but such changes do not necessarily indicate pain. Therefore, various pain scales have been designed for use in horses, incorporating many other behaviors and taking into account a range of behaviors. Alterations in facial expressions, for example, have been used as an indicator of nociception, and a facial expression–based pain-coding system, termed the Horse Grimace Scale, has been developed to facilitate objective evaluation of facial expressions.[58–60] However, that scale has not been completely validated, and the same facial expressions that indicate pain may overlap with facial expressions that indicate other conditions, such as fear and anxiety. Evaluation of facial expressions by use of the Horse Grimace Scale may have been useful in the present study for detecting pain or stress, but at the time of the study, the authors were unaware that this method existed for horses.

Overall, results of the present study suggested that application of stacked wedge pads to the forefeet of Tennessee Walking Horses and exercise with chains, which is considered to be humane under the Horse Protection Act[6] as applied in the study, resulted in no measured signs of an acute or subacute stress or nociceptive response. Although no evidence was found that these training devices, commonly applied to Tennessee Walking Horses, had an impact on horse welfare for the 5-day period of evaluation, the conditions under which the effects of these training devices were tested were not completely similar to those conditions used for Tennessee Walking Horses in training, and the results should not be generalized to the long-term use of these devices in horses performing the running walk. Indeed, the effects of these devices should be examined when horses are exercised with a rider at a speed and duration typical of those demanded while horses are in training. The long-term effects of these devices should also be evaluated. However, despite the aforementioned limitations, the study findings should be considered when making evidence-based decisions regarding the welfare implications of the common practice of applying stacked wedge pads and chains to Tennessee Walking Horses.

Acknowledgments

Supported by the Tennessee Equine Veterinary Research Organization of the College of Veterinary Medicine, University of Tennessee. This organization was not involved in study design, data analyses, or data interpretation or in drafting of the manuscript.

The authors declare that there were no conflicts of interest.

Footnotes

a. IceTag3D, IceRobotics Ltd, Edinburgh, Scotland.
b. Milacath extended-use catheter, Mila International Inc, Erlanger, Ky.
c. 8-inch large animal extension set, International WIN Ltd, Kennett Square, Pa.
d. St Croix keg shoe with heel, Tennessee Farrier Supply, Cumberland Furnace, Tenn.
e. Ironcraft Horseshoes Inc, Lewisburg, Tenn.
f. Santa Cruz Biotechnology Inc, Santa Cruz, Calif.
g. IceManager, IceRobotics Ltd, Edinburgh, Scotland.
h. IceReader, IceRobotics Ltd, Edinburgh, Scotland.

i. PROC MIXED, SAS, version 9.4, SAS Institute Inc, Cary, NC.
j. PROC GLIMMIX, SAS, version 9.4, SAS Institute Inc, Cary, NC.
k. PROC UNIVARIATE, SAS, version 9.4, SAS Institute Inc, Cary, NC.

References

1. Tennessee Walking Horse Breeders' and Exhibitors' Association. Tennessee Walking Horses: the basics. Available at: www.twhbea.com/cms/cmsfiles/PDFs/BasicsBrochure.pdf. Accessed Mar 6, 2017.
2. AVMA. Use of action devices and performance packages for Tennessee Walking Horses. Available at: www.avma.org/KB/Policies/Pages/Use-of-Action-Devices-Performance-Packages-for-Tennessee-Walking-Horses.aspx. Accessed Mar 6, 2017.
3. American Association of Equine Practitioners. AVMA and AAEP Position on the use of action devices and performance packages for Tennessee Walking Horses. Available at: aaep.org/avmaaaep-position-use-action-devices-and-performance-packages-tennessee-walking-horses#. Accessed Mar 6, 2017.
4. Clayton HM, White AD, Kaiser LJ, et al. Short-term habituation of equine limb kinematics to tactile simulation of the coronet. *Vet Comp Orthop Traumatol* 2008;21:211–214.
5. Bushe T, Turner TA, Poulos PW, et al. The effect of hoof angle on coffin, pastern, and fetlock joint angles, in *Proceedings*. 33rd Annu Meet Am Assoc Equine Pract 1987;33:729–738.
6. Horse protection regulations. *Fed Reg* 2017;15:1821–1831 (codified at 9 CFR §11.2).
7. Humane Society of the United States. What is soring? Important facts about the cruel abuse. Available at: www.humanesociety.org/issues/tenn_walking_horses/facts/what_is_soring.html. Accessed Mar 6, 2017.
8. US Equestrian Federation. *Rulebook*. Lexington, Ky: US Equestrian Federation, 2017;104–105.
9. Sapolsky RM. Social status and health in humans and other animals. *Annu Rev Anthropol* 2004;33:393–418.
10. Moberg GP. Biological response to stress: implications for animal welfare. In: Moberg GP, Mench JA, eds. *The biology of animal stress: basic principles and implications for animal welfare*. Wallingford, Oxfordshire, England: CABI Publishing, 2000;1–22.
11. Broom DM. Evolution of pain. In: Lord Soulsby EJL, Morton D, eds. *Pain: its nature and management in man and animals.* Vol 246. London: Royal Society of Medicine, 2001;246:17–25.
12. Coetzee JF, Lubbers BV, Toerber SE, et al. Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *Am J Vet Res* 2008;69:751–762.
13. Anderson DE, Muir WW. Pain management in cattle. *Vet Clin North Am Food Anim Pract* 2005;21:623–635.
14. Molony V, Kent JE. Assessment of acute pain in farm animals using behavioral and physiological measurements. *J Anim Sci* 1997;75:266–272.
15. Merskey H, Bogduk N. Part III: pain terms, a current list with definitions and notes on usage. In: Merskey H, Bogduk N, eds. *Classification of chronic pain, descriptors of chronic pain syndromes and definitions of pain terms.* 2nd ed. Seattle: International Association for the Study of Pain Press, 1994;207–214.
16. Mills PC, Ng JC, Kramer H, et al. Stress response to chronic inflammation in the horse. *Equine Vet J* 1997;29:483–486.
17. Raekallio M, Taylor PM, Bennett RC. Preliminary investigations of pain and analgesia assessment in horses administered phenylbutazone or placebo after arthroscopic surgery. *Vet Surg* 1997;26:150–155.
18. Pritchett LC, Ulibarri C, Roberts MC, et al. Identification of potential physiological and behavioral indicators of postoperative pain in horses after exploratory celiotomy for colic. *Appl Anim Behav Sci* 2003;80:31–43.
19. Sellon DC, Roberts MC, Blikslager AT, et al. Effects of continuous rate intravenous infusion of butorphanol on physiologic and outcome variables in horses after celiotomy. *J Vet Intern Med* 2004;18:555–563.
20. Bussières G, Jacques C, Lainay O, et al. Development of a composite orthopaedic pain scale in horses. *Res Vet Sci* 2008;85:294–306.
21. Baucus KL, Squires EL, Ralston SL, et al. Effect of transportation on the estrous cycle and concentrations of hormones in mares. *J Anim Sci* 1990;68:419–426.
22. James VHT, Horner MW, Moss MS, et al. Adrenocortical function in the horse. *J Endocrinol* 1970;48:319–335.
23. Thompson DL, Garza F, Mitchell PS, et al. Effects of short-term stress, xylazine tranquilization and anesthetization with xylazine plus ketamine on plasma concentrations of cortisol, luteinizing hormone, follicle-stimulating hormone and prolactin in ovariectomized pony mares. *Theriogenology* 1988;30:937–946.
24. Alexander SL, Irvine CHG, Livesey JH, et al. Effect of isolation stress on concentrations of arginine vasopressin, α-melanocyte-stimulating hormone and ACTH in the pituitary venous effluent of the normal horse. *J Endocrinol* 1988;116:325–334. PubMed
25. Allen BJ, Rogers SD, Ghilardi JR, et al. Noxious cutaneous thermal stimuli induce a graded release of endogenous substance P in the spinal cord: Imaging peptide action in vivo. *J Neurosci* 1997;17:5921–5927.
26. DeVane CL. Substance P: a new era, a new role. *Pharmacotherapy* 2001;21:1061–1069.
27. Murata H, Shimada N, Yoshioka M. Current research on acute phase proteins in veterinary diagnosis: an overview. *Vet J* 2004;168:28–40.
28. Thomas JS. Overview of plasma proteins and protein electrophoresis. In: Feldman BF, Zinkl JG, Jain NC, eds. *Schalm's veterinary hematology.* 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2000;891–898.
29. Roughan JV, Flecknell PA. Behavioural effects of laparotomy and analgesic effects of ketoprofen and carprofen in rats. *Pain* 2001;90:65–74.
30. Price J, Catriona S, Welsh EM, et al. Preliminary evaluation of a behaviour-based system for assessment of post-operative pain in horses following arthroscopic surgery. *Vet Anaesth Analg* 2003;30:124–137.
31. Ashley FH, Waterman-Pearson AE, Whay HR. Behavioural assessment of pain in horses and donkeys: application to clinical practice and future studies. *Equine Vet J* 2005;37:565–575.
32. Taylor PM, Pascoe PJ, Mama KR. Diagnosing and treating pain in the horse: where are we today? *Vet Clin North Am Equine Pract* 2002;18:1–19.
33. Baumgartner M, Zeitler-Feicht MH, Wohr AC, et al. Lying behaviour of group-housed horses in different designed areas with rubber mats, shavings and sand bedding. *Pferdeheilkunde* 2015;31:211–220.
34. Hoffmann G, Wagels E, Kraft S, et al. Comparative study of horses in tie stalls, individual housing in boxes and group housing. *Pferdeheilkunde* 2012;28:702–710.
35. Doherty TJ, Kattesh HG, Adcock RJ, et al. Effects of a concentrated lidocaine solution on the acute phase stress response to dehorning in dairy calves. *J Dairy Sci* 2007;90:4232–4239.
36. Millar HR, Simpson JG, Stalker AL. Evaluation of heat precipitation method for plasma fibrinogen estimation. *J Clin Pathol* 1971;24:827–830.
37. Endres MI, Barberg AE. Behavior of dairy cows in an alternative bedded-pack housing system. *J Dairy Sci* 2007;90:4192–4200.
38. Ross MW. Movement. In: Ross MW, Dyson SJ, eds. *Diagnosis and management of lameness in the horse.* Philadelphia: Saunders, 2003;60–73.
39. Mstl E, Palme R. Hormones as indicators of stress. *Domest Anim Endocrinol* 2002;23:67–74.
40. Munck A, Guyre PM, Holbrook NJ. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. *Endocr Rev* 1984;5:25–44.
41. Herskin MS, Munksgaard L, Andersen JB. Effects of social isolation and restraint on adrenocortical responses and hypoalgesia in loose-housed dairy cows. *J Anim Sci* 2007;85:240–247.

42. Herskin MS, Munksgaard L, Ladewig J. Effects of acute stressors on nociception, adrenocortical responses and behavior of dairy cows. *Physiol Behav* 2004;83:411–420.
43. Irvine CHG, Alexander SL. Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domest Anim Endocrinol* 1994;11:227–238.
44. Harrison S, Geppetti P, Substance P. *Int J Biochem Cell Biol* 2001;33:555–576.
45. Whitlock BK, Coffman EA, Coetzee JF, et al. Electroejaculation increased vocalization and plasma concentrations of cortisol and progesterone, but not substance P, in beef bulls. *Theriogenology* 2012;78:737–746.
46. Rumpler B, Riha A, Licka T, et al. Influence of shoes with different weights on the motion of the limbs in Icelandic horses during toelt at different speeds. *Equine Vet J Suppl* 2010;38:451–454.
47. Chateau H, Degueurce C, Denoix JM. Effects of 6 degrees elevation of the heels on 3D kinematics of the distal portion of the forelimb in the walking horse. *Equine Vet J* 2004;36:649–654.
48. Hultén C, Gronlund U, Hirvonen J, et al. Dynamics in serum of the inflammatory markers serum amyloid A (SAA), haptoglobin, fibrinogen and α(2)-globulins during induced noninfectious arthritis in the horse. *Equine Vet J* 2002;34:699–704.
49. Allen BV, Kold SE. Fibrinogen response to surgical tissue trauma in the horse. *Equine Vet J* 1988;20:441–443.
50. Nielsen LR, Pedersen AR, Herskin MS, et al. Quantifying walking and standing behaviour of dairy cows using a moving average based on output from an accelerometer. *Appl Anim Behav Sci* 2010;127:12–19.
51. Fries M, Montavon S, Spadavecchia C, et al. Evaluation of a wireless activity monitoring system to quantify locomotor activity in horses in experimental settings. *Equine Vet J* 2017;49:225–231.
52. DuBois C, Zakrajsek E, Haley DB, et al. Validation of triaxial accelerometers to measure the lying behaviour of adult domestic horses. *Animal* 2015;9:110–114.
53. White BJ, Coetzee JF, Renter DG, et al. Evaluation of two-dimensional accelerometers to monitor behavior of beef calves after castration. *Am J Vet Res* 2008;69:1005–1012.
54. Galindo F, Broom DM, Jackson PGG. A note on possible link between behaviour and the occurrence of lameness in dairy cows. *Appl Anim Behav Sci* 2000;67:335–341.
55. Galindo F, Broom DM. The relationships between social behaviour of dairy cows and the occurrence of lameness in three herds. *Res Vet Sci* 2000;69:75–79.
56. Blackie N, Bleach E, Amory J, et al. Impact of lameness on gait characteristics and lying behaviour of zero grazed dairy cattle in early lactation. *Appl Anim Behav Sci* 2011;129:67–73.
57. Chaplin SJ, Gretgrix L. Effect of housing conditions on activity and lying behaviour of horses. *Animal* 2010;4:792–795.
58. Dalla Costa E, Stucke D, F. D, et al. Using the Horse Grimace Scale (HGS) to assess pain associated with acute laminitis in horses (*Equus caballus*). *Animals (Basel)* 2016;6:E47.
59. Dalla Costa E, Minero M, Lebelt D, et al. Development of the Horse Grimace Scale (HGS) as a pain assessment tool in horses undergoing routine Castration. *PLoS One* 2014;9:e92281.
60. Gleerup KB, Forkman B, Lindegaard C, et al. An equine pain face. *Vet Anaesth Analg* 2015;42:103–114.