

Therapeutic Hypothermia (Cryotherapy) to Prevent and Treat Acute Laminitis

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KEYWORDS

• Equine • Laminitis • Cryotherapy • Distal limb

Laminitis is a debilitating disease of horses that causes significant morbidity and mortality. Laminitis lesions are generally considered irreversible, and there is currently no effective treatment. Prevention of laminitis in horses considered at risk and halting the progression of acute laminitis are therefore key areas for clinicians to focus their efforts. Most laminitis cases occur as a result of metabolic disturbances in conjunction with the consumption of carbohydrate-rich pasture or hay.¹ In these cases the laminitis is often insidiously progressive and episodic, making identification of the developmental period difficult. Severe, acute laminitis is a common sequel to numerous primary diseases, including colitis, pneumonia, metritis, and rhabdomyolysis. In these cases, the developmental period is more easily predictable; therefore, prevention or early interventional treatment may be possible. Digital hypothermia during the developmental phase has been shown to ameliorate experimentally induced acute laminitis,^{2,3} and has recently gained popularity in clinical cases for the prevention and treatment of acute laminitis.

Distal limb cryotherapy is commonly used in horses, particularly for the treatment of musculoskeletal injuries, although protocols are largely extrapolated from human medicine. The equine distal limb is highly resilient in the face of profound continuous hypothermia,⁴ providing a unique therapeutic opportunity. The appropriate protocols aimed at preventing and treating acute laminitis differ significantly from those that are traditionally used for the treatment of athletic injury. This article reviews the effects of hypothermia on tissue, and discusses the rationale and suggested protocols for the usage of distal limb cryotherapy in the prevention and treatment of laminitis based on current information.

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THE EFFECTS OF HYPOTHERMIA ON TISSUE

The physiologic effects of hypothermia on tissue are complex and still poorly understood. The major effects of hypothermia on most tissues are analgesia, hypometabolism, and a vascular response.⁵ Cold has a direct effect on the peripheral nerves; it reduces conduction velocity, increases the threshold for stimulation, and increases the refractory period after stimulation.⁶ Hypothermia has a profound inhibitory effect on tissue metabolism: tissue metabolic rate and oxygen consumption are directly related to temperature.⁷ Hypothermia has been best studied in brain tissue for its neuroprotective effects after traumatic and ischemic brain injury.⁸ Cerebral metabolism decreases by 6% to 10% for each 1°C decrease in body temperature.^{9–11} A reduced requirement of cooled tissue for oxygen, glucose, and other metabolites enhances the survival of cells during periods of ischemia.¹² This mechanism is believed to protect tissue on the periphery of an injury from secondary hypoxic damage,⁵ and is also the basis for the use of cryotherapy in organ transplant surgery.¹³ Hypothermia reduces apoptosis, mitochondrial failure, and inflammation after cerebral ischemia-reperfusion.⁸ A reduction in metabolic enzymatic activity of approximately 50% has been observed with a reduction in tissue temperature of 10°C.¹⁴ The activity of collagenases is significantly reduced at lower temperatures.¹⁵

Hypothermia exerts a profound anti-inflammatory effect through reduced production and activity of proinflammatory cytokines (interleukin [IL]-1 β , IL-2, IL-6, and IL-8)^{16–18}; increased production of anti-inflammatory cytokines (IL-10)^{16,19}; reduced rolling and adhesion of leukocytes^{20,21}; and reduced production of oxygen radicals by polymorphonuclear leukocytes.²² Cryotherapy has been traditionally used to reduce inflammation in musculoskeletal injury, particularly after surgery.^{5,12,23} Recently there has been interest in the profound anti-inflammatory effect of hypothermia on end-organ damage in models of sepsis and systemic inflammatory response syndrome.^{16,19,24,25} Preemptive hypothermia (10°C < normal) markedly reduced the severity of acute lung injury in a rat model of sepsis by reducing neutrophil emigration, inhibiting proinflammatory cytokine activity, and increasing anti-inflammatory cytokine activity.¹⁶ In a subsequent study, less profound hypothermia (5°C < normal), applied even after the lung was primed with neutrophilic inflammation, also decreased the severity of acute lung injury, suggesting a therapeutic role for hypothermia beyond its preventive effect.²⁵

Cryotherapy causes potent local vasoconstriction.²⁶ This is largely mediated by sympathetic nervous control; however, a direct constrictive effect on blood vessel walls may occur, particularly at lower temperatures.²⁷ Periods of transient vasodilation (cold-induced vasodilation [CIVD]) may occur when temperatures are reduced to below approximately 18°C.²⁸ A cyclic pattern of increasing and decreasing blood flow (the hunting reaction) may be noted.²⁹ CIVD has been studied mostly in the human hand; however, it has also been noted in the face, forearms, and feet.³⁰ In the human finger, CIVD seems to occur as a result of dilation of the arteriovenous anastomoses (AVAs), whereas cold-induced vasoconstriction seems to occur as a result of constriction of AVAs and the arteries supplying the finger.³¹ A recent study using direct microcirculatory observation showed marked arteriolar vasoconstriction with local cooling to 8°C for 30 minutes.³² It is generally accepted that the application of cryotherapy results in a marked net reduction in local perfusion.^{8,33}

Profound whole body hypothermia (>10°C below normal) can result in severe side effects associated with cardiac, endocrine, and metabolic function.⁸ Hypothermia is also associated with coagulopathy and increased risk of infection.⁸ Adverse effects of locally applied cryotherapy are rare, but may include frostbite and nerve palsy.^{5,34}

The temperatures and duration of exposure required to induce frostbite are unclear.³⁵ Nerve palsy is a rare complication of cryotherapy in human patients, and usually involves large superficial nerves.⁵ Prolonged exposure to the combination of cold and moisture has been associated with the development of immersion foot and trench foot in human patients.³⁶ These conditions cause local swelling and pain that may progress to blistering of the skin, nerve damage, and gangrene.³⁷ Cryotherapy is contraindicated in humans with peripheral vascular diseases, such as Raynaud phenomenon, because of its potent vasoconstricting effect.²⁶

THERAPEUTIC HYPOTHERMIA OF THE EQUINE DISTAL LIMB

Although cryotherapy is commonly used for the treatment and prophylaxis of musculoskeletal injuries in horses, there are few controlled studies evaluating the effects of cryotherapy on the equine distal limb, and treatment recommendations are largely based on extrapolation from protocols used in humans.^{38–40} Distal to the carpus and tarsus, the limbs of the horse are devoid of muscle and the major blood vessels are superficial; this is seemingly ideal for inducing deep hypothermia of the foot. However, the hair coat and hoof provide a barrier to effective conduction of heat out of the limb. Also, the presence of a rich vascular network within the corium, including numerous AVAs,^{37,38} means that rapid increases in net perfusion of the foot with warm blood can occur.

Numerous modalities are used for distal limb cryotherapy in horses; there are several commercially available devices for this purpose, although most are suited to short-term (30–60 minutes) applications. Published studies have used commercial cold gel wraps and cold cuffs as well as ice water immersion. A cold gel wrap (4°C) applied for 30 minutes to the metacarpal region of 10 horses resulted in a reduction in surface temperature over the dorsal metacarpal region that was sustained for 3 hours.⁴¹ Another study compared the effects of cold water immersion and cold-pack application to the equine metacarpal region for 30 minutes.⁴² This study showed a profound and sustained reduction in deep-tissue temperature during iced-water immersion (maximum reduction 16.3°C), that was far superior to cold-pack application. Continuous cryotherapy for 48 hours using iced-water immersion resulted in profound cooling of the digit (mean internal hoof temperature $5.3^{\circ} \pm 0.3^{\circ}\text{C}$) and was not associated with adverse clinical effects.⁴ Further studies have since confirmed the profound cooling effects of continuous cold-water immersion for 48 hours³ and 72 hours² on the digit, without apparent deleterious effects.

The application of cold to the equine distal limb generally results in profound vasoconstriction within digit. A scintigraphic study showed a significant reduction in soft-tissue perfusion when the equine digit was immersed in 4°C iced water for 30 minutes.⁴³ Based on hoof temperature (an indirect measure of digital perfusion),⁴⁴ digital vasoconstriction also predominates when distal limb cryotherapy is applied continuously for longer periods.^{2–4} Intermittent periods of increased internal hoof temperature (up to 12°C) were noted in some horses during 72 hours of cold-water immersion.² These 2- to 4-hour periods of increased hoof temperature occurred 12 to 24 hours apart and often the left and right forelimbs were asynchronous. This phenomenon is similar to that noted in horses standing in natural environments that are below freezing (Chris Pollitt, BVSc, PhD, unpublished data, 2000). The increases in hoof temperature represent transient increases in perfusion, metabolism, or both,⁴⁴ and may correspond with periods of hoof growth, or clearance of metabolic waste products. The phenomenon is dissimilar to CIVD (the hunting reaction), which involves oscillations over minutes, rather than hours.²⁹ In the human digit it is thought

to be a protective mechanism against cold-induced injury.³⁰ The hoof temperature fluctuations observed in the current study might be a variation of those seen in normal horses kept in climate-controlled environments.^{45,46}

The equine distal limb seems to be resilient to the effects of extreme continuous hypothermia. There are no reports in the literature of complications directly related to the clinical application of distal limb cryotherapy in horses. In addition, horses show no signs of adverse effects in arctic climates where their distal limbs are continuously immersed in snow. Cold-induced pain, observed in human patients when cryotherapy is applied at 5°C or less,²³ has not been noted in horse studies. Perhaps the primary concern associated with profound digital cooling is the potential for damage to tendons and ligaments of the equine distal limb. Petrov and colleagues⁴⁷ examined the effects of hypothermia on equine superficial digital flexor tendon (SDFT) cells in vitro, and on the core tendon temperature in vivo. The mean core SDFT temperature after 60 minutes of cooling, using a commercial cooling and compression device (set at 3°C), was $10.4 \pm 3.7^\circ\text{C}$, which was a mean decrease in temperature of 21.8°C over the starting point. No clinical detrimental effects were noted after the application of this protocol, and the viability of cultured tendon cells cooled to 10°C for 1 hour was not significantly different from that of cells incubated at 37°C.

USING DIGITAL HYPOTHERMIA TO HELP PREVENT LAMINITIS

Profound continuous digital hypothermia effectively ameliorates experimentally induced laminitis when applied throughout the developmental period.^{2,3} Although the pathophysiology of acute laminitis remains unclear, inflammatory and enzymatic processes seem to contribute to lamellar separation.^{48–56} Lamellar energy failure and ischemia-reperfusion injury are also likely to contribute to the pathogenesis of laminitis, regardless of whether they are primary or secondary events.^{57–63} The profound hypometabolic and anti-inflammatory effects of hypothermia may protect lamellar tissue from these processes during the developmental phase. Cryotherapy significantly reduced the upregulation of matrix metalloproteinase-2 mRNA³ and seems to reduce the expression of proinflammatory chemokines during the developmental phase of experimentally induced laminitis (James Belknap, DVM, PhD, unpublished data, 2009). Profound vasoconstriction may also prevent the hematogenous delivery of laminitis trigger factors.⁶⁴

Suggested Protocol

Ideally, cryotherapy should be applied for the duration of the developmental phase. Horses with conditions associated with a high risk of laminitis development (colitis, metritis, pneumonia, alimentary carbohydrate overload)^{65–69} should therefore be identified and treated preemptively whenever possible. Horses exhibiting clinical signs consistent with endotoxemia should be considered to be at a high risk of developing laminitis.⁶⁵ Continuous application is likely to yield the best results, although the effect of intermittent cooling has not been studied. Resolution of the primary disease may be used as an indicator for timing the cessation of cryotherapy in individual cases. The author prefers to continue cryotherapy for 24 to 48 hours after the resolution of clinical and laboratory signs of systemic inflammation. Rewarming should be gradual if possible (over 12–24 hours), as rapid rewarming after therapeutic hypothermia may lead to the reinitiation of deleterious processes and loss of the protective effect.⁸

Experimental data showed a preventive effect with internal hoof temperatures around or less than 5°C, representing a decrease of 20°C or more below normal. Recent human medical-related studies suggest a superior effect when mild to

moderate (10°C reduction) therapeutic hypothermia is used compared with traditional profound ($>10^{\circ}\text{C}$ reduction) hypothermia in various disease processes.⁸ A critical temperature for laminitis prevention has not been established, although it is likely that even mild decreases in lamellar temperature have some beneficial effect. Accurate measurement of the actual lamellar tissue temperature is problematic. During the application of distal limb cryotherapy, hoof wall surface temperature tends to be approximately 2° to 3°C less than that measured by temperature probes buried deep within the hoof wall, adjacent to the lamellae (van Eps, unpublished data, 2008). Based on currently available data, clinical cryotherapy application should be aimed at achieving hoof wall surface temperatures that are consistently less than 10°C . This necessitates cooling the hoof directly as well as cooling the blood that enters the foot. Immersion of the limb from the upper metacarpus and metatarsus distally in an ice and water mixture effectively achieves this, although constant ice replenishment is labor intensive. Commercially available wader-style devices can be modified to include direct cooling of the hoof itself (Fig. 1A). Commercially available ice-pack and cold-gel applications generally do not reduce internal hoof temperature below 20°C even with regular replenishment/exchange (van Eps, unpublished data, 2008). A prototype device (see Fig. 1B), consisting of a membrane that recirculates refrigerated coolant, consistently reduces hoof wall surface temperature below 10°C



Fig. 1. Successful induction of lamellar hypothermia requires cooling of the hoof capsule as well as the limb distal to the carpus or tarsus (in order to cool blood flowing to the digit). (A) Modification of a commercially available wader style ice boot (Ice boot [Jack's Inc, Washington Court House, OH, USA]) to include the foot. This type of boot necessitates regular ice replenishment (every 1–2 hours, depending on ambient temperature) to achieve a consistently low temperature. (Courtesy of Jane Axon, BVSc, DACVIM, Scone, NSW, Australia). (B) A prototype membrane cooling device containing recirculating refrigerated coolant (Apex 5C [Game Ready Equine, Alameda, CA, USA]) applied to the forelimbs. This type of device may provide a convenient and controlled means of prolonged continuous cryotherapy application. (Courtesy of Jane Axon, BVSc, DACVIM, Scone, NSW, Australia.)

(van Eps, unpublished data, 2009). Such a device may provide a convenient and effective means of inducing effective continuous digital hypothermia in the future.

USING DIGITAL HYPOTHERMIA FOR THE TREATMENT OF ACUTE LAMINITIS

Although there are no published data regarding the efficacy of cryotherapy for the treatment of laminitis, it is rational to assume that the hypometabolic effect of hypothermia may be beneficial during the acute phase in reducing inflammation and enzymatic activity. In the author's experience cryotherapy also provides some analgesia in horses with acute laminitis. Cryotherapy should be avoided in cases where infection is suspected within the foot (subsolar abscess, septic osteitis, or seedy toe) because hypothermia reduces the natural inflammatory response to infection.⁸ The author currently applies continuous distal limb cryotherapy to acute cases for up to 7 days after the first clinical signs of laminitis; however, it is unclear whether cryotherapy has any effect on the progression of laminitis, and research is required before any specific recommendations can be made.

REFERENCES

1. USDA. Lameness and laminitis in US horses. Fort Collins, Colorado, USA: USDA: APHIS:VS, CEAH, National Animal Health Monitoring System, 2000. Available at: <http://www.aphis.usda.gov/vs/ceah/ncahs/nahms/equine/equine98/eq98LAME.pdf>. Accessed January 5, 2010.
2. van Eps AW, Pollitt CC. Equine laminitis model: cryotherapy reduces the severity of lesions evaluated seven days after induction with oligofructose. *Equine Vet J* 2009;41:741–6.
3. van Eps AW, Pollitt CC. Equine laminitis: cryotherapy reduces the severity of the acute lesion. *Equine Vet J* 2004;36:255–60.
4. Pollitt CC, van Eps AW. Prolonged, continuous distal limb cryotherapy in the horse. *Equine Vet J* 2004;36:216–20.
5. Swenson C, Sward L, Karlsson J. Cryotherapy in sports medicine. *Scand J Med Sci Sports* 1996;6:193–200.
6. Douglas WW, Malcom JL. The effect of localized cooling on mammalian muscle spindles. *J Physiol* 1955;130:53–71.
7. Fuhrman GJ, Fuhrman FA. Oxygen consumption of animals and tissues as a function of temperature. *J Gen Physiol* 1959;42:715–22.
8. Polderman KH. Mechanisms of action, physiological effects, and complications of hypothermia. *Crit Care Med* 2009;37:S186–202.
9. Erecinska M, Thoresen M, Silver IA. Effects of hypothermia on energy metabolism in mammalian central nervous system. *J Cereb Blood Flow Metab* 2003;23:513–30.
10. Hagerdal M, Harp J, Nilsson L, et al. The effect of induced hypothermia upon oxygen consumption in the rat brain. *J Neurochem* 1975;24:311–6.
11. Palmer C, Vannucci RC, Christensen MA, et al. Regional cerebral blood flow and glucose utilization during hypothermia in newborn dogs. *Anesthesiology* 1989;71:730–7.
12. Knight KL. Metabolism and inflammation. In: Knight KL, editor. *Cryotherapy in sports injury management*. Champaign (IL): Human Kinetics; 1995. p. 77–84.
13. Griep RB, Stinson EB, Shumway NE. Profound local hypothermia for myocardial protection during open-heart surgery. *J Thorac Cardiovasc Surg* 1973;66:731–41.

14. Zachariassen KE. Hypothermia and cellular physiology. *Arctic Med Res* 1991; 50(Suppl 6):13–7.
15. Harris ED Jr, McCroskery PA. The influence of temperature and fibril stability on degradation of cartilage collagen by rheumatoid synovial collagenase. *N Engl J Med* 1974;290:1–6.
16. Lim CM, Kim MS, Ahn JJ, et al. Hypothermia protects against endotoxin-induced acute lung injury in rats. *Intensive Care Med* 2003;29:453–9.
17. Westermann S, Vollmar B, Thorlacius H, et al. Surface cooling inhibits tumor necrosis factor-alpha-induced microvascular perfusion failure, leukocyte adhesion, and apoptosis in the striated muscle. *Surgery* 1999;126:881–9.
18. Webster CM, Kelly S, Koike MA, et al. Inflammation and NFkappaB activation is decreased by hypothermia following global cerebral ischemia. *Neurobiol Dis* 2009;33:301–12.
19. Scumpia PO, Sarcia PJ, Kelly KM, et al. Hypothermia induces anti-inflammatory cytokines and inhibits nitric oxide and myeloperoxidase-mediated damage in the hearts of endotoxemic rats. *Chest* 2004;125:1483–91.
20. Prandini MN, Neves Filho A, Lapa AJ, et al. Mild hypothermia reduces polymorphonuclear leukocytes infiltration in induced brain inflammation. *Arq Neuropsiquiatr* 2005;63:779–84.
21. Kamler M, Goedeke J, Pizanis N, et al. In vivo effects of hypothermia on the microcirculation during extracorporeal circulation. *Eur J Cardiothorac Surg* 2005;28:259–65.
22. Novack TA, Dillon MC, Jackson WT. Neurochemical mechanisms in brain injury and treatment: a review. *J Clin Exp Neuropsychol* 1996;18:685–706.
23. Ohkoshi Y, Ohkoshi M, Nagasaki S, et al. The effect of cryotherapy on intraarticular temperature and postoperative care after anterior cruciate ligament reconstruction. *Am J Sports Med* 1999;27:357–62.
24. Fujimoto K, Fujita M, Tsuruta R, et al. Early induction of moderate hypothermia suppresses systemic inflammatory cytokines and intracellular adhesion molecule-1 in rats with caerulein-induced pancreatitis and endotoxemia. *Pancreas* 2008;37:176–81.
25. Chin JY, Koh Y, Kim MJ, et al. The effects of hypothermia on endotoxin-primed lung. *Anesth Analg* 2007;104:1171–8 [tables of contents].
26. Lehmann JF, de Lateur BJ. Cryotherapy. In: Lehmann JF, editor. *Therapeutic heat and cold*. 4th edition. Baltimore (MD): Williams & Wilkins; 1990. p. 590–631.
27. Perkins JF, Li MC, Hoffman F, et al. Sudden vasoconstriction in denervated or sympathectomized paws exposed to cold. *Am J Phys* 1948;155:165–78.
28. Clarke RS, Hellon RF, Lind AR. Vascular reactions of the human forearm to cold. *Clin Sci* 1958;17:165–79.
29. Lewis T. Observations upon the reactions of the vessels of the human skin to cold. *Heart* 1930;15:177–208.
30. Daanen HA. Finger cold-induced vasodilation: a review. *Eur J Appl Physiol* 2003; 89:411–26.
31. Bergersen TK, Hisdal J, Walloe L. Perfusion of the human finger during cold-induced vasodilation. *Am J Phys* 1999;276:R731–7.
32. Thorlacius H, Vollmar B, Westermann S, et al. Effects of local cooling on microvascular hemodynamics and leukocyte adhesion in the striated muscle of hamsters. *J Trauma* 1998;45:715–9.
33. Knight KL. Circulatory effects of therapeutic cold applications. In: Knight KL, editor. *Cryotherapy in sport injury management*. Champagne (IL): Human Kinetics; 1995. p. 107–26.

34. McGuire DA, Hendricks SD. Incidences of frostbite in arthroscopic knee surgery postoperative cryotherapy rehabilitation. *Arthroscopy* 2006;22:1141 e1–6.
35. Knight KL. Problems, precautions and contraindications in cold therapy. In: Knight KL, editor. *Cryotherapy in sport injury management*. Champagne (IL): Human Kinetics; 1995. p. 179–94.
36. DeGroot DW, Castellani JW, Williams JO, et al. Epidemiology of U.S. Army cold weather injuries, 1980–1999. *Aviat Space Environ Med* 2003;74:564–70.
37. Ungley CC, Channell GD, Richards RL. The immersion foot syndrome. 1946. *Wilderness Environ Med* 2003;14:135–41.
38. Ivers T. Cryotherapy: an in-depth study. *Equine Pract* 1987;9:17–9.
39. Blackwell RB. The use of cryotherapy in equine sports medicine. *Equine Athlete* 1991;4:1–5.
40. Ramey DW. Cold therapy in the horse. *Equine Pract* 1999;21:19–21.
41. Turner TA, Wolfsdorf K, Jourdenais J, et al. Effects of heat, cold, biomagnets, and ultrasound on skin circulation in the horse. In: *Proceedings of the Thirty-Seventh Annual Convention of the American Association of Equine Practitioners*. San Francisco (CA), 1991. p. 249–57.
42. Kaneps AJ. Tissue temperature response to hot and cold therapy in the metacarpal region of a horse. In: *Proceedings of the Forty-sixth Annual Convention of the American Association of Equine Practitioners*. San Antonio (TX), 2000. p. 208–13.
43. Worster AA, Gaughan EM, Hoskinson JJ, et al. Effects of external thermal manipulation on laminar temperature and perfusion scintigraphy of the equine digit. *N Z Vet J* 2000;48:111–6.
44. Hood DM, Wagner IP, Bru mbaugh GW. 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* 2001;62:1167–72.
45. Pollitt CC, Davies CT. Equine laminitis: its development coincides with increased sublamellar blood flow. *Equine Vet J Suppl* 1998;26:125–32.
46. Mogg KC, Pollitt CC. Hoof and distal limb surface temperature in the normal pony under constant and changing ambient temperatures. *Equine Vet J* 1992;24:134–9.
47. Petrov R, MacDonald MH, Tesch AM, et al. Influence of topically applied cold treatment on core temperature and cell viability in equine superficial digital flexor tendons. *Am J Vet Res* 2003;64:835–44.
48. Belknap JK, Giguere S, Pettigrew A, et al. Lamellar pro-inflammatory cytokine expression patterns in laminitis at the developmental stage and at the onset of lameness: innate vs. adaptive immune response. *Equine Vet J* 2007;39:42–7.
49. Blikslager AT, Yin C, Cochran AM, et al. Cyclooxygenase expression in the early stages of equine laminitis: a cytologic study. *J Vet Intern Med* 2006;20:1191–6.
50. Fontaine GL, Belknap JK, Allen D, et al. Expression of interleukin-1beta in the digital laminae of horses in the prodromal stage of experimentally induced laminitis. *Am J Vet Res* 2001;62:714–20.
51. Johnson PJ, Tyagi SC, Katwa LC, et al. Activation of extracellular matrix metalloproteinases in equine laminitis. *Vet Rec* 1998;142:392–6.
52. Kyaw Tanner M, Pollitt CC. Equine laminitis: increased transcription of matrix metalloproteinase-2 (MMP-2) occurs during the developmental phase. *Equine Vet J* 2004;36:221–5.
53. Mungall BA, Pollitt CC. Zymographic analysis of equine laminitis. *Histochemistry* 1999;112:467–72.

54. Mungall BA, Pollitt CC, Collins R. Localisation of gelatinase activity in epidermal hoof lamellae by in situ zymography. *Histochemistry* 1998;110:535–40.
55. Waguespack RW, Cochran A, Belknap JK. Expression of the cyclooxygenase isoforms in the prodromal stage of black walnut-induced laminitis in horses. *Am J Vet Res* 2004;65:1724–9.
56. Waguespack RW, Kempainen RJ, Cochran A, et al. Increased expression of MAIL, a cytokine-associated nuclear protein, in the prodromal stage of black walnut-induced laminitis. *Equine Vet J* 2004;36:285–91.
57. Bailey SR, Marr CM, Elliott J. Current research and theories on the pathogenesis of acute laminitis in the horse. *Vet J* 2004;167:129–42.
58. Hood DM, Grosenbaugh DA, Mostafa MB, et al. The role of vascular mechanisms in the development of acute equine laminitis. *J Vet Intern Med* 1993;7:228–34.
59. Moore RM, Eades SC, Stokes AM. Evidence for vascular and enzymatic events in the pathophysiology of acute laminitis: which pathway is responsible for initiation of this process in horses? *Equine Vet J* 2004;36:204–9.
60. Noschka E, Moore JN, Peroni JF, et al. Thromboxane and isoprostanes as inflammatory and vasoactive mediators in black walnut heartwood extract induced equine laminitis. *Vet Immunol Immunopathol* 2009;129:200–10.
61. Peroni JF, Harrison WE, Moore JN, et al. Black walnut extract-induced laminitis in horses is associated with heterogeneous dysfunction of the laminar microvasculature. *Equine Vet J* 2005;37:546–51.
62. Peroni JF, Moore JN, Noschka E, et al. Predisposition for venoconstriction in the equine laminar dermis: implications in equine laminitis. *J Appl Phys* 2006;100:759–63.
63. Robertson TP, Moore JN, Noschka E, et al. Evaluation of activation of protein kinase C during agonist-induced constriction of veins isolated from the laminar dermis of horses. *Am J Vet Res* 2007;68:664–9.
64. Pollitt CC. Equine laminitis: a revised pathophysiology. In: *Proceedings of the 45th Annual Convention of the American Association of Equine Practitioners*. Albuquerque (NM), 1999. p. 188–92.
65. Parsons CS, Orsini JA, Krafty R, et al. Risk factors for development of acute laminitis in horses during hospitalization: 73 cases (1997–2004). *J Am Vet Med Assoc* 2007;230:885–9.
66. Cohen ND, Parson EM, Seahorn TL, et al. Prevalence and factors associated with development of laminitis in horses with duodenitis/proximal jejunitis: 33 cases (1985–1991). *J Am Vet Med Assoc* 1994;204:250–4.
67. Cohen ND, Woods AM. Characteristics and risk factors for failure of horses with acute diarrhea to survive: 122 cases (1990–1996). *J Am Vet Med Assoc* 1999;214:382–90.
68. Slater MR, Hood DM, Carter GK. Descriptive epidemiological study of equine laminitis. *Equine Vet J* 1995;27:364–7.
69. Alford P, Geller S, Richardson B, et al. A multicenter, matched case-control study of risk factors for equine laminitis. *Prev Vet Med* 2001;49:209–22.