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

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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 (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.

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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

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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 (ρ_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 up to 48 h. Blood glucose concentration was determined immediately on fresh whole blood using a hand held 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 °C 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 DeFronzo et al. (1979). The HC technique induces hyperglycaemia (6.9 mmol/L above normal), for a period of 120 min, which suppresses 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 (10.9–13.3 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 × 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 × 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 and 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 400× magnification, with a minimum of 30 microscopic fields and eight primary epidermal lamellae (PELs) were 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.

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