Continuous intravenous infusion of glucose induces endogenous hyperinsulinaemia and lamellar histopathology in Standardbred horses

Melody A. de Laat [a], Martin N. Sillence [b], Catherine M. McGowan [c], Christopher C. Pollitt [a]

[a] *Australian Equine Laminitis Research Unit, School of Veterinary Science, The University of Queensland, Gatton, Queensland 4343, Australia.*
[b] *Faculty of Science and Technology, Queensland University of Technology, Brisbane, Queensland 4001, Australia.*
[c] *Institute of Ageing and Chronic Disease, Faculty of Health and Life Sciences, University of Liverpool, Neston, CH64 7TE, UK*

Keywords: Hyperglycaemia; Equine; Insulin; Laminitis

Abstract

Endocrinopathic laminitis is frequently associated with hyperinsulinaemia but the role of glucose in the pathogenesis of the disease has not been fully investigated. This study aimed to determine the endogenous insulin response to a quantity of glucose equivalent to that administered during a laminitis-inducing, euglycaemic, hyperinsulinaemic clamp, over 48 h in insulin-sensitive Standardbred racehorses. In addition, the study investigated whether glucose infusion, in the absence of exogenous insulin administration, would result in the development of clinical and histopathological evidence of laminitis. Glucose (50% dextrose) was infused intravenously at a rate of 0.68 mL/kg/h for 48 h in treated horses (n = 4) and control horses (n = 3) received a balanced electrolyte solution (0.68 mL/kg/h).

Lamellar histology was examined at the conclusion of the experiment. Horses in the treatment group were insulin sensitive (M value 0.039 ± 0.0012 mmol/kg/min and M-to-I ratio (x 100) 0.014 ± 0.002) as determined by an approximated hyperglycaemic clamp. Treated horses developed glycosuria, hyperglycaemia (10.7 ± 0.78 mmol/L) and hyperinsulinaemia (208 ± 26.1 µIU/mL), whereas control horses did not. None of the horses became lame as a consequence of the experiment but all of the treated horses developed histopathological evidence of laminitis in at least one foot. Combined with earlier studies, the results showed that laminitis may be induced by either insulin alone or a combination of insulin and glucose, but that it is unlikely to be due to a glucose overload mechanism. Based on the histopathological data, the potential threshold for insulin toxicity (i.e. laminitis) in horses may be at or below a serum concentration of ~200 µIU/mL.

Introduction

Endocrinopathic laminitis is a disease affecting the lamellar region of the horse's foot which arises secondary to hormonal dysfunction. Equine Cushing's disease, equine metabolic syndrome and excessive consumption of carbohydrate-rich pasture, have all been repeatedly implicated as predisposing factors for the development of lamellar failure (McGowan, 2010). Research has further defined endocrinopathic and pasture-associated laminitis as diseases primarily associated with elevated serum insulin concentrations (Hess et al., 2005; McGowan et al., 2004; Treiber et al., 2006a).

Although hyperinsulinaemia has been linked with the development of both naturally-occurring (Treiber et al., 2006b) and experimental (Asplin et al., 2007) forms of the disease, the mechanism by which an increase in circulating insulin can negatively impact on the lamellar region remains contentious. Previously, it has been demonstrated in horses that serum insulin concentrations > 1000 µIU/mL induce laminitis within 48 h (de Laat et al., 2010). However, the exogenous administration of insulin during a euglycaemic, hyperinsulinaemic clamp (EHC) necessitates the concurrent infusion of large amounts of glucose (DeFronzo et al., 1979). Although the subjects of a prolonged EHC remain euglycaemic at all times (de Laat et al., 2010), the effect of the infused glucose on lamellar tissues is unclear. Furthermore, pasture-associated laminitis is linked to hyperinsulinaemia and the consumption of pastures rich in non-structural carbohydrate (Geor, 2009).

Hyperglycaemia damages sensitive tissues, such as the kidney, in diabetic humans (Nishikawa et al., 2007). However, horses rarely develop type 2 diabetes, so

the consequences of hyperglycaemia and excessive glucose metabolism have received minimal attention in this species. The feedback relationship between insulin and glucose also means that determining the impact of one of these substances (in the absence of the other) is difficult to achieve in vivo. Moreover, the degree of hyperinsulinaemia associated with naturally-occurring endocrinopathic laminitis varies considerably between individuals, even those grazing the same pasture (Bailey et al., 2007; Carter et al., 2009), which makes the prediction of disease onset challenging. However, ponies with equine Cushing's disease, with a serum insulin concentration > 188 μIU/mL, are at an increased risk of laminitis (McGowan et al., 2004), which could suggest a primary role for insulin.

In the current study, we sought to investigate the endogenous insulin response to a prolonged glucose infusion (48 h). We infused a quantity of glucose equivalent to that administered during our previous prolonged EHCs (~ 0.32 g/kg/h) in insulin-sensitive, Standardbred horses (de Laat et al., 2010). We aimed to determine whether an increase in serum insulin concentration would occur in response to the glucose infusion and, if so, whether the resultant endogenous insulin concentrations would be as high as those recorded during an EHC (> 1000 μIU/mL). Our second objective was to determine whether the quantity of glucose administered during the EHC, and the accompanying endogenous insulin response, would result in clinical and histopathological evidence of laminitis. Our hypothesis was that endogenous hyperinsulinaemia of a lower magnitude than recorded during an EHC would develop secondary to a persistent glucose infusion over 48 h, and that this would not be sufficient to induce clinical or histopathological evidence of laminitis.

Materials and methods

The experimental protocol was approved by the Animal Ethics Committee of the University of Queensland (SVS/108/09/RIRDC) which ensured compliance with the Animal Welfare Act of Queensland (2001) and the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes (7th edition 2004). All horses were monitored by a registered veterinarian.

Subjects

Eight male, recently retired, Standardbred racehorses (417 ± 16 kg BW; 6.3 ± 0.74 years) were used. They were in moderate body condition (4.3/9; Henneke et al., 1983) and clinically normal on physical examination. Clinical and radiographic foot examination excluded horses with abnormalities associated with laminitis from the study. Heart rate, respiratory rate and rectal temperature were monitored daily prior to the study, and at 4 h intervals throughout the infusion period. All horses were subject to a pre and post-treatment lameness examination. Routine haematological and biochemical analyses were performed on blood samples (20 mL) drawn from all horses at the beginning and end of the study.

Urinalysis was performed at 8 h intervals during the infusion period to semi-quantitatively assess for glycosuria (Combur-9, Roche). The urine dipsticks were validated for equine urine against the hexokinase method using an automated clinical chemistry analyser ($\rho_c = 0.99$). All horses wore an equine nappy (Equisan) to facilitate urine collection.

Both forelimbs of all horses were fitted with pedometers, placed proximal to the carpus to determine if increased limb movement occurred secondary to shifting bodyweight, as a potential indicator of foot pain in treated horses. The horses were allocated at random to either a treatment or control group.

The experiment was conducted as controlled replicates within 2 weeks. Horses were housed at the research facility for 1 week prior to the study and were fed medium quality lucerne chaff and hay. Ad libitum access to water and the same food were provided throughout the study period.

Prolonged glucose infusion

Extended-use, IV catheters (MilaCath) were aseptically placed and sutured into both jugular veins of all horses. The infusion was administered into the right catheter while the left was used for blood collection.

Horses in the treatment group were administered a continuous infusion of glucose (50% dextrose, Baxter) over 48 h at a rate of 0.68 mL/kg/h. The rate was calculated from the quantity of glucose infused during previous EHCs (48 h) which resulted in clinical laminitis (de Laat et al., 2010). The glucose infusion rate was reduced in 10% increments if the blood glucose concentration exceeded 15 mmol/L, in order to avoid potential complications of marked hyperglycaemia. Control horses received a balanced electrolyte solution (Hartmanns, Baxter) at 0.68 mL/kg/h for 48 h.

Blood samples (10 mL) were drawn for the measurement of blood glucose and serum insulin concentration at the following time-points: 0 h, 15 min, 30 min, 1 h, 90

min, 100 min, 110 min, 2 h, then hourly until 6 h, once at 8 h, then every 4 h to 48 h. Blood glucose concentration was determined immediately on fresh whole blood using a handheld glucometer (Accucheck, Roche) that was calibrated against the hexokinase method, for these horses, using an automated clinical chemistry analyser (Lin's ρ_c = 0.95). The remaining blood was placed in plain Vacutainers, allowed to clot (30 min) and centrifuged at 3000 *g* for 10 min. Aliquots of serum (1 mL) were stored at -80 ºC until analysed. Serum insulin concentrations were measured using radioimmunoassay (Coat-a-count, Siemens) previously validated for use in horses (McGowan et al., 2008). Samples from treated horses, from the 3 h time-point onwards, were diluted 1:5 with insulin-free serum.

Determination of insulin sensitivity

Data from the initial 2 h of the infusion period was used as an approximated hyperglycaemic clamp (HC), to determine each treated horse's tissue sensitivity to endogenous insulin in accordance with De Fronzo et al., (1979). The HC technique induces hyperglycaemia (6.9 mmol/L above normal), for a period of 120 min, which suppressed basal hepatic glucose production and facilitates assessment of the sensitivity of the pancreatic beta cells to glucose. Glucose metabolism is calculated during the steady state period (90 – 120 min) when blood glucose concentrations are stable (10.9 to 13.3 mmol/L).

Although the infusion rate was not manipulated in the present study, a steady state period occurred and allowed insulin sensitivity values to be approximated for each treated horse. Thus, blood glucose and serum insulin concentrations taken during the steady state period (3 x 10 min) were used to calculate the amount of glucose

metabolised (M) and insulin sensitivity (M-to-I ratio), with allowances for urinary glucose loss and a space correction, using standard protocols (Rijnen and van der Kolk, 2003).

Lamellar histopathology

At the conclusion of the experiment the horses were humanely euthanased and necropsied. All four feet were immediately disarticulated at the metacarpo-phalangeal joint and cut into sagittal sections with a band saw. Lamellar tissue (5 mm x 5 mm) was dissected from the mid-dorsal region of each hoof with a scalpel, trimmed, rinsed and placed in 10% neutral buffered formalin for 24 h.

Following fixation, lamellar samples were processed routinely for histology and stained with haematoxylin and eosin (H & E) and periodic acid Schiff (PAS). Prepared sections were coded, randomised and examined independently via light microscopy (Olympus BX-50), by two authors (CCP and MAD) who were blinded to treatment type. Each foot was examined at 100, 200 and 400x magnification, with a minimum of 30 microscopic fields and eight primary epidermal lamellae (PELs) examined at each magnification. The lamellar histopathology was graded using the following scale: 0, negative; 1, secondary epidermal lamellar (SEL) lengthening and narrowing, nuclear disorientation, prominent nucleoli, loss of uniform basal cell architecture and apoptosis; 2, as above plus increased mitosis and dermal polymorphonucleocytes (PMNs); 3, marked cellular and structural changes with basement membrane dysadhesion and loss.

Histometric measurements of SEL length (SELL) and width (SELW) were made in the axial (tip) and abaxial (base) halves of eight PEL from both forefeet by one of the authors (MAD), who was blinded to the treatment group, according to a previously validated protocol (de Laat et al., 2011).

Statistical analysis

All data were normally distributed (Shapiro-Wilk test; $P > 0.05$). Blood glucose and serum insulin concentrations were compared within (paired) and between (unpaired) groups with a *t* test. Pedometer readings from both forelimbs of each horse were totalled and compared between groups using a Welch *t* test. The presence or absence of lamellar histopathology was assessed as an outcome using Fisher's exact probability test. Histometric measurements from each forefoot were averaged to obtain a single value for each horse and compared between groups using a Welch *t* test. All data are presented as means ± se and statistical significance was accepted at $P < 0.05$. Statistical analyses were performed using R, version 7.2.7.

Results

Subjects

Lameness was not detected in any horse. Routine blood haematology and biochemistry results did not differ between treatment and control groups either before or after the study. Demeanour, appetite and heart and respiratory rates did not change throughout the experiment. Rectal temperature was unchanged in seven of the horses however one control horse was withdrawn from the experiment with a transient fever. This resulted in the final replicate consisting of one control and two treated horses, and a sample size of seven.

Urinalysis results were unremarkable prior to the experiment. However, all of the treated horses developed mild glycosuria within 8 h of glucose infusion and this continued to increase over the 48 h period (Fig. 1). Glycosuria was not detected in any control horse. Pedometer readings did not differ between groups (control; 7.1 ± 2.1 steps/min and treatment; 5.5 ± 1.4 steps/min).

Prolonged glucose infusion

Glucose infusion was well tolerated by all of the treated horses and a reduction (10%) in infusion rate was only required in one horse between 14 h and 16 h. Basal blood glucose concentration did not differ between control (5.1 ± 0.13 mmol/L) and treated horses (5.7 ± 0.43 mmol/L) but blood glucose concentration (10.7 ± 0.78 mmol/L) increased ($P < 0.05$) above the baseline value in the treated horses during glucose infusion (15 min – 48 h; Fig. 2). The control group maintained basal blood glucose concentrations during the infusion (Fig. 2).

Basal serum insulin concentration was similar for treated (7.84 ± 0.29 µIU/mL) and control (8 ± 0.58 µIU/mL) horses and did not increase in the control group throughout the infusion (10.6 ± 1.36 µIU/mL). In contrast, serum insulin concentrations increased ($P < 0.05$) above basal levels during the glucose infusion in the treated group (24 h – 48 h; 208 ± 26.1 µIU/mL). A gradual increase in serum insulin concentration commenced within 15 min of the start of the infusion and was significantly elevated (121 ± 30 µIU/mL) above basal levels 4 h after the start of the experiment, reaching a peak concentration (268 ± 87 µIU/mL) by 32 h (Fig. 3). After

this there was no further increase in glucose and insulin concentration although glycosuria continued to increase up to 48 h (Fig. 1).

Determination of insulin sensitivity

During the steady state period (Fig. 2 inset) blood glucose and serum insulin concentrations for the treated horses were 12.5 ± 1.1 mmol/L and 41.7 ± 5.16 µIU/mL, respectively. The amount of glucose metabolised (M) during the steady state period for the treated horses was 0.039 ± 0.0012 mmol/kg/min and the M-to-I ratio (x 100) was 0.014 ± 0.002.

Lamellar histopathology

All four feet from control horses were normal (Table 1; Fig. 4a). In contrast, the treated horses developed histopathological evidence of laminitis in at least one foot ($P < 0.05$). The severity of lamellar histopathology varied among the treated horses with one horse sustaining lamellar pathology in one front foot only, compared to other horses with lamellar pathology in both front or all four, feet (Table 1).

Lesions in the treatment group included lengthening and tapering of the SELs (Fig. 4c), compared to control horses (Fig. 5). SELs at both the axial and abaxial ends of the PELs were narrower ($P < 0.05$) in treated horses (Fig. 5). Secondary dermal lamellae (SDLs) were frequently obliterated, resulting in confluence of SEL basal cells near the PEL axis (Fig. 4b). Cellular changes included rounded epidermal basal cell nuclei with prominent nucleoli, loss of uniform basal and parabasal cell architecture and increased evidence of mitotic figures (Table 1) and apoptosis (Fig. 4d). The basement membrane at the tips of the SELs was adjacent to the basal cell

layer but appeared irregular (Fig. 4b). Increased dermal presence of extravasated PMNs was seen around the axial tips of the PELs (Fig. 4d) in forefoot sections from three treated horses, compared to none in the control horses (Table 1).

Discussion

Our study has shown the endogenous insulin response of the equine pancreas to a prolonged glucose infusion in healthy, insulin-sensitive horses. It also demonstrated that hyperglycaemia and moderate hyperinsulinaemia can produce lamellar pathology consistent with laminitis in insulin-sensitive horses within 48 h. The results suggest that hyperinsulinaemic horses and ponies may have subclinical, endocrinopathic lamellar pathology that may progress to laminitis in the field. Considering that the quantity of glucose infused in this study was equivalent to the quantity that was administered during a laminitis-inducing EHC (de Laat et al., 2010), it appears unlikely that endocrinopathic laminitis is due to glucose overload alone. These results support the theory that endocrinopathic laminitis may arise secondary to hyperinsulinaemia in combination with an increased glucose load or hyperinsulinaemia alone.

The EHC technique has been criticised for the excessively high serum insulin concentrations attained, and there is little doubt that the model represents an exaggerated form of the naturally-occurring disease, with laminitis occurring within a 48 h period (de Laat et al., 2010). The current study has demonstrated that much lower (5-fold) serum insulin concentrations than those attained during the EHC are also capable of initiating lamellar damage, albeit less severe. This suggests that the severity of lamellar damage is closely linked with the magnitude of hyperinsulinaemia

and the time-frame over which it occurs, which can vary significantly between individuals. Insulin-resistant horses and ponies would be expected to produce a more exaggerated insulin response to carbohydrate ingestion and hyperglycaemia, and would therefore presumably be at risk of more severe lamellar injury than the insulin-sensitive horses used here.

An important aspect was to study the effects of hyperglycaemia and hyperinsulinaemia without the involvement of gastrointestinal variables, helping to make the study distinct from carbohydrate-overload laminitis. By administering the glucose intravenously (IV) we sought to mimic the glucose infusion component of the EHC, thereby developing a comparable technique, and we were also able to bypass the gastrointestinal tract. Carbohydrate-overload models of laminitis (and the naturally-occurring form of the disease that they seek to emulate) are associated with inappropriate hindgut fermentation and the systemic release of toxic factors that may initiate the syndrome (Garner et al., 1975). Insulin-induced laminitis is not associated with any clinical evidence of gastrointestinal involvement (Asplin et al., 2007; de Laat et al., 2010) and also appears to be a less inflammatory process than alimentary forms (de Laat et al., 2011).

By administering the glucose IV we were able to approximate an HC. Several tests for the determination of insulin and glucose metabolism have been adapted for use in the horse (Firshman and Valberg, 2007) and have improved our ability to diagnose insulin resistance in this species. While basal glucose and insulin concentrations and ratios are used most frequently in a clinical setting, more invasive tests, such as clamps, have been used to more accurately determine insulin sensitivity

in ponies and horses (Pratt et al., 2006; Rijnen and van der Kolk, 2003). In particular, the HC suppresses endogenous hepatic glucose production, which facilitates determination of the sensitivity of the pancreatic beta cells to glucose, and tissue sensitivity to endogenously produced insulin (DeFronzo et al., 1979). When used as a diagnostic test, the HC induces hyperglycaemia for only a short period (2 - 3 h). To our knowledge, the effects of prolonging this technique on glucose metabolism and insulin secretion have not been investigated in horses.

Although the HC involves varying the infusion rate to clamp blood glucose levels (the opposite approach to the one employed here) target blood glucose concentrations were comparable. Modification of the HC (i.e. to use fixed glucose infusion rates in the assessment of insulin secretion rates in humans) has been successful, with comparable results found between the two techniques (Kelley et al., 2010). Thus, calculation of the approximate insulin sensitivity of the treated horses was possible using steady state blood glucose concentrations (12.5 ± 1.1 mmol/L), providing further data in this scant field of knowledge.

The M value in the current study (0.039 ± 0.0012 mmol/kg/min) was higher than reported previously (Rijnen and van der Kolk, 2003) in Warmblood horses (0.011 ± 0.0045), and while it indicates a higher rate of metabolism of glucose (and sensitivity to insulin), is probably associated with the higher glucose infusion rate used in the current study (~270 mL/h vs. ~120 mL/h). However, the M-to-I ratio was similar in the two studies (0.017 ± 0.016 vs. 0.014 ± 0.002).

The M-to-I ratio calculated during a HC is an indicator of tissue sensitivity to endogenous (equine) insulin, in contrast to the EHC where the M-to-I ratio is a measure of tissue sensitivity to exogenously administered (human) insulin. The high serum insulin concentrations in the EHC also result in lower (100-fold) measures of insulin sensitivity due to the fact that increasing concentrations of insulin do not stimulate increased glucose metabolism but nevertheless do decrease the M-to-I ratio (DeFronzo et al., 1979). Thus, whereas the M values obtained from the two techniques are directly comparable, the M-to-I ratios are not (DeFronzo et al., 1979).

A study examining the effects of a prolonged glucose infusion on healthy humans found that hyperglycaemia (12.6 mmol/L) over 68 h resulted in reduced insulin secretion, decreased insulin clearance and reduced insulin-stimulated glucose uptake (Boden et al., 1996). This glucose desensitisation was compensated for (in part) by a decrease in the clearance rate of insulin from the body. Whether a reduction in insulin secretion and clearance occurred in the horses in the current study is unknown, and the use of a variable glucose infusion rate would reveal whether decreasing amounts of glucose would be required to maintain hyperglycaemia.

The infrequency with which horses develop hyperglycaemia may suggest that the equine pancreatic response to glucose is different to other species and is worthy of investigation. If insulin secretion remains at persistently high levels in horses in response to glucose intake, this may have implications for the pathogenesis of hyperinsulinaemic laminitis. However, the steadily increasing degree of glycosuria seen in our horses throughout the study supports the possibility that glucose desensitisation of peripheral tissues developed during the study. This finding may

suggest that horses are not able to continuously metabolise large amounts of glucose and that desensitisation occurs secondary to down-regulation or failure of glucose transporters.

Lamellar histopathological lesions observed in the present study were similar to those seen in horses with EHC-induced laminitis (de Laat et al., 2011), although less severe, and included increased evidence of apoptosis, mitosis and elongation and narrowing of the SELs. Variations in the length and shape of PELs and SELs can be normal (Kawasako et al., 2009), however lamellar pathology seen in our study differed considerably to control horses. The lesions more closely resembled those seen during the developmental stages of an EHC (M.A. de Laat., unpublished data) and this may suggest that lamellar pathology could have progressed had the hyperinsulinaemia persisted beyond 48 h. Temporal studies on the effect of mild-to-moderate hyperinsulinaemia on lamellar tissue are required.

Conclusions

The results of the current study demonstrate that a prolonged (48 h) glucose infusion induces moderate hyperinsulinaemia and subclinical lamellar pathology in insulin-sensitive horses. Although hyperglycaemia and glucose toxicity may be involved in the pathogenesis of endocrinopathic laminitis, it appears more likely that their primary role in disease pathogenesis is as a stimulus for insulin secretion. Moderate hyperinsulinaemia results in the development of lamellar compromise within 48 h, suggesting that lamellar pathology is potentially widespread in the hyperinsulinaemic equine population. An appreciation of the threshold for insulin toxicity may be important in the prevention of laminitis and our results suggest that

this threshold may be at or below ~ 200 µIU/mL. Swift and decisive management of even mild to moderate hyperinsulinaemia is therefore warranted.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

Acknowledgements

This study was funded by the Rural Industries Research and Development Corporation, Australia and the Animal Health Foundation, Missouri.

References

Asplin, K.E., Sillence, M.N., Pollitt, C.C., McGowan, C.M., 2007. Induction of laminitis by prolonged hyperinsulinaemia in clinically normal ponies. The Veterinary Journal 174, 530-535.

Bailey, S.R., Menzies-Gow, N.J., Harris, P.A., Habershon-Butcher, J.L., Crawford, C., Berhane, Y., Boston, R.C., Elliott, J., 2007. Effect of dietary fructans and dexamethasone administration on the insulin response of ponies predisposed to laminitis. Journal of the American Veterinary Medical Association 231, 1365-1373.

Boden, G., Ruiz, J., Kim, C.J., Chen, X.H., 1996. Effects of prolonged glucose infusion on insulin secretion, clearance, and action in normal subjects. American Journal of Physiology-Endocrinology and Metabolism 270, E251-E258.

Carter, R.A., Treiber, K.H., Geor, R.J., Douglass, L., Harris, P.A., 2009. Prediction of incipient pasture-associated laminitis from hyperinsulinaemia, hyperleptinaemia and generalised and localised obesity in a cohort of ponies. Equine Veterinary Journal 41, 171-178.

de Laat, M.A., McGowan, C.M., Sillence, M.N., Pollitt, C.C., 2010. Equine laminitis: Induced by 48 h hyperinsulinaemia in Standardbred horses. Equine Veterinary Journal 42, 129-135.

de Laat, M.A., van Eps, A.W., McGowan, C.M., Sillence, M.N., Pollitt, C.C., 2011. Equine Laminitis: Comparative Histopathology 48 hours after Experimental Induction with Insulin or Alimentary Oligofructose in Standardbred Horses. Journal of Comparative Pathology In Press, Corrected Proof. DOI: 10.1016/j.jcpa.2011.02.001

DeFronzo, R.A., Tobin, J.D., Andres, R., 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. American Journal of Physiology 237, E214-E223.

Firshman, A.M., Valberg, S.J., 2007. Factors affecting clinical assessment of insulin sensitivity in horses. Equine Veterinary Journal 39, 567-575.

Garner, H.E., Coffman, J.R., Hahn, A.W., Hutcheson, D.P., Tumbleson, M.E., 1975. Equine Laminitis of Alimentary Origin: An Experimental Model. American Journal of Veterinary Research 36, 441-444.

Geor, R.J., 2009. Pasture-Associated Laminitis. Veterinary Clinics of North America: Equine Practice 25, 39-50.

Henneke, D.R., Potter, G.D., Kreider, J.L., Yeates, B.F., 1983. Relationship between condition score, physical measurements and body-fat percentage in mares. Equine Veterinary Journal 15, 371-372.

Hess, T.M., Kronfeld, D.S., Treiber, K.H., Byrd, B.M., Staniar, W.B., Splan, R.K., 2005. Laminitic metabolic profile in genetically predisposed ponies involves

exaggerated compensated insulin resistance. Journal of Animal Physiology and Animal Nutrition 89, 431-431.

Kawasako, K., Higashi, T., Nakaji, Y., Komine, M., Hirayama, K., Matsuda, K., Okamoto, M., Hashimoto, H., Tagami, M., Tsunoda, N., Taniyama, H., 2009. Histologic evaluation of the diversity of epidermal laminae in hooves of horses without clinical signs of laminitis. American Journal of Veterinary Research 70, 186-193.

Kelley, D.E., Shankar, R.R., Keymeulen, B., Mixson, L., Mixson, D.L., Chung, C., Vandemeulebroucke, E., DeSmet, M., Cilissen, C., Herman, G.A., Beals, C.R., Steinberg, H.O., Shankar, S.S., 2010. The graded glucose infusion is comparable to the hyperglycaemic clamp as a tool for measurement of glucose-dependent insulin secretion. Diabetologia 53, 655.

McGowan, C.M., 2010. Endocrinopathic Laminitis. Veterinary Clinics of North America-Equine Practice 26, 233-237.

McGowan, C.M., Frost, R., Pfeiffer, D.U., Neiger, R., 2004. Serum insulin concentrations in horses with equine Cushing's syndrome: Response to a cortisol inhibitor and prognostic value. Equine Veterinary Journal 36, 295-298.

McGowan, T.W., Geor, R., Evans, H., Sillence, M., Munn, K., McGowan, C.M., 2008. Comparison of 4 assays for serum insulin analysis in the horse. Journal of Veterinary Internal Medicine 22, 115.

Nishikawa, T., Kukidome, D., Sonoda, K., Fujisawa, K., Matsuhisa, T., Motoshima, H., Matsumura, T., Araki, E., 2007. Impact of mitochondrial ROS production on diabetic vascular complications. Diabetes Research and Clinical Practice 77, S41-S45.

Pratt, S.E., Geor, R.J., McCutcheon, L.J., 2006. Effects of dietary energy source and physical conditioning on insulin sensitivity and glucose tolerance in Standardbred horses. Equine Veterinary Journal 38, 579-584.

Rijnen, K., van der Kolk, J.H., 2003. Determination of reference range values indicative of glucose metabolism and insulin resistance by use of glucose clamp techniques in horses and ponies. American Journal of Veterinary Research 64, 1260-1264.

Treiber, K.H., Kronfeld, D.S., Geor, R.J., 2006a. Insulin Resistance in Equids: a Possible Role in Laminitis. The Journal of Nutrition (Suppl.) 2094S-2098S.

Treiber, K.H., Kronfeld, D.S., Hess, T.M., Byrd, B.M., Splan, R.K., Staniar, W.B., 2006b. Evaluation of genetic and metabolic predispositions and nutritional risk factors for pasture-associated laminitis in ponies. Journal of the American Veterinary Medical Association 228, 1538-1545.

Tables

Table 1: Standardbred horses (*n = 7*) were treated with either an electrolyte solution (C1 – C3) or a continuous glucose infusion (G1 - G4), at a rate of 0.68 mL/kg/h for 48 h. Histological evidence of lamellar pathology was assessed in all four feet of each horse and each foot was graded on a scale of 0 to 3, to obtain a score out of 12. The number of mitotic figures and dermal polymorphonucleocytes (PMNs) in each high power (400x) field (hpf) were examined. Mean ± se serum insulin concentration was calculated for each horse.

Subject	*Histological grade of lamellar pathology (/12)*	*Feet affected*	*Number of mitotic figures/hpf*	*Number of PMNs/hpf in dermis*	*Serum insulin (µIU/mL)*
C1	0	-	0	0	8.6 ± 0.6
C2	0	-	0-1	0	9.1 ±1.0
C3	0	-	0	0	12 ± 1.6
G1	1	RF	0-2	> 5	182 ± 30
G2	3	LF, RF	0-15	> 10	135 ± 11
G3	2	LF, RF	0-5	0-1	287 ± 42
G4	4	all	0-10	> 5	231 ± 23

Key: The grading scale for each foot is as follows: 0, negative; 1, secondary epidermal lamellar (SEL) lengthening and narrowing, nuclear disorientation, prominent nucleoli, loss of uniform basal cell architecture and apoptosis; 2, as above plus increased mitosis and dermal polymorphonucleocytes; 3, marked cellular and structural changes with basement membrane dysadhesion and loss. RF, right fore; LF, left fore.

Figures

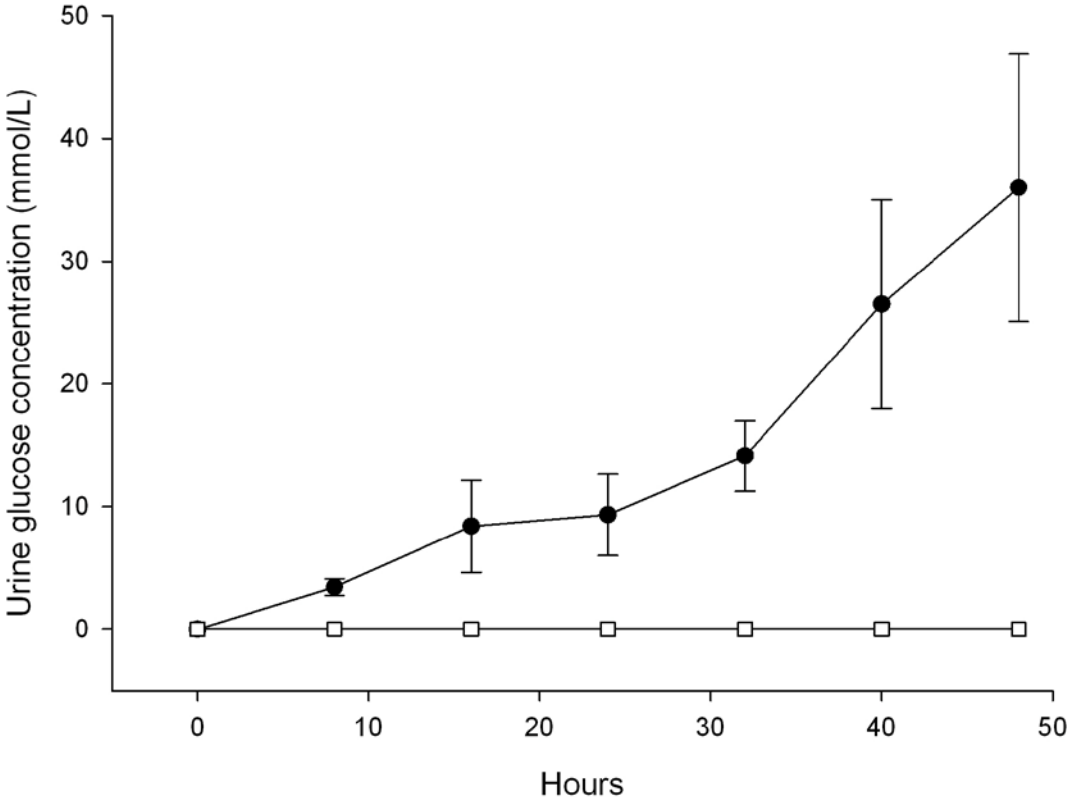

Figure 1: Urine glucose concentration (mmol/L) was measured semi-quantitatively in Standardbred horses treated with either an infusion of glucose (●, 50% dextrose, $n = 4$) or a balanced electrolyte solution (□, $n = 3$) at 0.68 mL/kg/h for 48 h. Mean ± se urine glucose concentration increased steadily over the 48 h period in the glucose treated horses while control horses did not develop glycosuria at any time.

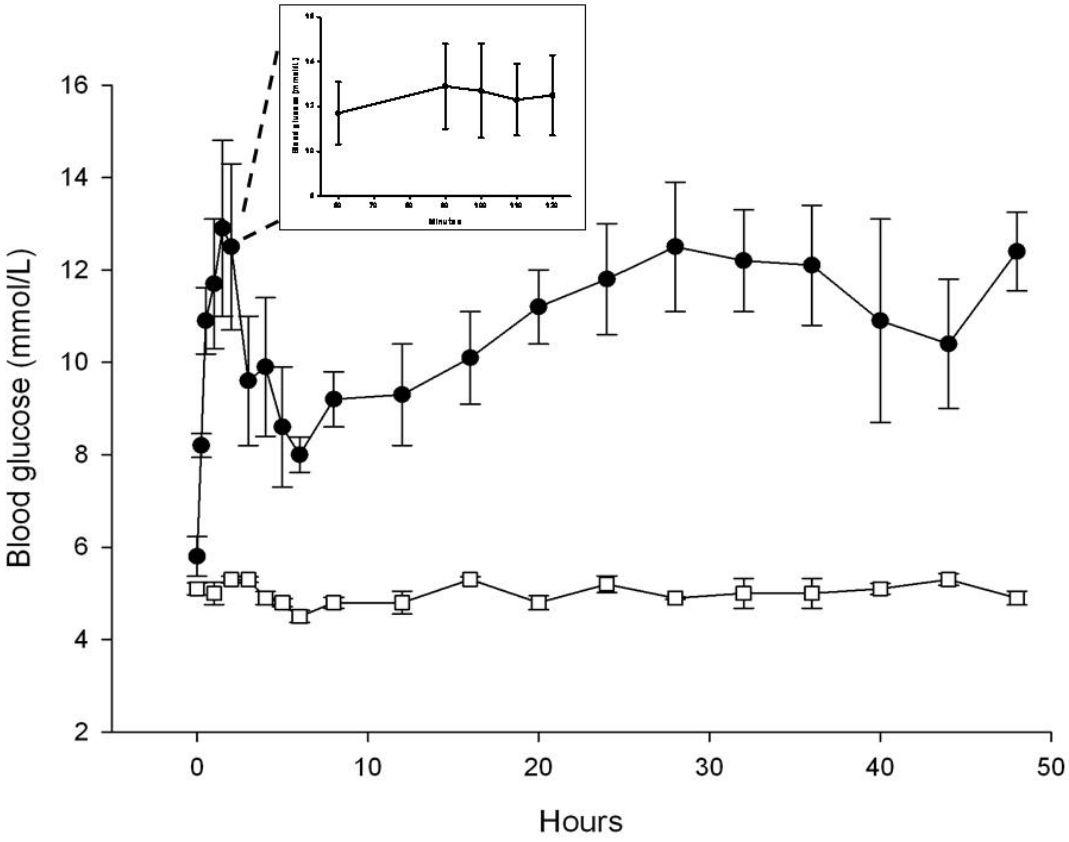

Figure 2: Mean ± se blood glucose concentration (mmol/L) was measured during a prolonged (48 h) glucose (50% dextrose; 0.68 mL/kg/h) infusion (●) in Standardbred horses (n = 4). Control horses (n = 3) received a prolonged (48 h) infusion of a balanced electrolyte solution (□). Blood glucose concentration remained in the normal range for the control horses but increased ($P < 0.05$) in treated horses. Blood glucose concentration during the steady state period (inset) was used to calculate insulin sensitivity.

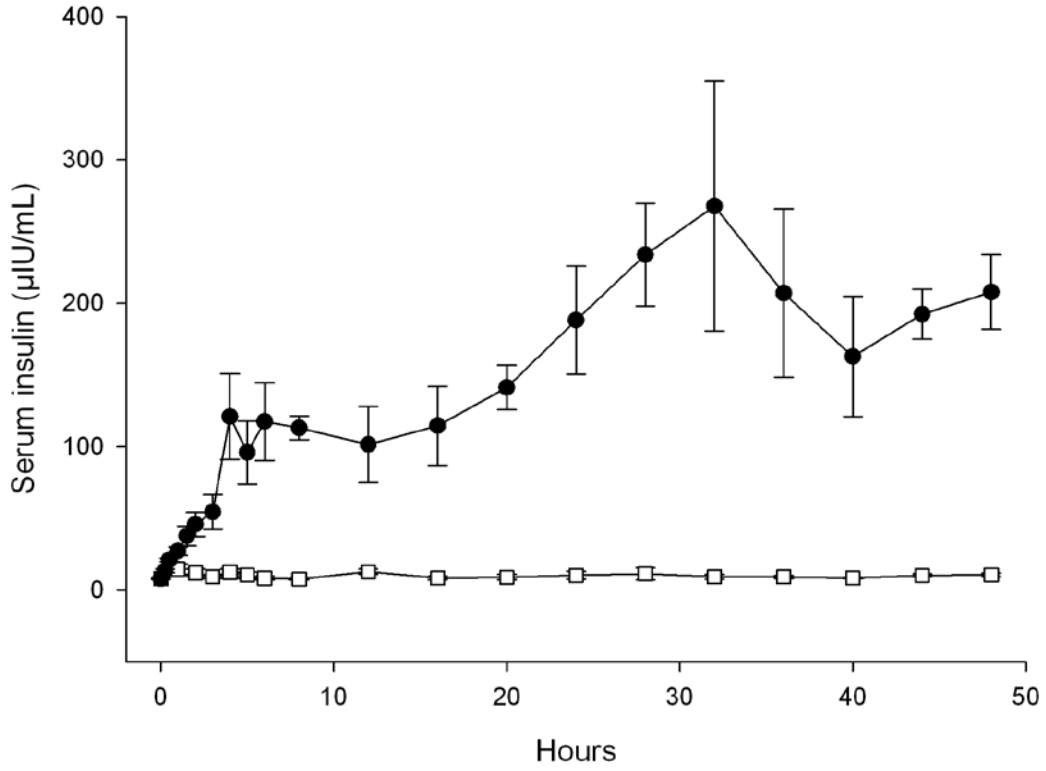

Figure 3: Mean ± se serum insulin concentration (µIU/mL) was measured in Standardbred horses treated with either a glucose (50% dextrose) infusion (n = 4) or a balanced electrolyte solution (n = 3) for 48 h. Serum insulin concentration increased ($P < 0.05$) in treated horses (●) during the infusion period, but remained normal in control horses (□).

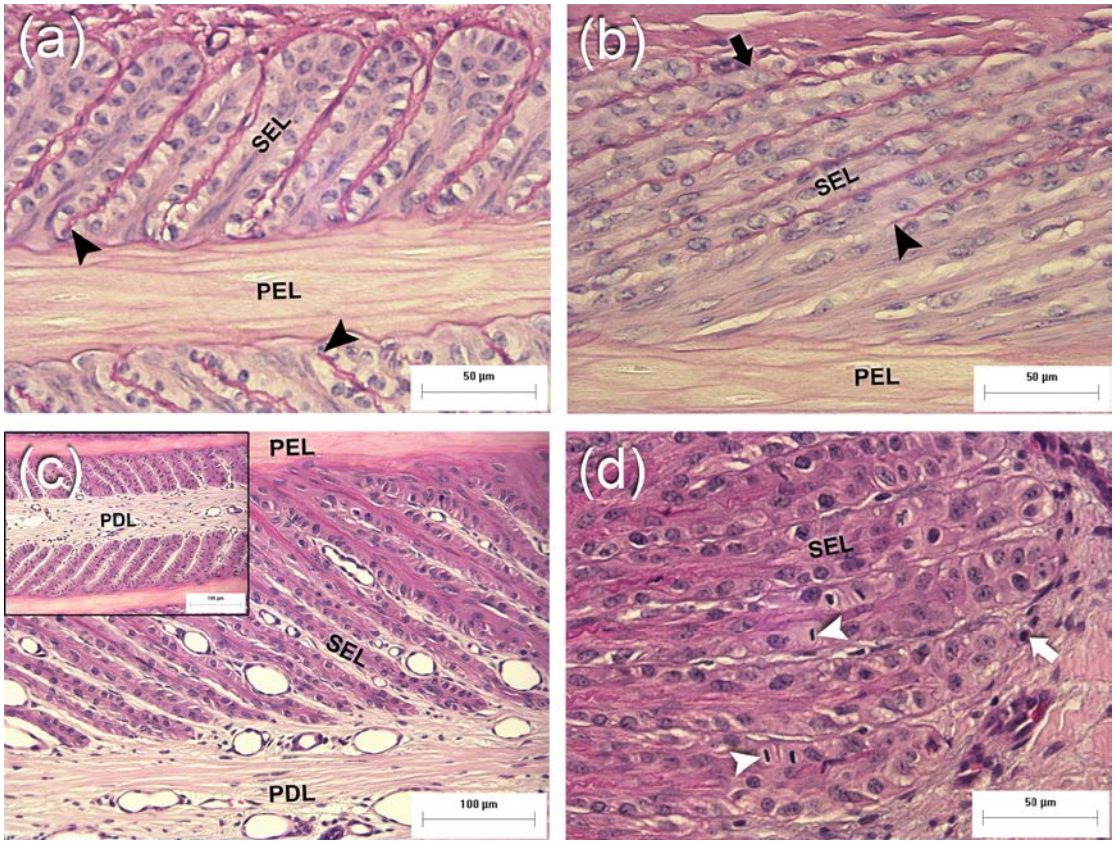

Figure 4: Representative photomicrographs from Standardbred horses receiving either an electrolyte (a, c inset) or glucose (50% dextrose) infusion (b, c, d) for 48 h. Control horses exhibited normal lamellar architecture (a) with tightly adhered PAS-stained basement membrane (BM) continuing the full length of the secondary epidermal lamellae (SEL) to the primary epidermal lamellar (PEL) axis (black arrowheads in a). Histopathological lesions in treated horses included loss of PAS-stained BM at the base of the SEL adjacent to the PEL axis (black arrowhead in b), patchy loss of definition of BM staining (black arrow in b), lengthening of SELs (c) and more frequent mitotic figures (white arrowheads in d). Infiltration of polymorphonuclear leucocytes around the axial tips of the PELs was seen in forefoot sections (white arrows in d). Stain = haematoxylin and eosin (a, b) or periodic acid Schiff (c, d).

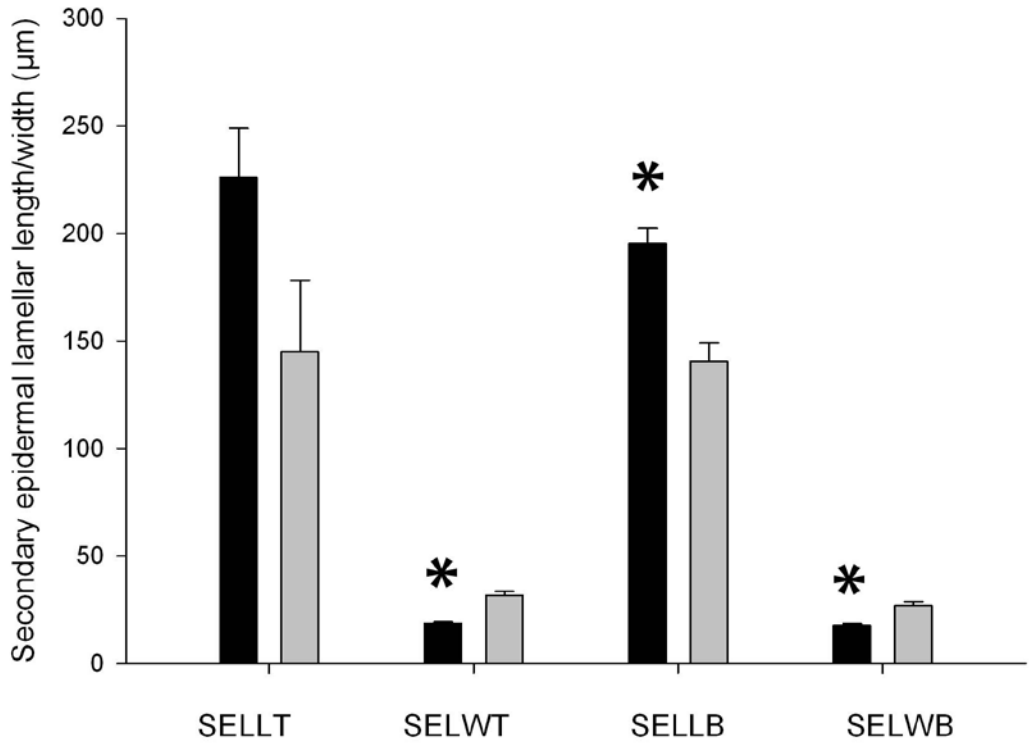

Figure 5: Mean ± se length (µm) of ten secondary epidermal lamellae (SELs) was measured in the axial (SELLT) and abaxial (SELLB) halves of eight primary epidermal lamellae (PEL) from both forefeet of horses treated with either an infusion of glucose (▉; 50% dextrose, $n = 4$) or an electrolyte solution (▒, $n = 3$) at 0.68 mL/kg/h for 48 h. SELs of treated horses were longer (*, $P < 0.05$) abaxially and narrower (*, $P < 0.05$) at both locations when compared to the control group.