

Equine laminitis: cryotherapy reduces the severity of the acute lesion

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Summary

Reasons for performing study: The hypometabolic and vasoconstrictive effects of cryotherapy could prevent the development of laminitis.

Objectives: To use distal limb cryotherapy to prevent laminitis induced by alimentary carbohydrate overload.

Methods: Laminitis was induced in 6 Standardbred horses that had one front limb continuously cooled in an ice/water mixture. Lameness evaluation, blinded lamellar histological grading and analysis for lamellar matrix metalloproteinase-2 (MMP-2) mRNA expression were used to evaluate the severity of laminitis.

Results: Cryotherapy was well tolerated and effective in cooling the feet. In each horse no lameness was observed in the treated limbs. Laminitis histology scores in the treated limbs were significantly less than those of the corresponding untreated forelimbs ($P < 0.05$). Laminitis histology scores in the treated limbs were also significantly less than those of the untreated limbs (fore- and hind) as a group ($P < 0.05$). Expression of MMP-2 mRNA in the iced feet was significantly ($P < 0.05$) less than that detected in the untreated feet.

Conclusions: Cryotherapy, when applied to one foot, markedly reduced the severity of acute laminitis in this study. We propose that vasoconstriction (preventing delivery of haematogenous trigger factors) and hypometabolism (reduction in lamellar MMP activity) were the primary therapeutic mechanisms.

Potential relevance: Although further research is needed, we suggest cryotherapy as a potentially effective prophylactic strategy in horses at risk of developing acute laminitis.

Introduction

Laminitis researchers remain divided as to the basic mechanisms that ultimately result in failure of the attachment apparatus between the hoof wall and distal phalanx (Pollitt 1996). There are 2 broad pathophysiological hypotheses accounting for laminitis that develops secondary to systemic disease (Hood 1999). The first proposes that digital hypoperfusion during the developmental stage leads to ischaemia of lamellar tissue. Support for decreased sublamellar perfusion during developmental and acute laminitis came from the studies of Coffman *et al.* (1970), Hood *et al.* (1978,

2001) and Adair *et al.* (2000). Proposed mechanisms of digital hypoperfusion include vasospasm (Hood *et al.* 1993), elevated digital interstitial pressure and oedema (Allen *et al.* 1990), microthrombus formation (Weiss *et al.* 1994, 1995), and shunting of blood through arteriovenous anastomoses (Robinson *et al.* 1976; Hood *et al.* 1978; Molyneux *et al.* 1994).

Excessive uncontrolled enzymatic degradation of lamellar attachments, caused by haematogenous 'laminitis trigger factors' delivered to the foot, forms the basis of the second hypothesis (Pollitt 1999a). Lamellar basement membrane (BM) destruction is an early and key event in the development of acute laminitis (Pollitt 1996). Inappropriate release of excess, activated matrix metalloproteinase (MMP), capable of initiating BM lysis and separation from lamellar epidermal cells, is thought to be responsible (Pollitt *et al.* 1998). Increased activated MMPs have been demonstrated in hoof tissue from horses with laminitis (Johnson *et al.* 1998; Pollitt *et al.* 1998). Up-regulation of the gene controlling matrix metalloproteinase-2 (MMP-2) activity has also been demonstrated in tissues affected by acute laminitis (Kyaw-Tanner and Pollitt 2004). If, as hypothesised, the haematogenous delivery of trigger factors is responsible for increasing lamellar MMP activity, then normal or enhanced blood flow would augment the delivery of these factors during the developmental stage of laminitis. Three studies (Robinson *et al.* 1976; Trout *et al.* 1990; Pollitt and Davies 1998) reported increased digital blood flow preceding and during acute laminitis induced by alimentary carbohydrate overload. Indeed, digital vasoconstriction during the developmental stage appeared to protect horses against laminitis (Pollitt and Davies 1998) and led Pollitt (1999b) to suggest that promoting digital vasoconstriction using cryotherapy may be an effective strategy to prevent laminitis.

Scalp cryotherapy prevents alopecia in human cancer patients undergoing chemotherapy (Katsimbri *et al.* 2000). Vasoconstriction apparently reduces delivery of the chemotherapeutic agent to the scalp, and cellular uptake and metabolism are reduced when residual drug reaches the hair follicles (Dean *et al.* 1979; Bulow *et al.* 1985). Cryotherapy could be used, therefore, during the developmental stage of laminitis to reduce delivery of laminitis trigger factors to the digit, as well as to reduce the activity of lamellar MMPs.

Since prolonged, continuous cryotherapy had no deleterious effect on normal horses (Pollitt and van Eps 2004), we evaluated its efficacy, when continuously applied to one limb, in preventing laminitis induced by alimentary carbohydrate overload.

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Fig 1: The horses were kept in stocks with the left forelimb in a rubber boot containing ice and water (a) to a level just below the carpus. Hoof temperature probes attached to plastic brackets (b) were inserted into predrilled holes in the dorsal hoof wall of both forelimbs and secured with screws (c).

Materials and methods

The experiments were conducted according to guidelines approved by the University of Queensland Animal Experimentation Ethics Committee, under the supervision of the Animal Welfare Officer.

Ten mature, Standardbred, horses (6 geldings and 4 mares) with normal feet and no lameness were used in the experiment. Four of the horses (3 geldings and 1 mare) were used exclusively as controls for the MMP-2 mRNA analysis, and were subjected to euthanasia, without further interference, by overdosing with barbiturate.

The remaining 6 horses (3 geldings and 3 mares) were selected for laminitis induction and were housed and fed in stables for 3 weeks prior to experimentation. Induction of laminitis was achieved using alimentary overload with the carbohydrate oligofructose. After administration of the bolus dose (10 g/kg bwt) of oligofructose (OF) in 4 l of water via nasogastric tube, each horse was confined to stocks for the duration of the 48 h experiment with free access to feed and water. The left forelimb of each horse was placed in a rubber boot (Bigfoot Ice boot)¹ containing a mixture of 50% cubed ice and 50% water (Fig 1a). The cryotherapy method of Pollitt and van Eps (2004) was modified; the level of ice and water was raised to just below the carpus to improve distal limb cooling efficiency. The boot was open at the top and the limb was free to move within the boot. The sole of the boot was 5 mm thick.

Forelimb hoof temperature (HT), ambient temperature, and internal ice boot temperature were logged continuously (Pollitt and van Eps 2004). However, the stainless steel brackets housing the HT thermistor probes were replaced with thicker plastic brackets to better insulate the HT probes (Figs 1b,c). Hindlimb HT was measured at 2 h intervals using an infrared scanning device (Infrared Temperature Scanner)². Rectal temperature was also

recorded at 2 h intervals. Appetite, demeanour, oral mucous membrane capillary refill time, faecal output, heart rate and pulse quality were also monitored every 2 h as indicators of clinical status (data are not included in the results).

Two hours prior to administration of the bolus dose of OF, and 10 mins after removal of the ice boot at 48 h, the horses were evaluated for lameness. All horses were walked toward and away from the observer and circled to the right and left. If mild or no lameness was detected at the walk then the horses were also trotted toward and away from the observer. Lameness of the forelimbs was graded using the system described by Stashak (1987). The presence or absence of hindlimb lameness was also recorded. Following the final lameness examination, all horses were subjected to euthanasia by overdosing with barbiturate, and the fore and hind feet removed and processed for histology (Pollitt 1996). Duplicate sections of the dorsal hoof lamellae from each foot, stained with haematoxylin and eosin and periodic acid-Schiff, were randomised, coded and examined using light microscopy by 4 blinded evaluators who graded the severity of laminitis using the scoring system of Pollitt (1996).

Samples of lamellar tissue from each hoof were rapidly frozen by immersion in liquid nitrogen and stored at -70°C . Samples of lamellar tissue from one hoof of each of the 4 control horses (2 forelimbs and 2 hindlimbs) were prepared similarly. All samples were later subjected to real-time polymerase chain reaction (PCR) analysis for MMP-2 mRNA (Kyaw-Tanner and Pollitt 2004). The magnitude of MMP-2 gene expression was normalised to the 4 controls.

Temperature data were analysed over time using one-way analysis of variance (ANOVA) on repeat measures, and at specific time points using paired *t* tests. Histological data were analysed using non-parametric tests applied to the median scores of the four

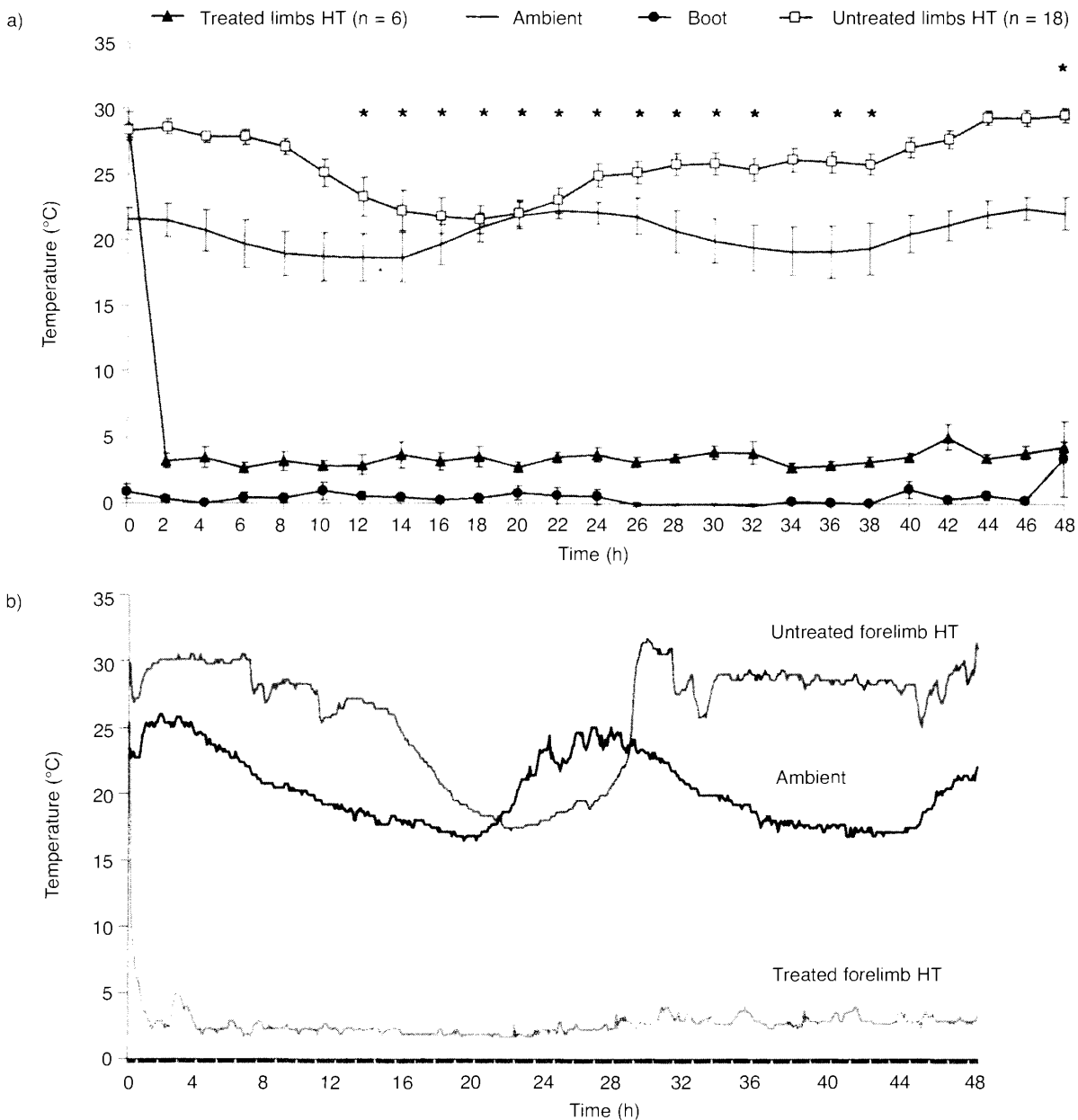


Fig 2: Temperature data. a) Mean \pm s.e. hoof temperature (HT) of the treated limbs compared to the mean \pm s.e. HT of the untreated fore- and hindlimbs. The mean \pm s.e. ambient and ice boot temperatures are also shown. The mean HT of the treated limbs was significantly less ($P < 0.05$) than that of all the untreated limbs by 2 h. The mean HT of all the untreated limbs at 12–32 h and 36–38 h after dosing was significantly less than that at 0 h, and at 48 h was significantly greater than that at 0 h ($P < 0.05$, indicated by *). b) A typical trace of the HT of the treated forelimb and untreated forelimb compared with ambient temperature in an individual horse (Cryo 4). Note the decreased HT of the untreated forelimb between 13 and 22 h followed by the rapid increase in HT between 28 and 31 h. After an initial rapid decrease, the treated limb HT remains below 5°C.

evaluators for each limb of each horse. Histological scores for the treated limbs ($n = 6$) were compared to those of the corresponding untreated forelimbs ($n = 6$) of each horse using a Wilcoxon signed ranks tests, and to those of all the untreated limbs as a group ($n = 18$) using Mann-Whitney analysis. Inter-evaluator agreement was tested using a weighted Kappa test. The real-time PCR results for the treated limbs ($n = 6$) were compared with the corresponding untreated forelimbs ($n = 6$) of each horse using paired t tests, and to that of all the untreated limbs as a group ($n = 18$) using an independent t test. The real-time PCR results from the 4 normal control hooves were compared with the treated limbs ($n = 6$) and all the untreated limbs as a group ($n = 18$) using independent t tests. The

subjective lameness data was not analysed statistically. A significance level of $P < 0.05$ was chosen. Results are expressed as the mean \pm s.e. Statistical analysis was conducted using computer software (Analyse-it, version 1.67)³.

Results

The horses tolerated restraint in the stocks and maintained the treated limbs within the ice boot voluntarily. Clinically, OF dosing was characterised by profuse, watery diarrhoea at 12–16 h that ceased by 36–44 h. All the horses developed mild to moderate depression and inappetance at 12–16 h that persisted until 28–36 h.

TABLE 1: Laminitis histology scores (0 = normal, 1 = mild, 2 = moderate, 3 = severe) for the 6 horses by evaluator

Horse ID	Evaluator	Histological score			
		Treated forelimb	Untreated forelimb	LH	RH
Cryo 1	A	0	2	1	1
	B	1	2	2	1
	C	1	2	1	1
	D	0	3	2	2
Median	0.5	2	1.5	1	
Cryo 2	A	0	2	1	1
	B	1	1	1	1
	C	0	2	1	1
	D	0	2	2	1
Median	0	2	1	1	
Cryo 3	A	0	3	2	3
	B	1	3	2	3
	C	1	3	2	2
	D	0	3	2	3
Median	0.5	3	2	3	
Cryo 4	A	0	2	2	2
	B	0	2	2	1
	C	0	3	2	2
	D	0	2	2	2
Median	0	2	2	2	
Cryo 5	A	0	1	0	0
	B	1	2	1	1
	C	1	3	1	1
	D	0	3	0	0
Median	0.5	2.5	0.5	0.5	
Cryo 6	A	0	1	2	1
	B	1	2	2	1
	C	1	1	2	1
	D	0	1	2	1
Median	0.5	1	2	1	

LH = Left hind. RH = Right hind.

One horse (*Cryo 6*) received 35 l of balanced polyionic fluid i.v. between 30 and 38 h because of deteriorating cardiovascular status (tachycardia with a weak pulse) and responded satisfactorily.

Lameness

No horse was lame at the initial evaluation prior to administration of the OF bolus. Following administration of the OF bolus, all horses appeared to distribute weight evenly between the forelimbs initially. Frequent, sometimes prolonged, lifting of the untreated fore- and hind feet occurred in all horses beginning between 28 and 46 h. After removal of the ice boot at 48 h, all horses were obviously lame in the untreated forelimb at the walk (head and neck lifting occurred during weight bearing on the untreated forelimb). All horses were given a lameness *grade* of 3/4 based on the lameness in the untreated forelimb. *Cryo 4* had the mildest lameness (though still appreciable at the walk) and was the only horse subjected to examination at the trot. *Cryo 5* had the most severe lameness in the untreated forelimb but could still bear weight on the limb. Lameness was not observed in the treated limb of any horse. Lameness was judged to be present in the hindlimbs of all horses except *Cryo 5*.

Temperature data

Rectal temperature increased gradually to a maximum at 28 h ($39.5 \pm 0.4^\circ\text{C}$) before returning to near starting values at 48 h

($38.5 \pm 0.3^\circ\text{C}$). Ambient temperature was uncontrolled and fluctuated diurnally (Fig 2a).

Ice boot temperature was $0.5 \pm 1.7^\circ\text{C}$. The HT of the treated limbs decreased rapidly within the first hour, and remained below 5°C for the remainder of the experimental period (mean $3.5 \pm 0.9^\circ\text{C}$). The HT of the untreated fore- and hindlimbs showed some fluctuation. At 12–32 h and 36–38 h after dosing the HT was significantly less ($P < 0.05$) than at 0, and at 48 h was significantly greater ($P < 0.05$) than at 0 h (Fig 2a). The HT of the untreated forelimb of each horse, except *Cryo 5*, initially decreased slowly, and the mean HT of the untreated forelimbs was not significantly different from ambient temperature between 12 and 28 h. Subsequently, there was a sharp HT increase (from below 25°C to above 30°C within 3 h) that restored the HT to or above that at 0 h, after which it persisted at or near that at 0 h (Fig 2b). The HT of the untreated forelimb of *Cryo 5* did not decrease to ambient temperature, and the HT increased gradually over the final 12–14 h of the experimental period.

Lamellar histology

Median laminitis histology scores of the treated feet of *Cryo 2* and *Cryo 4* were 0 (normal), with the remaining horses having median scores of 0.5 (Table 1). The untreated forefeet had median scores between 1 and 3. All hind feet had median scores of at least 1, with the exception of *Cryo 5* that had median scores of 0.5. Histological scores of the untreated forefeet were significantly greater ($P < 0.05$) than that of the treated forefeet. Histological scores in the untreated fore- and hindlimbs as a group ($n = 18$) were significantly greater ($P < 0.05$) than that of the treated limbs ($n = 6$).

Detachment of the BM from the secondary epidermal lamellae was present in all of the untreated feet except the hind feet of *Cryo 5*. Detachment of the BM was not seen in any of the treated feet.

Inter-evaluator agreement, expressed as a weighted Kappa statistic, ranged from 0.46 (between evaluators B and D) to 0.79 (between evaluators A and D). The mean agreement was 0.56.

Real-time PCR analysis

In all instances (except the right hind foot of *Cryo 1*) MMP-2 mRNA expression was greater in each of the untreated feet than the corresponding treated foot of each horse. Expression in the treated feet was consistently low, range 1.19–1.56 (mean 1.39 ± 0.06). Expression in the untreated feet was more variable, range 1.14–4.44 (mean 2.26 ± 0.20). MMP-2 mRNA expression in the treated feet was significantly less ($P < 0.05$) than that of the corresponding untreated forefeet. Mean MMP-2 mRNA expression magnitude in the treated feet ($n = 6$) was significantly less ($P < 0.05$) than that of all the untreated feet as a group ($n = 18$), but was significantly greater ($P < 0.05$) than that of the 4 normal control feet (mean 1.04 ± 0.02) (Fig 3).

Discussion

Application of the ice and water mixture was effective in cooling the treated foot and was well tolerated by the horses. The 5 mm thick floor of the ice boot affected neither stance nor, presumably, weight distribution. Interestingly, the foot lifting, characteristic of laminitis (Obel 1948), observed in the untreated limbs, was absent in the treated limbs. Raising the ice and water level resulted in

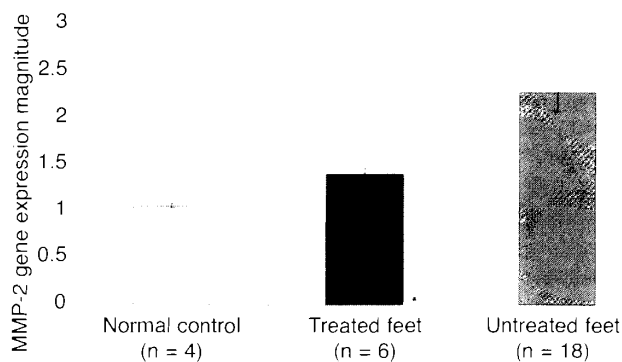


Fig 3: Real-time PCR analysis results. Mean \pm s.e. MMP-2 mRNA expression magnitude in the treated feet was significantly less ($P < 0.05$) than that of all the untreated feet as a group, but was significantly greater ($P < 0.05$) than that of the normal control feet.

more profound cooling of the digit compared with previous results (Pollitt and van Eps 2004). The apparent resilience of the equine distal limb to the potentially damaging effects of prolonged, extreme cold confirmed the results of Pollitt and van Eps (2004) and enabled the use of cryotherapy in the manner described.

Cryotherapy, when applied to one foot, markedly reduced the severity of acute laminitis in this study. Cryotherapy completely prevented the development of laminitis histopathology in the treated feet of 2 horses. The treated feet of the remaining 4 horses had some minor histopathological changes (slight elongation of the secondary epidermal lamellae and/or changes in basal cell nuclear morphology and position). However, the key lesion of laminitis (detachment of the BM from the secondary epidermal lamellae) was not present, and none of the treated feet had sufficient histopathology to be categorised as 'mild' within the grading system used. Histological scores of the treated feet were significantly less ($P < 0.05$) than those of the untreated feet. Genetic up-regulation of MMP-2, implicated in the pathogenesis of laminitis (Johnson *et al.* 1998; Pollitt *et al.* 1998; Kyaw-Tanner and Pollitt 2004), was also significantly reduced in the treated limbs ($P < 0.05$). Subjectively, cryotherapy also prevented development of the lameness typical of laminitis.

The absence of any marked variation in the HT of the treated limbs suggests that sublamellar perfusion and metabolic rate remained consistently low (Hood *et al.* 2001). The HT data from the untreated feet should be interpreted with caution due to the fluctuations in ambient temperature (Hood *et al.* 2001). Nevertheless, the period between 12 and 28 h when the untreated forelimb HT was not significantly different from ambient temperature likely represents a period of vasoconstriction and/or hypometabolism. Relative increases in digital perfusion and/or metabolic rate are difficult to detect via HT measurement at higher ambient temperatures such that were present in the current study (Hood *et al.* 2001). However, the sharp temperature rise (observed in the untreated forelimb of all but one horse), and the sustained increase in untreated limb HT that followed, likely represent a period of relative digital vasodilation and/or increased metabolic rate. A significant increase in the HT of all the untreated limbs over 0 h values, however, was present only at 48 h. Unfortunately, the time of onset of lameness relative to the HT changes could not be established due to the study design. The sharp HT rise observed in the untreated forelimbs would probably have been reflected in the mean HT of all the untreated limbs (Fig 2a) had normalisation of the temperature data to the time of onset of lameness been possible. Interestingly, the untreated forelimb of one horse in the study did

not experience a marked HT reduction, and the HT increase was more gradual. Despite this, moderate to severe laminitis histopathology developed in this limb.

The basic mechanisms of cryotherapy, namely the vascular and hypometabolic effects (Swenson *et al.* 1996), provide insight into the pathophysiology of acute laminitis. Substances delivered via the circulation to the digit, such as cytokines (Pollitt 1996) and bacterial products of hindgut origin (Mungall *et al.* 2001), have been proposed as potential initiators of MMP mediated lamellar separation. Profound, continuous vasoconstriction may have prevented the delivery of these haematogenous 'laminitis trigger factors' to the treated digit in this study. Such profound vasoconstriction would seem contraindicated if digital hypoperfusion was the primary mechanism involved in the development of laminitis (Hood *et al.* 1993). Indeed, cryotherapy is contraindicated in human patients with peripheral vascular diseases, such as Raynaud's phenomenon (Lehmann and de Lateur 1990).

The hypometabolic effect of cryotherapy is potent and complex (Zachariassen 1991). Metabolic rate and oxygen consumption are inversely related to tissue temperature (Fuhrman and Fuhrman 1959). Directly or indirectly, cryotherapy reduced the expression of MMP-2 mRNA in the lamellar tissue of the treated feet in this study and prevented the development of significant histological lesions. Cryotherapy has been shown to protect against tumor necrosis factor- α -induced microvascular perfusion failure, apoptosis, and leucocyte adhesion in the striated muscle of hamsters (Westerman *et al.* 1999). Polymorphonuclear leukocyte infiltration has been implicated as a contributor to BM separation and lamellar damage by Pollitt (1996). Preventing this with cryotherapy may be beneficial. Local mRNA expression of the potent cytokine interleukin-1 β during the prodromal stages of laminitis (Fontaine *et al.* 2001) could also be limited by cryotherapy.

Mild histological changes and slightly elevated MMP-2 mRNA expression were present in some of the treated limbs and a direct noxious effect of cryotherapy could possibly have caused this. However, we conclude that some circulatory delivery of laminitis trigger factors still occurred to activate lamellar MMPs in the cooled feet, albeit at a much reduced level. The mild increase in MMP-2 mRNA expression in the treated limbs in conjunction with mild histological changes supports the notion that these enzymes are involved early in the development of the laminitis lesion.

The design of the current study lacked absolute control measures for the rubber boot and the column of ice/water surrounding the treated limb. Although unlikely, it is possible that a factor other than the cooling effect may have contributed to reducing the severity of laminitis in the treated limbs. For ethical reasons the experiments were terminated at 48 h, soon after the foot pain of laminitis appeared. What was not determined is whether laminitis could still have developed after cryotherapy ceased. The lameness grading system traditionally used for laminitis (Obel 1948) is unsuitable for comparison between limbs and therefore the general lameness grading system of Stashak (1987) was used in this study. Although lameness was not observed in the treated limbs in this study, it is possible that mild lameness may have been present but was undetectable in the face of the moderate-severe lameness in the untreated forelimbs. The reliability of the histopathology grading system (Pollitt 1996) was assessed by measurement of the level of inter-evaluator agreement. The calculated Kappa statistic, which can vary from -1 (no agreement) through 0 (agreement by chance) to 1 (perfect agreement), can be interpreted on the scale developed by Landis and Koch (1977): 0.81–1.00, 'almost perfect';

0.61–0.80, 'substantial'; 0.41–0.60, 'moderate'; 0.21–0.40, 'fair'; 0.00–0.20, 'slight'; and <0.0, 'poor' agreement. The inter-evaluator agreement in this study ranged from moderate to substantial, with the mean agreement (0.56) marginally below what is considered substantial. This compares favourably with the agreement between pathologists evaluating neoplasia of the colon/rectum (Jensen *et al.* 1995) and prostate (Allam *et al.* 1996) in human patients, where Kappa statistics were 0.20–0.45. We conclude that the grading system of Pollitt (1996) was satisfactory in this study; however, improved evaluator training and more exact criteria for grading could improve the reliability of the system for future studies.

The results of this study suggest that the severity of acute laminitis may be reduced if distal limb cryotherapy is applied during the developmental stage of the disease. We propose that cryotherapy prevents haematogenous delivery of laminitis trigger factors to the lamellar tissue through vasoconstriction of the digital circulation. The low temperature achieved by the application of ice and water to the equine distal limb inhibits MMP production and activity, even if triggering factors are present. Although we suggest cryotherapy as a potentially effective prophylactic strategy in horses at risk of developing acute laminitis, we currently have no data to support cryotherapy in established acute or chronic laminitis cases.

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Manufacturers' addresses

¹Bigfoot Ice Boots, Esk, Queensland, Australia.

²Exergen Corporation, Natick, Massachusetts, USA.

³Analyse-it Software Ltd., Leeds, England, UK.

References

- Adair, H.S., 3rd, Goble, D.O., Schmidhammer, J.L. and Shires, G.M. (2000) Lamellar microvascular flow, measured by means of laser Doppler flowmetry, during the prodromal stages of black walnut-induced laminitis in horses. *Am. J. vet. Res.* **61**, 862–868.
- Allam, C.K., Bostwick, D.G., Hayes, J.A., Upton, M.P., Wade, G.G., Domanowski, G.F., Klein, M.A., Boling, E.A. and Stilmant, M.M. (1996) Interobserver variability in the diagnosis of high-grade prostatic intraepithelial neoplasia and adenocarcinoma. *Mod. Pathol.* **9**, 742–751.
- Allen, D., Jr., Clark, E.S., Moore, J.N. and Prasse, K.W. (1990) Evaluation of equine digital Starling forces and hemodynamics during early laminitis. *Am. J. vet. Res.* **51**, 1930–1934.
- Bulow, J., Friberg, L., Gaardsting, O. and Hansen, M. (1985) Frontal subcutaneous blood flow, and epi- and subcutaneous temperatures during scalp cooling in normal man. *Scand. J. Clin. Lab. Invest.* **45**, 505–508.
- Coffman, J.R., Johnson, J.H., Guffy, M.M. and Finocchio, E.J. (1970) Hoof circulation in equine laminitis. *J. Am. vet. med. Ass.* **156**, 76–83.
- Dean, J.C., Salmon, S.E. and Griffith, K.S. (1979) Prevention of doxorubicin-induced hair loss with scalp hypothermia. *N. Engl. J. Med.* **301**, 1427–1429.
- Fontaine, G.L., Belknap, J.K., Allen, D., Moore, J.N. and Kroll, D.L. (2001) Expression of interleukin-1beta in the digital laminae of horses in the prodromal stage of experimentally induced laminitis. *Am. J. vet. Res.* **62**, 714–720.
- Fuhrman, G.J. and Fuhrman, F.A. (1959) Oxygen consumption of animals and tissues as a function of temperature. *J. Gen. Physiol.* **42**, 715–722.
- Hood, D.M. (1999) The pathophysiology of developmental and acute laminitis. *Vet. Clin. N. Am.: Equine Pract.* **15**, 321–343.
- Hood, D.M., Wagner, L.P. and Brumbaugh, G.W. (2001) Evaluation of hoof wall surface temperature as an index of digital vascular perfusion during the prodromal and acute phases of carbohydrate-induced laminitis in horses. *Am. J. vet. Res.* **62**, 1167–1172.
- Hood, D.M., Grosenbaugh, D.A., Mostafa, M.B., Morgan, S.J. and Thomas, B.C. (1993) The role of vascular mechanisms in the development of acute equine laminitis. *J. vet. intern. Med.* **7**, 228–234.
- Hood, D.M., Amoss, M.S., Hightower, D., McDonald, D.R., McGrath, J.P., McMullan, W.C. and Scrutchfield, W.L. (1978) Equine laminitis I: Radioisotopic analysis of the haemodynamics of the foot during the acute disease. *J. equine med. Surg.* **2**, 439–444.
- Jensen, P., Krosgaard, M.R., Christiansen, J., Braendstrup, O., Johansen, A. and Olsen, J. (1995) Observer variability in the assessment of type and dysplasia of colorectal adenomas, analyzed using kappa statistics. *Dis. Colon Rectum* **38**, 195–198.
- Johnson, P.J., Tyagi, S.C., Katwa, L.C., Ganjam, V.K., Moore, L.A., Kreeger, J.M. and Messer, N.T. (1998) Activation of extracellular matrix metalloproteinases in equine laminitis. *Vet. Rec.* **142**, 392–396.
- Katsimbri, P., Bamias, A. and Pavlidis, N. (2000) Prevention of chemotherapy-induced alopecia using an effective scalp cooling system. *Eur. J. Cancer* **36**, 766–771.
- Kyaw-Tanner, M. and Pollitt, C.C. (2004) Equine laminitis: increased transcription of matrix metalloproteinase-2 (MMP-2) occurs during the developmental phase. *Equine vet. J.* **36**, 221–225.
- Landis, J.R. and Koch, G.G. (1977) The measurement of observer agreement for categorical data. *Biometrics* **33**, 159–174.
- Lehmann, J.F. and de Lateur, B.J. (1990) Cryotherapy. In: *Therapeutic Heat and Cold*, 4th edn., Ed: J.F. Lehmann, Williams & Wilkins, Baltimore, pp 590–631.
- Molyneux, G.S., Haller, C.J., Mogg, K. and Pollitt, C.C. (1994) The structure, innervation and location of arteriovenous anastomoses in the equine foot. *Equine vet. J.* **26**, 305–312.
- Mungall, B.A., Kyaw-Tanner, M. and Pollitt, C.C. (2001) *In vitro* evidence for a bacterial pathogenesis of equine laminitis. *Vet. Microbiol.* **79**, 209–223.
- Obel, N. (1948) *Studies of the Histopathology of Acute Laminitis*. Almqvist and Wileseells Bottrykeri Ab Uppsala (Thesis).
- Pollitt, C.C. (1996) Basement membrane pathology: a feature of acute equine laminitis. *Equine vet. J.* **28**, 38–46.
- Pollitt, C.C. (1999a) Equine laminitis: a revised pathophysiology. *Proc. Am. Ass. equine Practitrs.* **45**, 188–192.
- Pollitt, C.C. (1999b) Equine laminitis: Current concepts of inner hoof wall anatomy, physiology and pathophysiology. In: *Large Animal Proceedings of the Ninth Annual American College of Veterinary Surgeons Symposium*, San Francisco, pp 175–180.
- Pollitt, C.C. and Davies, C.T. (1998) Equine laminitis: its development coincides with increased sublamellar blood flow. *Equine vet. J., Suppl.* **26**, 125–132.
- Pollitt, C.C. and van Eps, A.W. (2004) Prolonged, continuous distal limb cryotherapy in the horse. *Equine vet. J.* **36**, 216–220.
- Pollitt, C.C., Pass, M.A. and Pollitt, S. (1998) Batimastat (BB-94) inhibits matrix metalloproteinases of equine laminitis. *Equine vet. J., Suppl.* **26**, 119–124.
- Robinson, N.E., Scott, J.B., Dabney, J.M. and Jones, G.A. (1976) Digital vascular responses and permeability in equine alimentary laminitis. *Am. J. vet. Res.* **37**, 1171–1176.
- Stashak, T.S. (1987) Diagnosis of lameness. In: *Adams' Lameness in horses*, 4th edn., Eds: O.R. Adams and T.S. Stashak, Lea & Febiger, Philadelphia, pp 100–156.
- Swenson, C., Sward, L. and Karlsson, J. (1996) Cryotherapy in sports medicine. *Scand. J. med. Sci. Sports* **6**, 193–200.
- Trout, D.R., Hornof, W.J., Linford, R.L. and O'Brien, T.R. (1990) Scintigraphic evaluation of digital circulation during the developmental and acute phases of equine laminitis. *Equine vet. J.* **22**, 416–421.
- Weiss, D.J., Trent, A.M. and Johnston, G. (1995) Prothrombotic events in the prodromal stages of acute laminitis in horses. *Am. J. vet. Res.* **56**, 986–991.
- Weiss, D.J., Geor, R.J., Johnston, G. and Trent, A.M. (1994) Microvascular thrombosis associated with onset of acute laminitis in ponies. *Am. J. vet. Res.* **55**, 606–612.
- Westermann, S., Vollmar, B., Thorlacius, H. and Menger, M.D. (1999) Surface cooling inhibits tumor necrosis factor-alpha-induced microvascular perfusion failure, leukocyte adhesion, and apoptosis in the striated muscle. *Surgery* **126**, 881–889.
- Zachariassen, K.E. (1991) Hypothermia and cellular physiology. *Arctic med. Res., Suppl.* **6**, 13–17.