Association between hyperinsulinaemia and laminitis severity at the time of pituitary *pars intermedia* dysfunction diagnosis

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Abstract

Background

Hyperinsulinaemia is the suspected component of insulin dysregulation having the strongest association with laminitis and occurs variably in equids with pituitary *pars intermedia* dysfunction (PPID).

Objectives

We hypothesised that magnitude of hyperinsulinaemia correlates with laminitis severity in PPID-affected equids. Furthermore, we hypothesised that owners can be unaware of chronic endocrinopathic laminitis.

Study design

Cross-sectional study.

Methods

Serum insulin concentrations, owner-reported laminitis history and radiographic evidence of laminitis were determined in 38 client-owned horses and ponies with confirmed PPID. Laminitis severity was

classified into four categories (normal [nonlaminitic], mild, moderate or severe laminitis) based on degree of distal phalangeal rotation. Animals were also categorised as normoinsulinaemic (<20 μU/ml), mildly hyperinsulinaemic (20–50 μU/ml) and severely hyperinsulinaemic (>50 μU/ml). One-way ANOVA, *t* tests and Fisher's exact tests were performed.

Results

While owners reported laminitis in 37% of animals, 76% were laminitic based on study criteria (P = 0.01). Owners reported laminitis more frequently in hyperinsulinaemic vs. normoinsulinaemic animals; recognition increased with severity of hyperinsulinaemia (P = 0.03). Mean insulin concentrations were higher in equids with moderate to severe radiographic laminitis (geometric mean 74.1, 95% confidence interval (CI) 38.4–143.1 uU/ml) vs. those classified radiographically as normal to mild (31.9, 95% CI 21.1–48.1 uU/ml P = 0.03).

Main limitations

Dynamic insulin testing was not performed; some normoinsulinaemic animals might have had subtle insulin dysregulation.

Conclusions

Although radiographic abnormalities were present in most animals at the time of PPID diagnosis, chronic laminitis remained unrecognised by many owners. Owner awareness of laminitis increased with severity of hyperinsulinaemia and higher insulin concentrations were detected in association with more severe radiographic changes.

The Summary is available in Chinese – See Supporting Information.

Introduction

Aged equids increasingly comprise a larger segment of the equine population as medical advances maintain their athletic abilities and quality of life well into their 20s and beyond. Up to 30% of aged animals develop pituitary *pars intermedia* dysfunction (PPID), which generally manifests in animals over 15 years old 1, 2. The *pars intermedia* is normally under tonic dopaminergic inhibition by hypothalamic neurons; neurodegeneration leads to *pars intermedia* hyperplasia, microadenoma or macroadenoma formation and uninhibited release of hormones from melanotropes 3. The diseased *pars intermedia* secretes abnormally large quantities of proopiomelanocortin-derived peptides, of which adrenocorticotropic hormone (ACTH), α-melanocyte stimulating hormone (α-MSH), corticotropin-like intermediate lobe peptide and β-endorphin appear most important to the pathogenesis of PPID. Many of these hormones alter insulin and glucose dynamics, along with other aspects of metabolism.

Metabolic abnormalities associated with PPID place affected animals at a high risk of developing laminitis and most cases of laminitis are associated with underlying

endocrinopathies 4. Laminar pain can end a horse's athletic career and, sometimes, become severe enough to necessitate euthanasia. Endocrinopathic laminitis is often insidious in onset and can go unnoticed by owners until laminar damage is severe. Therefore, identification of early-stage laminitis in PPID-affected equids is imperative to limit laminar damage and distal phalanx displacement.

While not universally present, insulin dysregulation (ID) occurs in 30–60% of PPID-affected equids 2, 5, 6; its presence may therefore have prognostic and therapeutic implications, as equids with concurrent PPID and ID (specifically, hyperinsulinaemia) appear to be at the highest risk of developing laminitis 7. Insulin dysregulation encompasses any combination of fasting hyperinsulinaemia, postprandial hyperinsulinaemia, exaggerated insulin responses to oral/i.v. carbohydrates, tissue insulin resistance and dyslipidaemia 8. Of these components, persistent hyperinsulinaemia is believed to have the strongest association with laminitis, by virtue of its ability to directly induce laminar damage 9-12.

The current study was undertaken to determine if the presence of hyperinsulinaemia is suggestive of subclinical laminitis and if the degree of hyperinsulinaemia is predictive of laminitis severity. We hypothesised that magnitude of hyperinsulinaemia correlates with laminitis severity in PPID-affected equids. Furthermore, we hypothesised that owners can be unaware of mild endocrinopathic laminitis.

Methods

Animals

Thirty-eight client-owned nonpregnant mares (17) and geldings (21) were enrolled in November 2008, December 2008 and January 2009. All animals were diagnosed with PPID based on the presence of pathognomonic hypertrichosis and endocrine test abnormalities. Eleven animals had both an abnormal overnight dexamethasone suppression test (ODST; plasma cortisol ≥1 µg/dl at 19 h post dexamethasone administration 13) and high resting endogenous plasma ACTH concentration (≥45 pg/ml; reference range 9–45 pg/ml1); 24 had an abnormal ODST alone; and 3 had high plasma endogenous ACTH concentration alone. Additional eligibility criteria were a hypertrichosis score ≥1 (0 = normal [no unusual hair growth]; 1 = regional [long hair growth restricted to discrete areas]; 2 = generalised [slightly to moderately long haircoat and failure of normal shedding]; 3 = severe generalised [markedly long and/or curly

haircoat over entire body]) and no treatment for PPID or ID for at least 60 days prior to study enrolment. Horses ranged in age from 14 to 34 years (mean 24 ± 4.2 [s.d.] years), weighed 117–623 kg (mean 434 ± 108 kg), and had body condition scores of 3.5–7.0 (mean 5.0 ± 1.0) on the scale of 1–9 developed by Henneke *et al*. 14. Breeds included Arabian (6), Arabian-cross (6), Missouri Fox Trotter (1), Morgan (4), Morgan-cross (2), mixed-breed (2), Norwegian Fjord-cross (1), Paso Fino (1), pony (4), pony-cross (1), Quarter Horse and Quarter Horse-type (4), Standardbred (1), Tennessee Walking Horse (1), Thoroughbred (3) and Warmblood (1).

Study design

Horses were admitted to the Veterinary Medical Centre and housed in box stalls throughout the 2-day testing period. Animals were in stalls for 0.5–4 h before physical and lameness examinations were performed and blood (20 ml) was collected by jugular venipuncture into ethylenediaminetetraacetic acid (EDTA)-containing and serum tubes for measurement of serum insulin, plasma ACTH and ODST baseline plasma cortisol concentrations. Dexamethasone2 (40 µg/kg bwt i.m.) was administered at 17.00 on Day 1 and plasma cortisol concentration was measured again 19 h later (12:00pm on Day 2). Lateral hoof radiographs of both thoracic limbs were obtained. Owners were asked whether or not their horse had a history of laminitis; veterinary confirmation of historical laminitis was not required. Grass hay and water were provided ad libitum at all times. Concentrate feed was withheld throughout testing and for a minimum of 6 h prior to blood collection on Day 1.

Hormone assays

After blood collection, EDTA tubes were processed immediately and serum tubes were allowed to clot at room temperature for 1 h. Tubes were centrifuged at 1000× ***g*** for 10 min and plasma or serum was harvested and stored at −20°C until analysed. Serum insulin (DSL-1600 insulin radioimmunoassay)3 and cortisol (Coat-A-Count Cortisol direct radioimmunoassay)4 concentrations were measured using radioimmunoassays that have been validated for use with equine serum in our laboratory and by others 15, 16. Plasma ACTH concentration was measured with a human-specific immunoradiometric assay5previously used to analyse equine samples 17.

Hyperinsulinaemia classification

Horses were classified as either normoinsulinaemic or hyperinsulinaemic (serum insulin ≥20 μU/ml) 18. Because no fasting protocol was followed and testing in the fed state could conceivably alter serum insulin concentration, the hyperinsulinaemic category was further subdivided into mildly hyperinsulinaemic (20–50 μU/ml) and severely hyperinsulinaemic (>50 μU/ml) groups 19.

Laminitis classification

Horses were classified as either nonlaminitic (no history or current hoof morphological or radiographic evidence of laminitis) or laminitic (if any of the following were present: historical laminitis, abnormal hoof morphology [dorsal hoof capsule concavity or divergent growth rings] or radiographic evidence of laminitis [rotation of the third phalanx (P3) within the hoof capsule, P3 tip osteophytes, P3 remodelling or lysis or subcapsular gas shadows]). Animals were also classified according to severity of radiographic P3 rotation relative to the dorsal hoof capsule wall (normal, ≤2°; mild, 3–5°; moderate, 6–10°; severe, ≥11°); if hooves differed, the more severe score was used.

Data analysis

One-way ANOVA and *t* tests were performed using computer software (SigmaStat, version 3.5)6 to compare serum insulin concentration between laminitic and nonlaminitic groups and among groups classified according to radiographic laminitis severity. Serum insulin concentrations required logarithmic transformation to fit ANOVA normal distribution assumptions and are reported as geometric means with 95% confidence intervals. Mean separation was performed using Fisher's protected least significant difference and significance was set at $P<0.05$.

Two-tailed Fisher's exact tests (SAS, version 9.4)7 were used to compare how frequently owner-reported historical laminitis agreed with classification of horses as either laminitic or nonlaminitic. Agreement was further compared within normoinsulinaemic, mildly hyperinsulinaemic and severely hyperinsulinaemic groups. Significance was set at $P<0.05$ for all comparisons.

Results

Laminitis and physical characteristics

Overall, 29 horses were classified as laminitic at the time of study enrolment. Of these, 19 had radiographic P3 rotation, with or without other radiographic abnormalities (P3 tip

osteophytes, P3 remodeling or lysis or subcapsular gas shadows) and abnormal hoof morphology; these animals were assigned a laminitis severity score based on the degree of P3 rotation (mild = 7, moderate = 6 and severe = 6; Table 1). Another nine animals were included in the laminitis group based on abnormal hoof morphology and/or radiographic abnormalities other than P3 rotation; although classified as laminitic for statistical comparisons of laminitic vs. nonlaminitic, these animals were assigned a radiographic laminitis severity score of 'normal', based on lack of P3 rotation. One horse was included in the laminitis group solely because it had a clinical history of laminitis; this was the only animal in the laminitis group not to have radiographic or hoof morphological abnormalities. The animal had no other known cause of lameness and was considered to have had a mild laminitic episode that did not cause permanently detectable abnormalities.

Table 1. Geometric mean (95% confidence interval) insulin concentrations in 38 horses grouped accordingly to radiographic laminitis severity. Severity classification was assigned according to the degree of distal phalanx rotation; the normal category therefore includes both nonlaminitic animals (n = 9) and laminitic animals without radiographic distal phalanx rotation (n = 10)

Radiographic laminitis classification (n)	Insulin (µU/ml)	P value	Combined groups (n)	Insulin (µU/ml)	P value
Severe (6)	123.1 (61.2–248.0)	0.07	Severe and Moderate (12)	74.1 (38.4–143.1)	0.03
Moderate (6)	44.6 (16.4–121.6)				
Mild (7)	29.4 (12.1–71.7)		Mild and Normal (26)	31.9 (21.1–48.1)	
Normal (19)	32.8 (20.4–				

Radiographic laminitis classification (n)	Insulin (μU/ml)	P value	Combined groups (n)	Insulin (μU/ml)	P value
	52.7)				

Groups did not differ significantly with respect to age, body weight or body condition score for any classification scheme (laminitic or nonlaminitic; normal, mild, moderate or severe radiographic laminitis severity; and normoinsulinaemic, mildly hyperinsulinaemic or severely hyperinsulinaemic). At the time of enrolment, a trained observer (J.F.) judged five horses in the laminitic group to be lame due to laminitis; five additional animals appeared lame from other musculoskeletal disorders. The remaining 19 animals in the laminitic group had historical laminitis, abnormal hoof morphology and/or radiographic evidence of laminitis, but were not lame at the time of evaluation. Two horses classified as nonlaminitic appeared lame from causes other than laminitis.

Owner awareness of laminitis

Although 29/38 (76%) of horses were classified as laminitic at the time of study enrolment, owners reported a history of laminitis in only 14/38 (37%) of these animals (P = 0.01). Owners were likely to report a history of laminitis in horses that were hyperinsulinaemic compared with normoinsulinaemic, and recognition of laminitis increased with the severity of hyperinsulinaemia (P = 0.03). In severely hyperinsulinaemic horses, owners reported that 10/16 (63%) animals had a history of laminitis compared with 14/16 (88%) that were classified as laminitic based on the study criteria. This number decreased to 2/11 (18%) horses with an owner-reported history of laminitis in the mildly hyperinsulinaemic group compared with 8/11 (73%) identified as laminitic by the investigators. In normoinsulinaemic horses, owners reported historical laminitis in 2/11 (18%) animals compared with 7/11 (64%) meeting the laminitic classification criteria. The percentage of horses defined as laminitic based on study criteria did not differ among insulin classification groups. Radiographic evidence and/or hoof morphological abnormalities were present in all but one horse with an owner-reported history of laminitis.

Relationship between serum insulin concentrations and laminitis

Insulin concentrations tended to be higher as the radiographic severity of laminitis increased but this was not statistically different (P = 0.07; Table 1). Differences reached statistical significance when groups were combined into normal and mild compared with moderate and severe radiographic classifications (P = 0.03).

Discussion

Our hypotheses that magnitude of hyperinsulinaemia correlates with laminitis severity and that owners can be unaware of mild endocrinopathic laminitis were both supported. Laminitis was identified by the investigators in 76% of the animals, but it went undetected by owners in nearly half of those cases. Owners were likely to have recognised laminitis in horses that were hyperinsulinaemic than those that were normoinsulinaemic; furthermore, recognition of laminitis increased with the severity of hyperinsulinaemia. Horses and ponies with moderate to severe hoof radiographic changes had higher serum insulin concentrations than those with either mild changes or normal radiographs.

Endocrinopathic laminitis appears to have an insidious onset that often goes unrecognised by owners until severe cumulative laminar damage has occurred. Previous studies have shown that the severity of histological laminar pathology does not correlate with the duration of clinical laminitis; advanced lesions are often present in horses with a reportedly recent onset of laminar pain 7, 12. In this study, owners recognised laminitis in only half of the animals that were classified as laminitic by a veterinarian; laminitis was more under-recognised in normoinsulinaemic than hyperinsulinaemic horses. An important opportunity therefore exists for educating owners about the importance of routine screenings for both PPID and ID, as well as laminitis, as part of geriatric wellness examinations. Owners can also be taught to identify laminitis earlier in PPID-affected animals. All equids with PPID should be tested for ID and hoof radiographs should be considered. Early recognition can facilitate therapeutic interventions to mitigate laminar damage before it becomes severe.

Hyperinsulinaemia and ID are established risk factors for laminitis 8, 20, 21. Of all ID components, hyperinsulinaemia appears to be the most detrimental, as insulin administration at supraphysiological doses to healthy ponies 9 and Standardbred horses 10induces laminitis within 48–72 h. Pathologically high circulating insulin concentrations are believed to aberrantly activate the laminar epidermal epithelial cell insulin-like growth factor-1 receptor 22, 23; this receptor shares close structural

homology with the insulin receptor and is responsive to supraphysiological insulin levels 24. Among its many functions, insulin-like growth factor-1 regulates cell growth, adhesion, proliferation, differentiation and apoptosis 25. Alterations in tissue growth and repair due to abnormal laminar keratinocyte proliferation and maturation, cytoskeletal dysfunction and changes in the extracellular matrix 26, 27 are believed to underlie laminar weakening, stretching and final disadhesion of the basal epithelium from the basement membrane. In the current study, the fact that moderate to severe radiographic laminitis was associated with higher serum insulin concentrations would be consistent with hyperinsulinaemia as an important contributing factor.

One question that arises is whether hyperinsulinaemia directly causes laminitis in PPID-affected equids, or if it is simply a marker of PPID chronicity. Evidence is mounting for hyperinsulinaemia as the causative factor of endocrinopathic laminitis in both PPID and Equine Metabolic Syndrome. When laminitis develops in horses with PPID, the histological lesions share many features with those seen in both experimental hyperinsulinaemia models and naturally occurring hyperinsulinaemic laminitis 7, 12. These include elongation and widening of primary epidermal laminae; increased tapering and fusion of primary and secondary epidermal laminae; presence of mitotic figures in secondary epidermal laminae; apoptotic cells; and proliferation, keratinisation and separation of the epidermis from the underlying dermis. On the other hand, laminar histology in horses with PPID that are normoinsulinaemic is often normal 7. This could suggest that PPID alone, in the absence of hyperinsulinaemia, does not cause laminar damage and that only PPID-affected equids with concurrent ID develop laminitis. In support of this, one study of PPID-affected horses found that concurrent hyperinsulinaemia was present in all laminitic animals and absent in the nonlaminitic group 7. However, that study was not designed to identify nonlaminitic animals that were hyperinsulinaemic. Results of the current study would suggest that normoinsulinaemic horses are as likely as hyperinsulinaemic animals to have laminitis at the time of PPID diagnosis, albeit the laminitis is less severe and probable to be noticed by owners. However, because dynamic insulin testing was not performed, it is possible that some normoinsulinaemic animals had subtle ID, without overt hyperinsulinaemia; this could have contributed to development of laminitis in the normoinsulinaemic group. If so, it would underscore the importance of using sensitive dynamic insulin tests to identify mild ID as early as possible in PPID-affected equids.

There remains debate about the relationship between PPID and ID, as not all PPID horses are affected by ID 6. One possibility is that they are independent conditions that can occur either individually or concurrently; identifying them together may simply represent the coexistence of two common endocrinopathies in the same animal. The prevalence of obesity, which is linked closely with ID, can be as high as 50% in some equine populations 28. Inevitably, some of these metabolically unhealthy animals also develop PPID with age. Alternatively, PPID may induce or exacerbate ID and hyperinsulinaemia, particularly in animals that already have predisposing genetic or environmental risk factors. Pathologically high pituitary hormone concentrations can act as insulin secretagogues in pancreatic β-cells 29, 30 and hepatic insulin clearance might be reduced as a result of mild hepatic dysfunction associated with obesity, PPID and/or ageing 31. Heterogeneity in the severity of pituitary lesions, as well as individual variability in the specific proopiomelanocortin-derived peptide profile secreted by the diseased *pars intermedia*, might explain why PPID only induces ID in a subset of affected animals.

An important dimension that has recently gained attention is considering what happens when pathological conditions that induce hyperinsulinaemia are superimposed on the normal age-related decline in insulin sensitivity. As in other species, insulin sensitivity decreases with age in horses and insulin responses to oral and i.v. glucose challenges are exaggerated 32, 33. Mechanisms implicated in age-related insulin resistance include inflammation (‘inflammaging’), oxidative stress, mitochondrial dysfunction, increased visceral adiposity, decreased lean muscle mass and reduced physical activity 34. Because geriatric horses are already prone to heightened insulin responses, pathological conditions that exacerbate ID might greatly increase their risk of developing hyperinsulinaemia and endocrinopathic laminitis.

Hyperinsulinaemia may have prognostic and therapeutic implications when managing PPID. In a study of 20 horses with PPID, serum insulin concentrations <62 μU/ml predicted survival and >188 μU/ml predicted nonsurvival at 1–2 years following diagnosis, with both sensitivity and specificity >90% 35. In that cohort, most animals that did not survive succumbed to laminitis 36. Another study of horses with endocrinopathic laminitis attributable advanced PPID, marked ID or both showed a significant positive correlation between laminitis grade and the initially measured plasma insulin concentration 37. Furthermore, decreasing plasma insulin concentration over approximately 1 year correlated with decreasing laminitis grade. In the current study,

71% of PPID-affected equids were hyperinsulinaemic. Although this is higher than in some reports 5, 6, it is in keeping with others 38, 39; a possible explanation is that animals enrolled in this study had more advanced PPID, as inclusion criteria included hypertrichosis.

The main limitation of this study is that it may not be generalisable to equids with early/mild endocrinopathies and laminitis. The inclusion criteria that horses had to have hypertrichosis, in addition to abnormal endocrine test results, selected for animals with moderate to advanced PPID. Laminitis might be less prevalent, milder, or show a different relationship with hyperinsulinaemia in horses with early PPID. In this study, laminitis was diagnosed based on historical reporting by owners, gross changes in hoof morphology and radiography. Histopathology was not performed, as laminar biopsies are not a routine procedure in client-owned animals. It is conceivable that animals classified as nonlaminitic had histological evidence of laminitis that was not yet severe enough to cause gross morphological changes. Finally, dynamic insulin testing was not performed to identify ID; thus, some animals classified as normoinsulinaemic might have been insulin dysregulated, albeit without overt hyperinsulinaemia. Therefore, it remains possible that ID is a necessary factor for laminitis in all PPID-affected horses.

In conclusion, our results demonstrate that the magnitude of hyperinsulinaemia correlates with radiographic laminitis severity and that owners can remain unaware of mild endocrinopathic laminitis; this is particularly evident in normoinsulinaemic horses, as the likelihood of owner recognition increases with the degree of hyperinsulinaemia. Because of its irreversible nature, early recognition of horses with subclinical laminitis is imperative. Based on results of this study, hyperinsulinaemia in any PPID-affected equid should raise suspicion of laminitis, even without prior history or current lameness consistent with laminitis. When identified in PPID-affected animals, ID might also be among the most important components to target therapeutically in order to prevent laminitis.

Authors' declarations of interests

Dr Tadros and Dr Schott have consulted for Boehringer Ingelheim. Dr Fowlie, Dr Marteniuk and Dr Refsal declare no competing interests.

Ethical animal research

The study protocol was approved by the Michigan State University Institutional Animal Care and Use Committee and signed informed consent was obtained from owners prior to study enrolment.

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Authorship

All authors contributed to and approved the final manuscript. Dr Tadros contributed to study design, performed data analysis and interpretation and prepared the manuscript. Dr Schott, Dr Fowlie and Dr Marteniuk designed and performed the study and contributed to data analysis and interpretation. Dr Refsal contributed to study design and collected data.

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- 3 Diagnostic Systems Laboratories Inc, Webster, Texas, USA.
- 4 Diagnostic Products Corporation, Los Angeles, California, USA.
- 5 DiaSorin, Stillwater, Minnesota, USA.
- 6 Systat Software, San Jose, California, USA.
- 7 SAS Institute Inc, Cary, North Carolina, USA.