Measurement of digital laminar and venous temperatures as a means of comparing three methods of topically applied cold treatment for digits of horses

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Objective—To compare effects of 3 methods of topically applied cold treatment (cryotherapy) on digital laminar and venous temperatures in horses.

Animals—9 healthy adult Thoroughbreds.

Procedures—Thermocouples were placed in palmar digital veins and digital laminae of both forelimbs of horses. Three methods of cryotherapy were applied to the distal aspects of the limbs: wader boot (63-cm-tall vinyl boot filled with ice and water [ice slurry]), ice bag (5-L fluid bag filled with ice slurry), and a gel pack boot (boot containing frozen gel packs). Gel packs and ice slurries were replenished every hour during cryotherapy. The forelimb that received the first treatment was randomly assigned; thereafter, control and treated forelimbs were alternated for each treatment. For each treatment, temperatures were recorded every minute during 15-minute pretreatment, 2-hour treatment, and ≥30-minute rewarming periods. Once temperatures had returned to within 3°C below pretreatment values, the experiment was repeated in a similar manner for other cryotherapy methods.

Results—Digital venous temperatures were similar to laminar temperatures during each treatment. Ice bag and wader boot treatments caused similar cooling of digits. Gel boot treatment did not cause substantial cooling of digits.

Conclusions and Clinical Relevance—Ice bag treatment caused laminar and digital venous cooling equivalent to that of wader boot treatment. Cryotherapy by use of 5-L fluid bags with an ice slurry may be a readily available, practical, and efficient method for prevention of laminitis in horses. Digital laminar and venous temperatures were similar in forelimbs of horses before and during cryotherapy. (Am J Vet Res 2012;73:860–866)
nar thermocouples directly into the epidermal–dermal laminar interface, in a manner similar to a described technique, was chosen for measurement of laminar temperatures in the study reported here. To the authors’ knowledge, direct in vivo measurement of digital venous temperature has not been reported in horses.

We hypothesized that cryotherapy of horse limbs by means of a boot filled with ice and water that extended proximally to the distal aspect of the antebrachium (wader-style boot) would cause more substantial cooling of limbs than a 5-L fluid bag filled with ice and water or a commercially available frozen gel pack boot. We also hypothesized that laminar temperatures of horses would attain lower minimum values than digital venous temperatures during cryotherapy.

Materials and Methods

Animals—Nine healthy adult Thoroughbreds (5 mares and 4 geldings) ranging in age from 2 to 23 years (median, 8 years) that did not have signs of lameness or laminitis (as determined on the basis of physical and lameness examinations) were enrolled in the study. Each horse was placed in a stocks in a standing position and sedated with detomidine hydrochloride (0.01 mg/kg, IV) and butorphanol tartrate (0.01 mg/kg, IV) for placement of thermocouples. Horses tolerated standing in stocks; however, additional doses of detomidine hydrochloride (0.05 mg/kg, IV) and butorphanol tartrate (0.05 mg/kg, IV) were administered as needed to some of the horses to keep them standing with a forelimb in a wader boot or 5-L fluid bag filled with ice and water. The amount of sedation administered to horses was recorded, and the degree of sedation of horses was subjectively scored by 1 investigator (HLR) as light, moderate, or heavy. Horses were offered free-choice water and timothy hay during the experiment. All experimental procedures were performed at Cornell University. The study was approved by the Cornell University Institutional Animal Care and Use Committee.

Thermocouple placement—For each horse, distal aspects of both forelimbs were clipped of hair and scrubbed with chlorhexidine gluconate. A low 4-point nerve block was performed for both forelimbs; 1 mL of 2% lidocaine hydrochloride was injected at each nerve block site. In addition, 1 mL of lidocaine hydrochloride was injected SC on the dorsal midline aspect of limbs approximately 0.5 cm proximal to the coronary band. A 10-cm-wide rubber tourniquet was applied to the proximal aspect of the coronary band and advanced distally to the hub of the needle. The needle was removed, and the thermocouple was secured to skin with n-butyl cyanoacrylate.

A 38-mm hypodermic needle that was placed on the dorsal midline aspect of limbs 0.5 cm proximal to the coronary band and advanced distally to the hub of the needle. The needle was removed, and the thermocouple was secured to skin with n-butyl cyanoacrylate.

Radiography—Thermocouple positioning was confirmed following probe placement by acquisition and evaluation of lateral-medial and dorsolateral-palmaromedial oblique radiographic images of both forelimb feet of each horse. Evaluation of radiographic images revealed that there was more variability in positioning of the venous thermocouple than the laminar thermocouple. Position of venous thermocouples was categorized into 1 of 2 groups for each limb: within the hoof or within the region between the metacarpophalangeal joint and the coronary band (ie, pastern).

Study design and cryotherapy—A crossover study design was used to assess 3 methods of cryotherapy of digits of horses, which included a gel pack boot (hook-and-loop fastener boot with frozen gel packs placed on the distal aspects of limbs), an ice bag (5-L fluid bag filled with a mixture of crushed ice and water [ice slurry] extending from the hoof to the proximal aspect of the pastern), and a wader boot (vinyl boot filled with ice slurry extending from the hoof to the distal aspect of the antebrachium). The order in which the gel boot and ice bag treatments were applied was randomized, and the forelimb to which the first treatment was applied was randomized. Because of protracted rewarming time of digits following application of wader boot treatment for the first 2 horses in the study, it was applied as the third treatment for subsequent horses. Treated forelimb and forelimb exposed to control conditions were alternated for each cryotherapy method. Gel packs were replaced and ice slurries were replenished every hour during each cryotherapy period. Ambient, rectal, and pastern surface temperatures were measured prior to initiation of cryotherapy and every hour during the experiment. Ambient temperature was not controlled and ranged from 13.9°C to 25.9°C during experiments; however, the largest range in ambient temperature for any of the horses during an experiment was 3.4°F. Rectal temperature was measured with a digital thermometer, and ambient and pastern temperatures were measured with a thermocouple (similar to the digital laminar and venous thermocouples) and a microprobe thermometer. Digital laminar and venous temperatures were simultaneously recorded to 2 data logging devices for treated limbs and limbs exposed to control conditions at 1-minute intervals during the experiment.

For each cryotherapy method, digital laminar and venous temperatures were measured in both forelimbs of each horse every minute during the 15-minute pre-treatment period (baseline), 2-hour treatment, and ≥ 30 minute rewarming period. The ≥ 30 minute-rewarming period after each treatment was intended to allow the temperature of both digits of each horse to return to within 3°C below baseline temperature prior to beginning another treatment.

After completion of experiments, thermocouples were removed and hemostatic pressure bandages were applied to digital vasculature of horses for 10 minutes. Distal aspects of limbs were bandaged (standing wraps)
overnight (12 hours). Horses were monitored by physical examination and palpation of the distal aspect of the limbs for 48 hours following experiments to detect swelling, lameness, or other complications.

**Statistical analysis**—Mean ± SE minimum digital laminar and venous temperatures and mean ± SE digital laminar and venous temperature changes (mean pretreatment [baseline] temperature – minimum temperature during treatment) were calculated for each cryotherapy method. Prior to logistic regression model fitting, all potential covariates were screened for significance at $P < 0.10$. The following covariates with a value of $P > 0.10$ were excluded from the model: limb (left or right), digital venous thermocouple position (hoof or pastern), experiment location (standing stocks in one of two separate barns), order of treatment (first, second, or third), date on which experiment was performed, sex, age, level of sedation (light, moderate, or heavy) required for completion of experiment, and ambient temperature during the experiment.

A 1-way ANOVA was performed with a Tukey-Kramer honestly significant difference post hoc test to detect differences in minimum temperatures and temperature changes among treatments. The effect of cryotherapy among groups was analyzed for data collected at 15-minute intervals during the 120-minute treatment period via repeated-measures 1-way ANOVA. A 2-sample $t$ test for the difference in population means was used to compare minimum laminar hoof temperature data from the present study with data reported by other authors. Pearson product-moment correlation coefficients were calculated to determine relationships among variables. Statistical analyses were performed with statistical software. Values of $P < 0.05$ were considered significant.

**Results**

**Clinical observations**—All horses tolerated laminar and venous thermocouples well, and all horses tolerated standing in stocks. No swelling, lameness, or other complications associated with placement of thermocouples were detected. Level of sedation was not significantly correlated with minimum digital laminar temperature ($P = 0.66$) or minimum digital venous temperature ($P = 0.55$). Data were recorded for a minimum of 30 minutes after wader boot treatments; however, because of scheduling constraints, data were not collected for sufficient duration to enable digital temperatures to return to within $3^\circ$C below baseline digital temperatures after this treatment in all horses. For 6 horses, complete data (data for 15 minutes of baseline temperature; data for 120 minutes of treatment, and data for $\geq 30$ minutes ofrewarming) were recorded for all 3 cryotherapy methods. Data were incomplete for 3 horses (thermocouple failure for 1 horse, data logging device failure for 1 horse, and failure of digital temperature to return to within $3^\circ$C below baseline temperature within 5 hours of rewarming in 1 horse). Complete data were obtained for 6 gel pack boot treatments, 7 wader boot treatments, and 8 ice bag treatments.

**Radiography and thermocouple location**—Radiography revealed that there was no detectable rotation of the distal phalanx away from the dorsal aspect of the hoof wall in any of the horses (ie, there were no radiographic signs of laminitis). Radiography revealed that there was more variability in position of digital venous thermocouples versus position of digital laminar thermocouples. Nine digital venous thermocouples were within the lateral proper palmar digital vein in the pastern, and 9 digital venous thermocouples were within the hoof capsule, most (7/9) of which had been advanced to the level of the lateral aspect of the distal sesamoid (navicular) bone. One thermocouple had been advanced into the coronal vein, and 1 thermocouple had been advanced into the bulbous ramus of the lateral proper palmar digital vein. Digital venous thermocouple location (hoof vs pastern) did not have a significant ($P = 0.86$) effect on digital venous temperature. Digital laminar thermocouple position was similar between forelimbs of each horse and consistent among all horses; these thermocouples were approximately 25 to 30 mm distal to the coronary band and 0 to 5 mm dorsal to the dorsal radiographic border of the distal phalanx (Figure 1).

**Cryotherapy temperature data**—There were no significant differences between mean baseline laminar...
(P = 0.17) or venous (P = 0.33) temperatures for control or treated limbs prior to beginning each treatment (gel boot, ice bag, and wader boot). Ambient temperature was significantly correlated with baseline digital venous (P = 0.009) and laminar (P < 0.001) temperatures but was not significantly correlated with posttreatment digital venous (P = 0.65) or laminar (P = 0.72) temperatures. Minimum digital laminar and venous temperatures were closely correlated (r = 0.98), and pastern surface temperatures were highly correlated with digital laminar (r = 0.86) and venous (r = 0.87) temperatures.

Wader boot and ice bag treatments caused significant (P < 0.001) digital laminar (mean ± SE temperature change, –24.44 ± 1.9°C and –23.29 ± 1.4°C, respectively) and venous (mean ± SE temperature change, –22.04 ± 1.6°C and –19.49 ± 1.5°C, respectively; Figure 2) cooling. Mean ± SE minimum digital laminar temperatures of 11.14 ± 1.7°C and 11.41 ± 1.6°C were achieved for wader boot and ice bag treatments, respectively. Mean ± SE minimum digital venous temperatures of 13.27 ± 1.6°C and 15.43 ± 1.5°C were obtained for wader boot and ice bag treatments, respectively. Digital laminar and venous temperatures were not significantly different for wader boot and ice bag treatments; however, they were significantly lower than temperatures for control or gel boot treatments. Mean minimum laminar temperature for the wader boot treatment (11.14 ± 1.7°C) was not significantly (P = 0.36) different from mean laminar temperatures achieved by Pollitt et al2 in clinically normal horses by use of a similar method of cryotherapy.

Wader boot and ice bag treatments caused similar pastern surface cooling (mean ± SE temperature change, –14.8 ± 1.1°C and –11.9 ± 1.0°C, respectively); these values were significantly (P < 0.001) different from values for gel boot treatment and control conditions (mean ± SE temperature change, –1.2 ± 1.1°C and –1.6 ± 0.6°C, respectively). Mean ± SE minimum pastern surface temperatures were 16.7 ± 1.3°C (wader boot), 17.6 ± 1.2°C (ice bag), 28.2 ± 0.7°C (gel boot), and 29.0 ± 1.3°C (control conditions).

Wader boot and ice bag treatments typically caused marked cooling in treated limbs and minimal cooling in contralateral untreated control limbs (Figure 3). Analysis of pooled data for control conditions indicated that digital laminar temperature decreased slightly

Figure 2—Mean ± SE minimum digital laminar and venous temperatures in forelimbs of 9 healthy horses that underwent 3 methods of topically applied cold treatment (cryotherapy) and in contralateral forelimbs simultaneously exposed to control (no treatment) conditions. Temperatures were measured with thermocouples placed in digital laminae of the dorsal aspect of a hoof (black bars) and in a lateral palmar digital vein (gray bars). Methods of cold treatment included a gel pack boot (gel; hook-and-loop fastener boot with frozen gel packs placed on the distal aspect of limbs), a 5-L ice bag (ice bag; 5-L fluid bag filled with ice and water [ice slurry] extending proximally from the digit to the distal aspect of the antebrachium), and a wader boot (wader; vinyl boot filled from the digit to the proximal aspect of the region between the metacarpophalangeal joint and the coronary band [ie, pastern], and a wader boot). Each treatment was applied for 120 minutes. Order in which gel boot and ice bag treatments were applied was randomized. Values for limbs exposed to control conditions during each treatment (gel control, ice bag control, and wader control) were determined. Each treatment was applied for 120 minutes. Order in which gel boot and ice bag treatments were applied was randomized. The forelimb to which the first treatment was applied was randomized, and treated forelimb and control forelimb were alternated for each subsequent cryotherapy method. Because of protracted rewarming time of digits following application of wader boot treatment for the first 2 horses in the study, it was applied as the third treatment in subsequent horses. *Values with different letters are significantly (P < 0.05) different.

Figure 3—Representative temperature profile of forelimbs of a horse that was treated with the 3 methods of topically applied cold treatment (cryotherapy) described in Figure 2. The profile depicts digital laminar (solid black line) and digital venous (dotted black line) temperatures in the treated limb, digital laminar (solid gray line) and digital venous (dotted gray line) temperatures in the contralateral limb exposed to control conditions, and rectal temperatures (black circles). The order, timing, and duration (120 minutes) of each treatment are indicated by the horizontal bars at the top of the graph. Notice there was marked cooling of digits during ice bag and wader boot treatments and minimal cooling during gel boot treatment.
from the beginning to the end of the study (mean ± SE temperature change, –2.22 ± 0.9°C); mean ± SE digital laminar temperature of limbs during control conditions was 32.59 ± 1.0°C. Digital venous temperature during control conditions also decreased slightly from the beginning to the end of the study (mean ± SE temperature change, –2.05 ± 0.9°C); mean ± SE digital venous temperature of limbs during control conditions was 32.75 ± 1.0°C. Gel boot treatment caused a slight decrease in digital laminar temperature (mean ± SE temperature change, –2.08 ± 1.7°C) in treated limbs; mean ± SE laminar temperature was 33.40 ± 1.8°C. Gel boot treatment caused a similar decrease in digital venous temperature (mean ± SE temperature change, –2.48 ± 1.7°C) in treated limbs; mean ± SE digital venous temperature was 32.67 ± 1.8°C (Figure 2). There were no significant differences among data for limbs during control conditions for any of the 3 cryotherapy methods (gel boot control, ice bag control, and wader control conditions) or for gel boot-treated limbs. Rewarming time of digits (time to return to within 3°C below baseline temperature) was not determined after the final treatment for all horses because of scheduling constraints. Temperatures were monitored for a minimum of 30 minutes and a maximum of 305 minutes after the final treatment. Rewarming to within 3°C of baseline was not observed within 30 to 305 minutes after final wader and ice bag treatments.

Discussion

Results of the present study indicated that ice bag and wader boot treatments, both of which included use of ice slurry, caused similar significant reductions of digital laminar and venous temperatures in horses; digital laminar temperatures were reduced > 23°C and digital venous temperatures were reduced > 19°C, compared with temperatures during control conditions. Gel boot treatment did not cause significant cooling of digital laminar or venous temperatures. To the authors’ knowledge, continuous application of cryotherapy to the distal aspect of limbs is the only treatment that ameliorates severity of lesions attributable to acute oligofructose-induced laminitis in horses. Results of the present study support the continuous application of cryotherapy to the distal aspect of limbs of horses via a 5-L fluid bag or a 63-cm-tall vinyl wader boot filled with ice slurry (replenished at hourly intervals).

A crossover study design was used in the present study; all 3 methods of cryotherapy were investigated in each horse. The rationale for use of the same horses to investigate all 3 treatments was to control for potential variability among horses by use of a forelimb of each horse as a control limb during cryotherapy of contralateral forelimbs. This approach increased the statistical power of the study without requiring an increase in sample size, thus minimizing the number of horses used in the experiment. The order in which treatments were applied and forelimb (left or right) to which the first treatment was applied were randomized for each horse; limbs contralateral to those undergoing cryotherapy were not treated (control conditions). Subsequent treatments were not applied until digital laminar and venous temperatures returned to within 3°C below baseline temperatures in treated limbs. The minimum duration of the rewarming period was 30 minutes; however, the time required for temperatures in treated limbs to return to within 3°C below baseline temperatures was substantially longer following application of cryotherapy methods that resulted in substantial cooling.

In most horses, the minimum digital laminar temperature during wader boot and ice bag treatments was lower than the minimum digital venous temperature during those treatments. The mean difference between digital laminar and venous temperatures was greater for ice bag treatment (4°C) than for wader boot treatment (2°C). Immersion of an entire metacarpus in ice slurry (wader boot treatment) may increase the surface area available for counter-current heat exchange between digital arteries and veins, thereby resulting in lower digital venous temperatures than may be achieved with other treatments. No evidence of vasodilation or hyperthermia was detected in digits during or immediately after application of cryotherapy methods in the present study, although the 120-minute period of cryotherapy may have been too short to elicit such a response in the horses. Other authors detected periodic increases in hoof temperature in 4 of 6 control horses undergoing cryotherapy; there was an increase in hoof temperature approximately once per day in those horses. Recurrent periods of vasodilation were also detected in horses in which laminitis was induced by administration of carbohydrate; however, this response was not detected until 3 days after induction of laminitis.

Cryotherapy has been recommended for prevention of laminitis in horses affected by systemic diseases such as colitis, metritis, and pleuropneumonia. The mechanism of action by which cryotherapy may reduce severity of laminitis is undetermined. Potential mechanisms include a hypometabolic effect in tissues, leading to reduced digital laminar tissue requirements for energy metabolites; inhibition of proteases capable of degrading components of laminar extracellular matrix (eg, matrix metalloproteinase-2, matrix metalloproteinase-9, and a disintegrin and metalloproteinase with thrombospondin motifs-4); inhibition of inflammation within laminar tissues; and vasocorrection in digits causing reduction in delivery of circulating substances (as yet unidentified) capable of inducing laminitis.

Other authors have reported that cryotherapy of digits prevents upregulation of cytokine, chemokine, and cyclooxygenase-2 mRNA in digital laminar tissues during the developmental and acute phases of oligofructose-induced laminitis in horses.

Authors of another study found that cooling of isolated equine cutaneous digital arteries and veins from 30°C to 22°C enhances their contractile response to acetylcholine in vitro; this response is mediated by postsynaptic α2-adrenoceptors. Rho kinase activation and production of reactive oxygen species contribute to cooling-enhanced contractile responses in isolated digital cutaneous veins of horses. Cooling from 30°C to 22°C enhances serotoninergic-mediated contraction but inhibits α-adrenoceptor response in vitro preparations of digital small laminar arteries of horses; contraction is most evident in endothelium-denuded small lami-
nlar arteries, suggesting that endothelium may play an important role in preventing excessive vasoconstriction and maintaining blood flow during conditions of cool environmental temperature. An abundance of contradictory information exists regarding whether development of laminitis is predominantly associated with vasoconstriction, vasodilation, or formation of microthrombi. However, it appears that vasoconstriction develops more commonly than arterial constriction during laminitis. Vascular dysregulation and disruption of vascular homeostasis may be more appropriate terms for describing events during the developmental phase of laminitis because these events likely develop concurrently. Because digital venous or microvascular temperatures may indicate the efficacy of cryotherapy as accurately or more accurately than temperatures at the dermal-epidermal digital laminar interface, implantable thermocouples were placed in equine digital veins for comparison of those temperatures with laminar temperatures in the present study.

The digital temperature necessary to prevent development of laminitis in horses is not known. van Eps and Pollitt have reported that maintaining a hoof temperature < 5°C, as measured with a thermoprobe inserted into the stratum medium of the dorsal aspect of a hoof wall, is effective in preventing severe laminitis in horses that received an overload of carbohydrate via nasogastric intubation. In that study, cryotherapy of the distal aspect of limbs was performed continuously for 48 or 72 hours by means of a vinyl boot containing ice slurry or a continuously circulating water bath. Cryotherapy by use of these methods effectively prevents signs of lameness and results in reduced severity of digital laminar histologic scores and reduced expression of laminar matrix metalloproteinase-2 mRNA. A digital laminar temperature < 5°C was achieved in only 1 horse in the present study. Minimum laminar temperature during wader boot treatment was 11.14 ± 1.7°C in the present study, which was substantially warmer than the laminar temperature achieved in the study of van Eps and Pollitt. However, the present study included clinically normal horses, and the mean laminar temperatures achieved were similar to laminar temperatures reported by Pollitt and van Eps in clinically normal horses as application of ice slurry to a region that includes the hoof to the distal aspect of the antebrachium. In addition to being less cumbersome to apply than wader boots, 5-L fluid bags were more affordable and could be readily applied to all 4 limbs in horses affected by systemic conditions that place them at risk for developing laminitis.

Results of this study provided evidence that application of ice slurry to a region including the hoof and pastern may be as effective in lowering laminar and venous temperatures in the distal aspect of limbs of clinically normal horses as application of ice slurry to a region that includes the hoof to the distal aspect of the antebrachium. In addition to being less cumbersome to apply than wader boots, 5-L fluid bags were more affordable and could be readily applied to all 4 limbs in horses affected by systemic conditions that place them at risk for developing laminitis.

References


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