

Equine laminitis: its development coincides with increased sublamellar blood flow

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Summary

The effect of alimentary carbohydrate overload on hoof temperature was investigated to determine the state of the sublamellar vasculature preceding the onset of equine laminitis. Hoof, core and ambient temperatures and heart rate were logged continuously in 21 mature Standardbred horses kept in an environmental chamber set at 10°C. Recording hoof temperature was a successful, noninvasive, method to measure indirectly, shifts in digital blood flow against a background of cold induced, physiological, vasoconstriction. High hoof temperatures were assumed to indicate digital vasodilation and low hoof temperatures digital vasoconstriction. Seven horses were either untreated or sham treated controls. A slurry of ground wheat flour (17.5 g/kg) was administered via nasogastric tube to 13 horses all of which were humanely killed 48 h later. Histological sections of the lamellar tissues were examined for evidence of laminitis. Analysis of mean hoof temperature graphs showed that horses judged laminitis positive had experienced a period of prolonged digital vasodilation 16–40 h after carbohydrate overload. Laminitis negative horses experienced no such period of vasodilation and never had hoof temperatures significantly (except once, at 28 h) above that of controls. The only parameter which significantly differentiated the laminitis positive from laminitis negative horses, between 12 and 32 h after carbohydrate overload, was hoof temperature, which was significantly higher in laminitis positive horses ($P < 0.05$). Therefore, a period of sublamellar vasodilation, 12 to 40 h after alimentary carbohydrate overload precedes the onset of laminitis. If the digital circulation sustains vasoconstriction during this period then laminitis does not occur. We propose that the period of increased digital blood flow in laminitis positive horses, concomitant with the severe metabolic crisis brought on by the alimentary carbohydrate overload, may expose the lamellar tissues to a concentration of blood borne factors sufficient to trigger lamellar separation.

Introduction

Shunting of blood away from the nutrient capillaries of the hoof lamellae via inappropriately dilated arteriovenous anastomoses (AVAs) has been proposed as a pathophysiological mechanism for

developmental equine laminitis (Robinson *et al.* 1976; Hood *et al.* 1978). Detailed information on the anatomy of the AVAs in the dermis of the equine digit (Pollitt and Molyneux 1990; Molyneux *et al.* 1994) lent weight to the proposal that AVA shunting could be a mechanism involved in the development of laminitis. A normal function of dilated AVAs may be to maintain foot temperature above the tissue freezing point (about -1.0°C) when extremely low environmental temperatures could cause damage (frostbite) to horses standing, for long periods, in ice and snow. For this to occur it is assumed that a dual circulation exists: a slow nutrient capillary circulation supporting the metabolism of the lamellar tissues and a fast AVA circulation periodically delivering warm arterial blood to the dermis of the foot when the foot reaches a critically low temperature. A circulatory arrangement like this has been described in the feet of cold adapted mammals, the arctic fox and grey wolf (Henshaw *et al.* 1972). Skin AVAs are also heat dissipating structures and dilate in response to rising core temperature therefore increasing the rate of surface heat loss. The high density of AVAs located in the entire skin surface of the Weddell seal is believed to be important in dissipating heat when the animal is out of water (Molyneux and Bryden 1975). The AVAs in the digit of the equine foot may also be involved in heat dissipation, dilating in response to rising core temperatures. Partitioning of blood from one circulation to the other implies the existence of sophisticated reflex and local control mechanisms reviewed by Hales and Molyneux (1988). Sensory, afferent nerves relay information from the foot to the hypothalamus about the thermal status of the foot and in turn vasomotor, efferent nerves regulate AVA tone via catecholamine and peptidergic fibres innervating smooth muscle cells in the AVA wall. Superimposed on this centrally mediated control of AVA tone are active neurogenic, vasodilatory, mechanisms and local axon reflexes which under certain circumstances can increase the vasodilatory capacity of skin type tissues. Therefore, AVAs are the target of reflexly (central) evoked thermoregulatory responses and capillary flow is principally the target of direct (local) temperature effects (Hales and Molyneux 1988). Flow rates through skin capillaries are lower than through the wider diameter AVAs but capillaries are nevertheless more efficient dissipators of heat and are a major site of heat exchange. There is no significant difference between the maximum total heat loss through capillaries and AVAs in the metatarsal skin of sheep (Rubsamen and Hales 1984).

The laminitis literature is divided on the subject of sublamellar perfusion (Hood *et al.* 1993). The angiographic

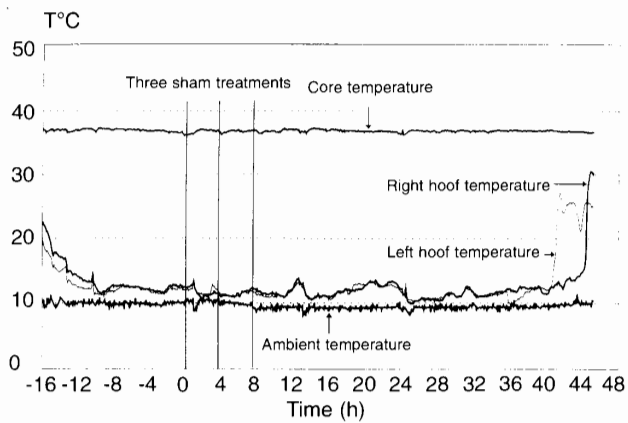


Fig 1: Graphs of right and left fore hoof, core and ambient temperatures in a sham treated control horse. Hoof temperatures remained close to ambient for most of the experimental period whereas core temperature was in the normal range throughout.

studies of Coffman *et al.* (1970) and scans of the digit obtained after arterial injection of radiolabelled aggregates of albumin (Hood *et al.* 1978) supported hypoperfusion and laminar ischaemia as causes of laminitis. Presumably the hoof would be cool if perfusion of the sublamellar vasculature was reduced prior to the appearance of laminitis. Direct measurement of digital blood flow in acute laminitis by Robinson *et al.* (1976) showed an increase in blood flow as a result of decreased vascular resistance (vasodilation). Similarly Trout *et al.* (1990) could not support lamellar ischaemia as a primary cause of laminitis as their noninvasive scintigraphic studies of the digital circulation showed a statistically significant elevation of sublamellar blood flow prior to lameness. A warm hoof would result if digital AVAs were dilated.

Laminitis may result from a failure of the connective tissue junction between the inner hoof wall and the distal phalanx because fresh arterial blood is preferentially shunted through dilated AVAs instead of through nutrient capillaries normally supporting key epidermal structures of the inner hoof wall. Prolonged inappropriate AVA dilation may cause stagnation of the nutrient capillary circulation, ischaemia of the hoof epidermis and eventually destruction of the lamellar anatomy (Pollitt 1991). If prolonged AVA dilation is indeed the cause of lamellar pathology then, as Mogg (1991) noted, a period of hoof warming must precede the appearance of the clinical foot pain and lamellar pathology of acute laminitis.

To determine if hoof warming and, by inference, a vasodilatory event, precedes the onset of laminitis we investigated the effect of alimentary carbohydrate overload on hoof temperature. Previous attempts to monitor digital blood flow during the developmental stage of laminitis required either anaesthesia, cannulation of digital arteries or use of irritating drugs and the resultant observations have been criticised as possibly artifactual (Robinson 1990). Therefore, this study used a noninvasive method to measure hoof temperature and therefore indirectly measure digital blood flow. Since the hoof, core and ambient temperature were to be monitored continuously for 48 h, against a background of cold induced vasoconstriction, other clinical data were collected, at 8 hourly intervals, to seek temporal correlations between clinical signs and changes in hoof temperature. Appetite, thirst, demeanor, foot behaviour, digital pulse, faecal consistency, gut sounds, rectal and core temperature and faecal pH were assessed and recorded.

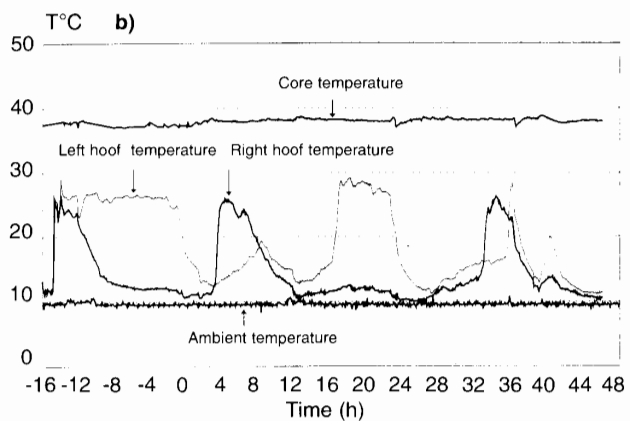
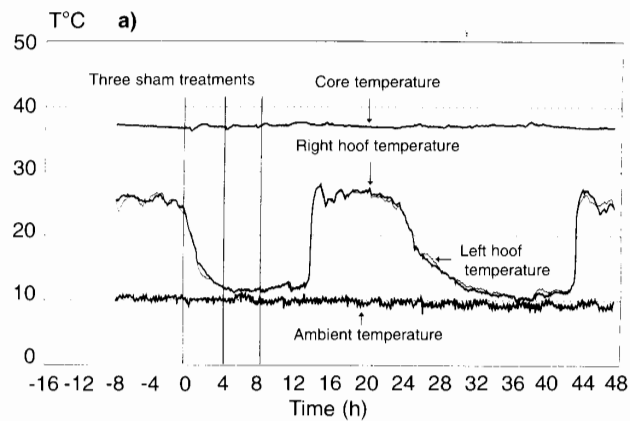


Fig 2a: Graphs of right and left fore hoof, core and ambient temperatures in a sham treated control horse. Hoof temperatures rose and fell synchronously through 25°C while core temperature remained in the normal range. Fig 2b: Untreated control horse. The right and left hoof temperatures rose and fell independently of each other.

Materials and methods

The experiments were conducted according to guidelines approved by the University of Queensland Animal Experimentation Ethics committee. All horses under experimentation were inspected by the Animal Welfare Officer.

Experiments were carried out in an environmental chamber with an ambient temperature set at 10°C. A total of 22 horses (17 geldings and 5 mares) were used, who were all Standardbred racehorses, recently retired from the track, age 2–9 years. Two horses were in the chamber for each experiment, one in the carbohydrate treatment group, the other in the sham treated control group. Fourteen horses were dosed with carbohydrate, 7 were sham dosed and one was an untreated control. Each horse was tethered to the wall and could move freely in an arc of 180°. Fresh water at room temperature (about 25°C) was offered to the horses at 3 hourly intervals. Fodder, in the form of a pelleted working horse mix and lucerne chaff, was available at all times.

Each horse was fitted with a harness consisting of a girth, crupper and a breastplate with an attached saddlebag. In the saddlebag was an 8 channel data logger. Six channels recorded hoof temperature and ambient temperature. The 7th channel was connected to a jugular catheter with a built in thermistor for measuring core temperature. The 8th channel was an event marker.

The hoof temperature sensors were NPN silicon transistors,

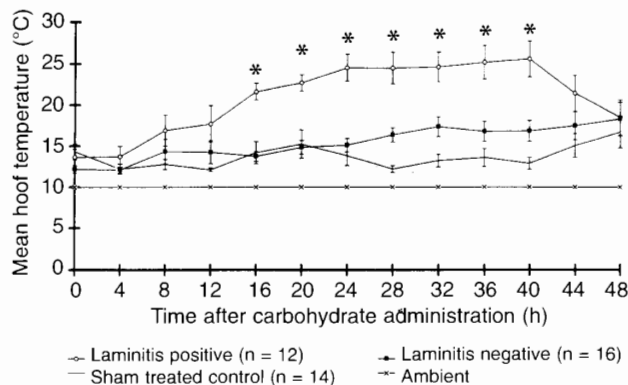


Fig 3: Mean \pm s.e. hoof temperatures of the sham treated control horse group ($n = 14$) compared to the mean \pm s.e. hoof temperatures of the laminitis positive ($n = 12$) and the laminitis negative horse group ($n = 16$). The hoof temperature of the laminitis positive group was significantly higher than the laminitis negative group and the sham treated controls between 16 and 40 h after the first administration of carbohydrate (time 0). The indicators of statistical significance refer only to the differences between the laminitis positive and negative groups. * $P < 0.05$.

type BC846. These are a surface mount type, used because of their small size. The sensors were connected to oscillator circuits, with a pulse train output, the frequency of which varied with the temperature of the sensor. The microprocessor circuit of the datalogger, type CMOS 6805, counted and logged the pulses over a preprogrammed period of 5 min. Hoof temperature was measured using the sensors calibrated for temperature. Each sensor was soldered to the end of an insulated cable and embedded in epoxy resin (Araldite)¹. The cables were 2 m in length. The embedded sensor was inserted into a hole drilled into the dorsal hoof wall of the fore feet 15 mm below the hairline of the coronet and taped to the hoof with PVC electrical tape. Drilling of the holes caused no pain response and no haemorrhage. The holes were 4 mm diameter and 7 mm deep, and were perpendicular to the hoof wall. There were two sensors in each fore foot. The temperature at the hoof surface of all the horses was checked with a thermometer operating on a different principle (Infrared Temperature Scanner)² and always agreed with the temperature displayed by the implanted transistors. Heat sink paste was placed in the drilled holes before insertion of the sensors. The cables connecting the sensors to the data loggers, were taped to the legs of the horses at the mid pastern, mid cannon and proximal carpal regions. The tape was applied loosely to prevent skin pressure and possible oedema formation. Enough cable was left at each joint to allow full flexion and extension. Two sensor cables were taped to the harness so that the sensors hung free on either side of the abdomen to record ambient temperature.

Core temperature was measured with a sterilised thermistor catheter (Swan-Ganz thermodilution catheter)³ inserted into the right atrium of the heart via a 10G cannula inserted into the left jugular vein at a site anaesthetised with local anaesthetic. The free end of the thermistor catheter was sutured to the skin and a collar of adhesive tape (Elastoplast)⁴ was applied to the neck of the horse to secure the 10G cannula, the thermistor catheter and its data logger connecting cable. The 6 sensors and the thermistor catheter were all calibrated beforehand in set temperature water baths.

Heart rate was measured using a heart rate monitor (Equine Electronics equine heart rate computer)⁵ with recording electrodes placed in contact with clipped skin, under the girth, at

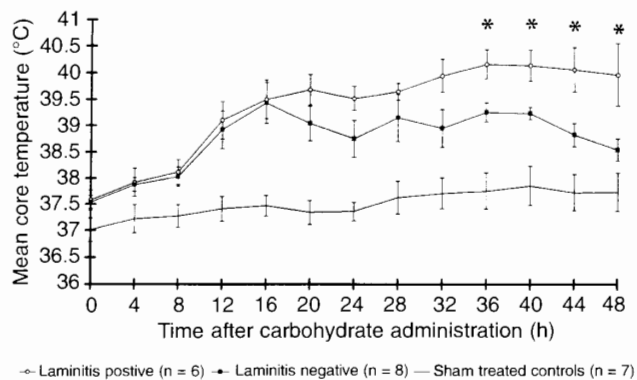


Fig 4: Mean \pm s.e. core temperatures of all horse groups. The laminitis positive group ($n = 6$) and laminitis negative group ($n = 8$) developed and maintained mean core temperatures significantly above the control group ($n = 7$) which remained within normal limits. The mean core temperatures of the laminitis positive horses was significantly higher than the laminitis negative horses 36 h after carbohydrate overload. The indicators of statistical significance refer only to the differences between the laminitis positive and negative groups. * $P < 0.05$.

the withers and sternum. A second data logger was connected to the heart rate monitor earphone output via a filtering and rectifying circuit which turned the tone burst (beeping) output of the monitor into a pulse train. This recorded the computed heart rates over a 60 s period and logged the average heart rate/min.

The information stored in both the temperature and heart rate data loggers was dumped to a notebook computer every 8 h via an RS232 cable. Software, specially written for this project, transformed the temperature data into graph form and stored the data in files for further analysis.

The horses were acclimatised in the environment chamber for 16 h prior to dosing with carbohydrate. After the acclimatisation period, the treated horse was dosed with 3 lots of wheat flour (17.5 g/l) mixed to a slurry in 8 litres of water. Each dose was administered via nasogastric tube, every 4 h. The control horses were sham treated with the same volume of room temperature water given at the same times. During the 48 h experimental period observations were made regarding appetite, drinking, general demeanour, foot behaviour, oral mucous membrane capillary refill time, digital pulse, faecal consistency, faecal pH, gut sounds (left colon and ileo-caecal sounds) and rectal temperature. Horses showing signs of colic were treated with appropriate doses of detomidine HCl 10 mg/ml (Dormosedan)⁶ and butorphanol tartrate 10 mg/ml (Dolorex)⁷. Forty-eight hours after the first administration of the carbohydrate, or before if there was evidence of colic or foot pain, the experiment was stopped. Five horses failed to reach the 48 h end point and were not included in the data analysis (2 mares, 3 geldings). Three of these developed severe electrolyte and fluid disturbances, became recumbent 16–30 h after carbohydrate dosing and were promptly subjected to euthanasia. The remaining 2 were subjected to euthanasia because of colic unresponsive to treatment.

Treated horses were killed with an overdose of barbiturate and both front feet removed by disarticulation at the metacarpal/phalangeal joint. The foot was cut on a bandsaw to harvest the lamellae of the dorsal hoof wall into 10% neutral buffered formalin according to the method of Pollitt (1996). Stained sections of the hoof wall lamellae were examined with a light microscope and the severity of the laminitis was graded using the scoring system of Pollitt (1996). Horses with laminitis

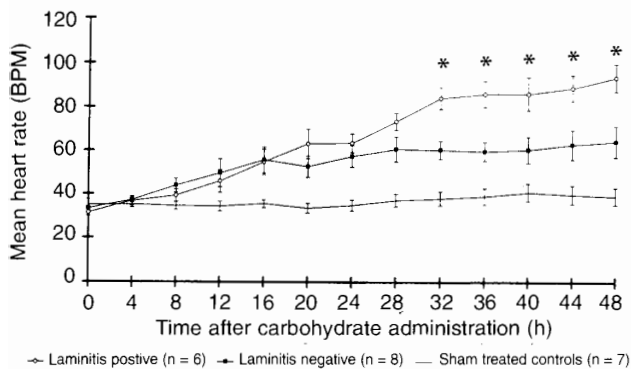


Fig 5: Mean \pm s.e. heart rates of all horse groups. The mean heart rates of the 7 sham treated control horses was normal throughout the 48 h experimental period. The 6 laminitis positive and 8 laminitis negative horses developed and maintained elevated mean heart rates. The mean heart rates of the laminitis positive horses became significantly greater than the laminitis negative horses after 32 h. The indicators of statistical significance refer only to the differences between the laminitis positive and negative groups. * $P < 0.05$, BPM = beats per min.

in either fore foot were grouped as laminitis positive. Horses with no lamellar lesions attributable to laminitis were grouped as laminitis negative. The control horses, not dosed or dosed only with water, were not killed at 48 h but rested at pasture for 3 or 4 weeks and then reused in the carbohydrate treatment group.

The data stored in the files of the laptop computer were joined and converted into graphical form. The 4 hourly means \pm s.e. of the right and left foot temperatures of the fore feet, core temperature, heart rate and faecal pH, of the laminitis positive horses were graphed and compared to the means of the laminitis negative and the control horses.

Since the hoof temperature, core temperature and heart rate data was recorded every 5 min and the data were to be analysed at 4 hourly intervals, an average of 5 hourly time points was calculated for each 4 hourly time point. The 2 hourly time points prior to and following the actual time point, and the time point itself were used. For the statistical analysis, a repeated measures analysis of variance (MANOVA) was performed for the time factor. The Greenhouse-Geisser epsilon test was applied to adjust the degrees of freedom in the F-tests, to compensate for sphericity in the covariance matrix due to correlated errors over time. A significant time effect was followed up with a series of cross-sectional analysis of variance (ANOVA) at each 4 or 8 hourly time point (depending on which set of data). The 2 front hooves were treated as a split unit within each horse, which were the whole units in the analysis of variance (ANOVA). The analysis of variance was performed using a computer software programme (Anon 1994).

Results

Acclimatisation period

During the 16 h acclimatisation period the horses accepted the harness and the data collecting apparatus with no signs of discomfort. For the most part the horses stood quietly and became animated only when anticipating the supply of fresh rations. They frequently changed position and showed no sign of foot discomfort while barefoot on the concrete floor of the environmental chamber. Some of the horses shivered slightly in the 10°C environment, especially those with short summer hair

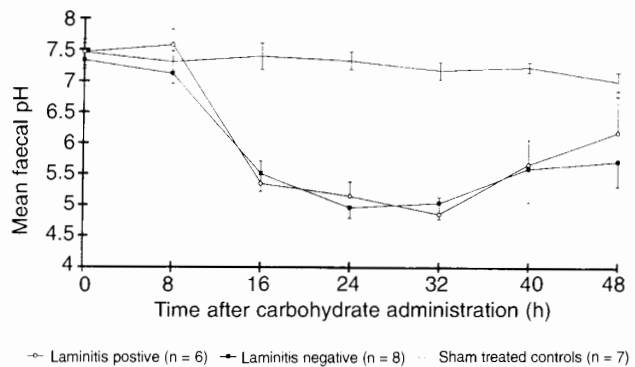


Fig 6: Mean \pm s.e. faecal pH of all horse groups. The mean faecal pH of the 7 sham treated control horses remained neutral, while the 6 laminitis positive and 8 laminitis negative horses had rapid, similar, falls in faecal pH.

coats. All horses had core temperatures within the normal range. By the end of the acclimatisation period (time zero) the mean \pm s.e. foot temperature (combined data of 42 hooves) of all horses was $13.3 \pm 0.46^\circ\text{C}$, close to the ambient temperature (10°C). The mean core temperature of all horses, at time 0, was $37.38 \pm 0.12^\circ\text{C}$. The mean faecal pH was 7.45 ± 0.21 .

Hoof temperature

In 4 sham treated controls hoof temperature remained close to 10°C ambient (Fig 1). The remaining 3 horses and an untreated control developed spontaneous rapid rises in hoof temperature (up to 25°C) which lasted 1.4–11.4 h. Increases in left and right hoof temperature were synchronised in some horses (Fig 2a) whereas in others both rise and fall of temperature were independent of each other (Fig 2b). After the warm period, the temperature of the hooves returned to the previous low level although in some horses, temperature fell rapidly whereas in others it fell slowly (Figs 2a and b). The graph of mean hoof temperatures of control horses ($n = 7$) was, therefore, not flat but showed random changes (Fig 3).

In laminitis positive horses ($n = 6$) increases and decreases were synchronised (Fig 7). At time zero, temperatures were close to the ambient temperature but increased in all cases 4–15 h after the administration of carbohydrate. Generally, temperatures remained elevated throughout the experimental period, except for a period of low temperature which corresponded to the initial increase in core temperature (Fig 7).

Usually, hoof temperatures increased sharply to $25\text{--}32^\circ\text{C}$. One horse was an exception, its hoof temperatures rose slowly, but continuously, and at the end of the experiment its temperatures were approximately 25°C . Temperature of some horses remained high at the end of the experiment, while in others it dropped to near ambient temperature (Fig 7).

During the first 24 h mean \pm s.e. hoof temperatures increased gradually from $13.63 \pm 1.00^\circ\text{C}$ to $24.97 \pm 1.62^\circ\text{C}$ and then plateaued (Fig 3). Forty hours after first administration of carbohydrate mean hoof temperature was $25.62 \pm 2.15^\circ\text{C}$ and then decreased sharply. At 16 h mean hoof temperature of the 6 laminitis positive horses ($21.63 \pm 1.02^\circ\text{C}$) was significantly higher ($P < 0.05$) than that of the 8 laminitis negative horses ($13.73 \pm 0.6^\circ\text{C}$) and the 7 sham controls ($14.21 \pm 1.37^\circ\text{C}$), remaining significantly high until 40 h after time zero (Fig 3).

The hoof, core and ambient temperature graph of an

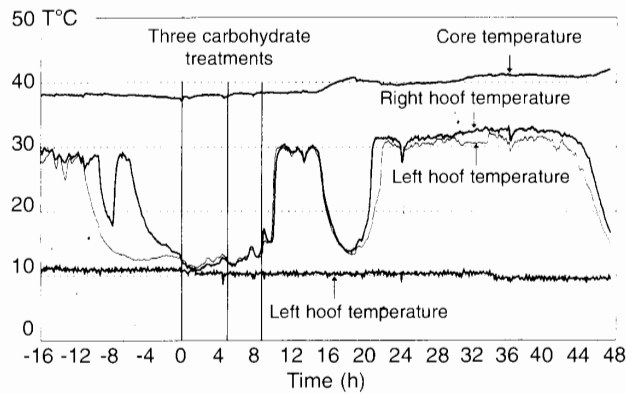


Fig 7: Graphs of left and right fore hoof, core and ambient temperatures in an individual laminitis positive horse. Both left and right hoof temperatures rose and fell synchronously. Twenty-two h after alimentary carbohydrate overload the hooves of both forefeet reached and maintained their maximum temperature for 20 h. The development of laminitis appeared to be linked to this prolonged period of digital vasodilation. Core temperature rose above 40°C at 18 h and again at 30 h.

individual laminitis negative horse is shown in Figure 8. Left and right hoof temperatures in the laminitis negative horses were not as synchronous as hoof temperatures of the laminitis positive horses. In 5 horses, they were synchronous, but in the 3 others, they appeared independent of each other. All laminitis negative horses started with hoof temperatures close to ambient temperature of 10°C. Hoof temperature graphs of individual laminitis negative horses did not resemble each other, which is in contrast with those of laminitis positive horses. One horse had hoof temperatures that remained close to ambient for the whole experiment. Most of the hoof temperature increases that occurred were random, rapid, and in the range 25–32°C. However, these warm hoof temperature periods were short and lasted 3–12 h. Some warm hoof temperature periods consisted of a cluster of peaks. When the hoof temperatures fell they returned to near ambient temperature either rapidly or slowly.

Overall, mean hoof temperature of laminitis negative horses did not rise to any extent over the course of the experiments, and remained within 5°C of the ambient temperature. The mean hoof temperature of the laminitis negative horses are compared to the mean hoof temperature of the laminitis positive horses in Figure 3.

Mean \pm s.e. hoof temperature of laminitis negative horses ($16.39 \pm 0.87^\circ\text{C}$) was briefly significantly higher ($P < 0.05$) than the mean hoof temperature of the sham treated control horses ($12.23 \pm 0.41^\circ\text{C}$) 28 h after first administration of carbohydrate (Fig 3).

Core temperature

In sham treated controls ($n = 7$) mean \pm s.e. core temperature was normal ($37.27 \pm 0.22^\circ\text{C}$) throughout the 48 h experimental period (Figs 1, 2 and 4).

There was a rapid rise in core temperature in laminitis positive ($n = 6$) and laminitis negative horses between 8 and 20 h after first administration of carbohydrate. Increased core temperature persisted for the remainder of the experiment in most horses (Figs 4, 7 and 8). However, one horse had a second rise in core temperature at approximately 42 h (Fig 7).

During the first 8 h mean \pm s.e. core temperatures of laminitis positive and laminitis negative horses increased slowly, between 8 and 16 h they increased rapidly and then plateaued until

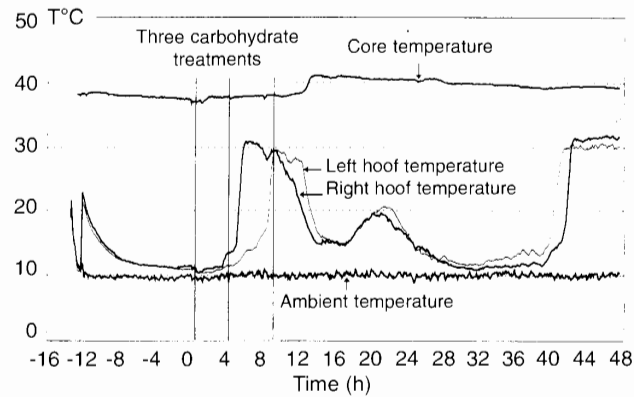


Fig 8: Graphs of left and right fore hoof, core and ambient temperatures in an individual laminitis negative horse. The right and left hoof temperatures rose and fell fairly synchronously but 13 h after carbohydrate overload remained below 20°C and close to ambient for 27 h. This digital vasoconstriction apparently prevented the development of laminitis. Fourteen hours after carbohydrate overload the core temperature rose above 40°C for 14 h.

approximately 28 h (Fig 4). In laminitis positive but not laminitis negative horses they rose again until 36 h and then dropped slowly for the remainder of the experiment. The mean core temperature of laminitis positive horses ($38.12 \pm 0.24^\circ\text{C}$) became significantly higher ($P < 0.05$) than control horses ($37.27 \pm 0.22^\circ\text{C}$) 8 h after first administration of carbohydrate whereas in laminitis negative horses ($37.88 \pm 0.13^\circ\text{C}$) it was significantly higher than control horses at 4 h ($37.22 \pm 0.27^\circ\text{C}$) and remained significantly higher until 44 h after first administration of carbohydrate.

Mean \pm s.e. core temperatures of laminitis positive horses was not significantly higher than laminitis negative horses until 36 h after carbohydrate overload. At 36 h mean core temperature of the laminitis positive horses was $40.17 \pm 0.27^\circ\text{C}$ and laminitis negative horses was $39.26 \pm 0.18^\circ\text{C}$ ($P < 0.05$). This significant difference remained until the end of the experiment (Fig 4).

Heart rates

The 7 sham treated controls and the untreated control had normal heart rates throughout the 48 h experimental period (Fig 5). The laminitis positive horses all developed increased heart rates whereas only 4 of the laminitis negative horses increased. The heart rate of one laminitis positive horse increased at 8 h while another did not increase until 20 h. The laminitis negative horses had varying increases in heart rates. One horse had no increase in heart rate and 3 others had only slight increases in heart rate.

The mean \pm s.e. heart rates of laminitis positive horses (Fig 5) gradually increased from 31.4 ± 1.44 beats/min after first administration of carbohydrate to 94.00 ± 6.23 beats/min at the end of the experiment. Sixteen hours after the first administration of carbohydrate, mean heart rates of laminitis positive horses (54.6 ± 5.93 beats/min) and the laminitis negative horses (55.5 ± 5.88 beats/min) was significantly higher ($P < 0.05$) than mean heart rates of control horses (35.5 ± 1.80 beats/min) and remained so throughout the experiment (Fig 5).

The mean heart rates of laminitis positive horses (84.2 ± 5.00 beats/min) was not significantly higher than the heart rates of laminitis negative horses (60.25 ± 4.28 beats/min) until 32 h after first administration of carbohydrate. This significant difference ($P < 0.05$) remained until the end of the experiment (Fig 5).

Faecal pH

In the laminitis positive and laminitis negative horses, mean faecal pH remained approximately neutral for the first 8 h, and then dropped sharply (Fig 6) so that 16 h after first administration of carbohydrate, mean faecal pH was 5.35 ± 0.36 and 5.51 ± 0.29 respectively. Faecal pH remained near this low value between 16 and 32 h and then slowly rose for the remainder of the experiment. At 16 h, mean faecal pH of laminitis positive horses (5.35 ± 0.36) and laminitis negative horses (5.51 ± 0.29) was significantly lower ($P < 0.05$) than mean faecal pH of controls (mean \pm s.e. = 7.4 ± 0.21). This significant decrease in faecal pH remained until 40 h.

At no time was mean \pm s.e. faecal pH of laminitis positive horses significantly different from laminitis negative horses (Fig 6).

Clinical appearance

There were no changes in clinical appearance in either the laminitis positive or the laminitis negative horses until 8–16 h after the first administration of carbohydrate. The first clinical sign to change was the volume and frequency of ileocaecal (IC) sounds. At 8 h, most horses had louder, more frequent, sounds. However between 16 and 24 h IC sounds disappeared and in most horses remained absent until around 40 h. Similar changes were detected in left colon (LC) sounds except that they occurred approximately 8 h after the changes in IC sounds. All horses dosed with carbohydrate lacked gut sounds at some stage during the 48 h experimental period.

Decreased appetite and drinking, depression, prolongation of capillary refill time and diarrhoea occurred in all horses dosed with carbohydrate. Two of the laminitis positive and 4 of the laminitis negative horses developed mild to moderate, intermittent, colic 30–32 h after dosing. Signs of colic lasted 4–6 h and by 40 h had usually ceased. Neither of the laminitis positive horses were judged to need analgesia or sedation. However the 4 laminitis negative horses with colic received i.v. injections of 20 mg butorphanol and 10 mg detomidine to establish analgesia and sedation, respectively. After injections, heart rate and core temperature decreased for 2–4 h but no consistent change in hoof temperature resulted. In previous trials 10 mg of detomidine injected i.v. into 3 normal Standardbred horses did not effect consistent changes in hoof temperature (C.C. Pollitt and C.T. Davies, unpublished data). The treatment regime effectively abolished signs of colic for periods of 60–90 min but if colic returned the treatment was repeated. Two of the 4 horses that developed colic required only single treatments. Of the remainder one was treated 4 times, the other 5 times.

A pronounced digital pulse was detected in most horses at 24–32 h, which usually disappeared after this time. Only one of the 6 laminitis positive horses had an exaggerated digital pulse at 48 h and all of these horses exhibited some weight shifting in either front or hind feet between 40 and 48 h.

One of the laminitis positive horses, apparently less affected by the carbohydrate than the others, began to eat and drink again at 40 h.

Although laminitis negative horses showed similar clinical signs to the laminitis positive horses they were slightly less severe. Near the end of the experimental period, they were usually less depressed, ate, drank and had shorter oral mucous membrane capillary refill times.

Discussion

Mean heart rates and body temperatures recorded in the 6 laminitis positive horses were significantly higher than in the 7 sham treated control horses which is consistent with previous reports of laminitis induced by alimentary carbohydrate overload (Garner *et al.* 1975; Harkema *et al.* 1978; Kirker-Head *et al.* 1987). However, in this study, similar significant increases in mean heart rate and body temperature occurred in 8 laminitis negative horses. Furthermore the fall in pH of the faeces of both laminitis positive and negative horses was virtually identical and it was difficult to identify any differences in the spectrum of clinical signs: they appeared equally ill, particularly around 24 h after administration of carbohydrate. At this time it was impossible, on the basis of clinical signs, to predict which horses would subsequently develop laminitis. Hoof temperature was the only significant difference between laminitis positive and negative groups. Sixteen hours after carbohydrate administration mean hoof temperature of laminitis positive horses showed a highly significant rise of almost 8°C above mean hoof temperatures of the laminitis negative group: The mean \pm s.e. hoof temperature of the laminitis positive horses reached a maximum of $25.62 \pm 2.15^\circ\text{C}$ and remained significantly above that of laminitis negative horses until 40 h after the first administration of carbohydrate. At 48 h hoof temperatures were similar at around 18°C.

We make the assumption here that changes in hoof temperature reflect changes in the underlying dermal blood flow as has long been accepted in studies using a variety of other animal models (Henshaw *et al.* 1972; Hales 1985). The calibrated transistors placed in holes in the hoof wall probably did not measure the temperature of the lamellar dermis with absolute accuracy, but they appeared to measure successfully, temperature shifts. Therefore, a high hoof temperature would be associated with sublamellar vasodilation and a low hoof temperature with some degree of vasoconstriction. The plateaux of maximum hoof temperatures occurring, during the development of laminitis in the laminitis positive horses, were not significantly higher than the spontaneous high hoof temperatures occurring in the same horses (and in some sham treated controls) during the acclimatisation period. Our results suggest that a hoof temperature of around 30°C represents the maximum degree of vasodilation achievable in an environmental chamber with an ambient temperature set at 10°C. This hoof temperature was periodically reached in normal sham treated control horses and laminitis positive and laminitis negative horses during the acclimatisation period. Therefore, it is not the degree of vasodilation that appears to be the critical factor in determining if a horse develops laminitis, but the timing and duration of the vasodilation in relation to the concomitant metabolic events induced by the carbohydrate overload. Prolonged foot vasodilation alone does not cause laminitis as we maintained foot temperatures averaging 28°C for 30 h using repeated injections of bupivacaine HCl (Marcain)⁸ to the palmar digital nerves, with no ill effect (C.C. Pollitt and C. T. Davies, unpublished data).

Our data suggest that for laminitis to result from the alimentary administration of carbohydrate a period of sublamellar vasodilation must occur. If the circulation remains constricted then laminitis does not occur. The critical period during which vasodilation and laminitis concur is 12–40 h after the first administration of carbohydrate. Clinical laminitis, manifest as weight shifting from one foot to the other, appeared

32–40 h after carbohydrate administration. Histopathology confirmed the existence, in laminitis positive horses, of the lesions typical of acute laminitis 48 h after alimentary carbohydrate overload as described previously (Pollitt 1996).

Pyrexia was a feature of both the laminitis positive and negative groups of horses. Mean core temperatures of both groups were virtually identical (Fig 4) for the first 16 h after the first administration of carbohydrate. However, after 16 h, mean core temperature of laminitis positive horses increased above that of the negative horses for the remainder of the experimental period, although the difference only reached significance after 36 h. The graphs of core temperature for both laminitis positive and negative horses were biphasic. The first peak occurred between 16 and 20 h and the second, 16 h later at about 36 h. Circulating endotoxin is a potent pyrogen in horses and single i.v. injections of *E. coli* endotoxin cause increases in core temperature proportional to the dose administered (Wisniewski *et al.* 1992). Endotoxaemia following alimentary carbohydrate overload in horses was a feature of 11 of 12 horses developing Obel grade 3 laminitis (Sprouse *et al.* 1987). In 5/11 (45%) of these horses the endotoxaemia was biphasic with peaks, separated by an interval of 16 h, occurring at 32 and 48 h. The rapid production of high concentrations of Gram-negative endotoxin in the caecum of horses after alimentary carbohydrate overload (Moore *et al.* 1979) appears to account for the endotoxaemia verified by Sprouse *et al.* (1987) in horses treated similarly. Although endotoxin was not measured in the blood of the 13 horses, given alimentary overload of carbohydrate in this study, it seems probable that fluctuating levels were responsible for the pattern of pyrexia recorded from both laminitis positive and negative horses. Higher mean core temperatures developed by the laminitis positive group could have been due to higher blood concentrations of endotoxin.

Capillary and AVA blood flow in the skin have been manipulated experimentally in sheep by inducing fever with i.v. pyrogen and by heating the spinal cord (Hales *et al.* 1982; Rubsamen and Hales 1984). Skin heat conductance increased as blood flow increased in either the capillaries or the AVAs, but the highest levels of heat conductance were only achieved when both capillary and AVA blood flow were at their highest levels. A feature of the hoof temperature graphs of the laminitis positive horses was a prolonged period of high temperature (Figs 3 and 7) which appeared to be the maximum hoof temperature achievable in the 10°C environment. If this high hoof temperature is occurring because all available digital capillaries and AVAs are fully dilated, as seems probable (Hales 1985), then it follows that the tissues of the foot were being perfused by more blood than would be the case if the digital blood vessels were constricted.

Blood flow through AVAs is controlled by specific central thermoregulatory reflexes, whereas capillary flow is the target of local temperature effects (Hales 1985). Even if AVAs were the only hoof vessels to dilate initially, the delivery of hot arterial blood to the dermal tissues would soon raise the local temperature and cause capillary blood flow to increase. This principle has been well demonstrated in the hind leg skin of sheep using intra-arterial injections of capillary sized radioactive microspheres (Hales 1981). If this occurs during the development of laminitis it suggests a more passive role for the digital circulation than has previously been proposed. We speculate that, in response to rising core temperature, AVAs in the digits dilate via the central thermoregulatory reflex pathway and raise local temperature. The high local temperature causes capillaries to dilate via the local

reflex and sublamellar tissues are perfused with maximum possible flow of blood. This may expose the lamellar tissue, be it dermis or epidermis, to blood borne factors which trigger lamellar separation of acute laminitis.

The presence of a pronounced digital pulse has been noted by many authors as an important clinical sign of acute laminitis (Robinson 1990; Hood *et al.* 1993). In our study, all the laminitis positive horses developed a pronounced digital pulse in one or both front feet, at 24 or 32 h. This occurred in conjunction with warm hooves. In 3 of these horses the pronounced digital pulse persisted for 8 h then disappeared. One horse had a pronounced digital pulse at 24 h, which had disappeared at 32 h but was detectable again at 48 h. In most of the laminitis negative horses, a pronounced digital pulse occurred only once when hoof temperatures were either warm or cold.

Shunting of blood through AVAs has been proven to occur (Hood *et al.* 1978) at the time of clinical lameness (about 48 h after carbohydrate overload) when pulsing of the digital arteries is invariably present. The transient pronounced digital pulse recorded before clinical lameness in the horses of this study could correlate with a period of AVA dilation. The pulse disappears when the foot warms and initiates high capillary flow therefore relieving the AVAs of the 'need' to remain fully dilated. Confirmation of this hypothesis awaits further study.

Based on these data the use of vasodilatory therapy during the developmental phase of laminitis would be contraindicated. Paradoxically, the peripheral vasodilatory agent isoxsuprine hydrochloride was declared beneficial in the treatment of acute laminitis by Kirker-Head *et al.* (1987). Laminitis was induced by the alimentary carbohydrate overload method but the drug was not administered until 48 h had elapsed when clinical lameness had appeared. There is a proven correlation between clinical lameness and the severity of lamellar pathology (Pollitt 1996); and since the critical vasodilatory, laminitis associated, phase had passed, isoxsuprine could have had no influence on the pathogenesis of the laminitis in these horses. The drug and the vasodilation it induced may well have improved the state of the treated horses by relieving painful post developmental ischaemia and promoting healing of lamellae already damaged at the time of its administration. Similar logic applies to the report of Hinckley *et al.* (1996) that laminitis in grass founded ponies responded to vasodilation induced by topical application of glyceryl trinitrate to the pasterns.

The decrease in capillary perfusion and significant arteriovenous shunting present in horses with acute laminitis after alimentary carbohydrate overload shown by Hood *et al.* (1978) are also data obtained after lamellar pathology had occurred and, therefore, cannot be used to imply pathogenesis. In the experiments of Hood *et al.* (1978) foot blood flow increased until just prior to the onset of clinical laminitis which is in agreement with the sequence of events presented here. Similarly, the deduction by Coffman *et al.* (1970) that acute laminitis involved decreased lamellar perfusion was based on angiovenograms, interpreted to demonstrate reduced or obliterated terminal arteries, made when clinical laminitis was already underway. The vessel constriction and reduced digital blood flow they demonstrated was probably the result of lamellar injury rather than the cause of it.

This, the first study to evaluate hoof temperature continuously and, hence, sublamellar blood flow during the developmental phase of laminitis confirms the conclusion of Robinson *et al.* (1976) and Trout *et al.* (1990) that laminitis is preceded by an increased flow of blood in the lamellar region.

This is at variance with the results of Coffman *et al.* (1970), Garner *et al.* (1975), Hood *et al.* (1978), Hood *et al.* (1990) and Hinckley *et al.* (1996) who suggested that the reduced digital blood flow involved was a cause of laminitis. In this study there was no evidence that decreased digital blood flow was associated with the onset of laminitis and the contrary appeared to be the case: it was increased sublamellar blood flow that was associated with laminitis. The experiments, conducted on horses with short summer hair coats and therefore not physiologically adapted to a low environmental temperature (10°C), appear to have serendipitously revealed that digital vasoconstriction conferred protection during the developmental phase of carbohydrate induced laminitis.

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Manufacturers' addresses

¹Selleys Chemical Co., Padstow, NSW 2211, Australia.

²Exergen Corporation, Natick, Massachusetts, USA.

³Baxter Healthcare Corporation, California, USA.

⁴Smith and Nephew Pty Ltd, Clayton, Victoria 3168, Australia.

⁵Equine Electronics, PO Box 255, East Brisbane, Queensland 4169, Australia.

⁶Ranvet Pty Ltd, Beaconsfield, NSW 2015, Australia.

⁷Intervet, (Australia) Pty Ltd, Lane Cove, NSW 2066, Australia.

⁸Astra Pharmaceuticals Pty Ltd, North Ryde, NSW 2113, Australia.

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