Domestic Animal Endocrinology xxx (2017) 1–9

Contents lists available at ScienceDirect

Domestic Animal Endocrinology

journal homepage: www.domesticanimalendo.com

The oral glucose test predicts laminitis risk in ponies fed a diet high in nonstructural carbohydrates

A.D. Meier [a], M.A. de Laat [a], D.B. Reiche [b], C.C. Pollitt [c], D.M. Walsh [d], J.M. McGree [e], M.N. Sillence [a,*]

[a] Earth, Environmental and Biological Sciences School, Queensland University of Technology, Queensland, Australia
[b] Boehringer Ingelheim Vetmedica, Ingelheim am Rhein, Germany
[c] Australian Equine Laminitis Research Unit, School of Veterinary Science, University of Queensland, Queensland, Australia
[d] Animal Health Foundation, Pacific, Missouri, USA
[e] School of Mathematical Sciences, Queensland University of Technology, Queensland, Australia

ARTICLE INFO

Article history:
Received 29 August 2017
Received in revised form 24 October 2017
Accepted 25 October 2017

Keywords:
Equine metabolic syndrome
Horse
Hyperinsulinemia
Insulin dysregulation
Pituitary *pars intermedia* dysfunction

ABSTRACT

The aim of this study was to investigate the relationship between laminitis development in ponies and insulin/glucose concentrations in response to the oral glucose test (OGT) and a dietary challenge high in nonstructural carbohydrates (NSCs). After undergoing an OGT (1 g dextrose/kg BW in feed), 37 ponies with 2-h serum insulin concentrations ranging from 22 to 1,133 μIU/mL were subjected to a diet challenge period (DCP), consuming 12 g NSC/kg BW/d for up to 18 d. Insulin and glucose responses were measured on day 2 of the DCP. Clinical laminitis was diagnosed by blinded experts and confirmed radiographically. Basal ACTH levels and clinical signs were assessed to investigate concurrent putative pituitary pars intermedia dysfunction (PPID). The diet induced Obel grade 1 or 2 laminitis in 14 ponies (38%). The ponies that developed laminitis had higher maximum concentrations of blood glucose ($P = 0.04$) and serum insulin ($P = 0.02$) in response to the diet. The geometric mean (95% CI) blood glucose concentration for laminitis cases was 14.9 (12.9–17.2) mM, compared to 10.7 (9.2–12.5) mM for ponies who did not develop laminitis. Similarly, the geometric mean (95% CI) for serum insulin was 396 (301–520) μIU/mL for laminitis cases, compared to 216 (148–316) μIU/mL for ponies who did not develop laminitis. Laminitis incidence was likewise associated with insulin concentrations measured during the OGT. Laminitis occurred at frequencies of 0% (0/7) if postdextrose insulin (μIU/mL) was <50; 35% (8/23) if insulin was 50 to 195; and 86% (6/7) if insulin was >195 μIU/mL. Basal ACTH concentrations were above seasonally accepted reference ranges in 16/37 ponies, and 8 of these animals (50%) developed laminitis. This included all 5 ponies in the study that had clinical signs of PPID (100%). In contrast, hyperinsulinemia and laminitis occurred in only 3/11 ponies (27%) with elevated ACTH concentrations and no clinical signs of PPID ($P = 0.009$). Thus, laminitis occurrence was associated with higher glucose and insulin responses to both the OGT and challenge diet, and the frequency of laminitis can be predicted based on insulin and glucose hyperresponsiveness to these oral carbohydrate challenges.

1. Introduction

Laminitis is the most serious disease of the equine foot, resulting in pathology with long-lasting functional effects [1]. The disease is complex, with 3 main etiologies [2]; the most common of which is endocrinopathic, including insulin dysregulation and pituitary pars intermedia dysfunction (PPID) [3]. The evidence for a link between insulin dysregulation, hyperinsulinemia, and laminitis is strong, as experimental insulin infusion can induce laminitis in healthy horses and ponies [4,5], and

* Corresponding author. Tel.: +61 7 3138 2565; fax: ■■■.
E-mail address: martin.sillence@qut.edu.au (M.N. Sillence).

0739-7240/$ – see front matter © 2017 Elsevier Inc. All rights reserved.
https://doi.org/10.1016/j.domaniend.2017.10.008

hyperinsulinemia has been shown to be a prospective risk factor for laminitis [6]. Diets high in nonstructural carbohydrates (NSCs), such as certain pastures and sweet-feeds, may stimulate hyperinsulinemia and trigger laminitis in insulin-dysregulated horses [7].

Accordingly, various tests have been developed to diagnose insulin dysregulation. Initially, these were based on assessing insulin resistance using intravenous methods [8], but as the importance of the enteroinsular axis has become apparent, oral tests are now preferred [9]. Threshold values have been established to diagnose insulin dysregulation, using tests such as the oral glucose test (OGT) [10]. However, the precise relationship between immediate laminitis risk, the OGT, and dietary glucose and insulin concentrations has yet to be determined. The present study aimed to investigate that relationship, in ponies that were fed a challenge diet high in NSCs.

Our first hypothesis was that laminitis can be incited in a predictable way, using a combination of animal selection based on an OGT, and the feeding of a standardized high NSC diet. Our second hypothesis was that the speed and/or the severity of laminitis can be predicted based on the size of the insulin and glucose response to the high NSC challenge diet.

2. Materials and methods

2.1. Selection of animals

This experiment was part of a larger study approved by The Animal Care and Ethics Committees of the University of Queensland (Approval # QUT/SVS/114/14) and Queensland University of Technology (Approval # 1400000575).

Seventy-five ponies were purchased from local dealers and acclimatized for 3 wk before undergoing an initial OGT for screening purposes [10]. The ponies were fasted overnight, and at 8 AM the following d, they were fed 1-g dextrose/kg BW (Sigma-Aldrich, Castle Hill, Australia) mixed with 200-g wheat bran and 0.15% BW lucerne chaff. Blood samples were taken immediately before the feed, then 2 h later, to measure blood glucose and serum insulin concentrations.

Fifty ponies with the highest insulin concentrations at 2 h were retained. Thirty-eight of these were randomly selected and enrolled, although 1 mare was subsequently lost due to a fractured leg. The final cohort included 37 ponies, and a second OGT performed within 5 d of study commencing, revealed that they had a wide range of 2 h insulin concentrations (21–1,133 μIU/mL). This was unexpected but proved to be beneficial for correlation and regression analysis.

The cohort included 16 ponies with elevated ACTH concentrations, based on seasonally adjusted locality norms of >27.8 pg/mL in nonautumn months and >77.4 pg/mL in autumn months [11]. However, only 5 of these ponies had clinical signs of PPID including both hirsutism and polydipsia/polyuria. These ponies were included because they represented a significant proportion of the hyperinsulinemic population enrolled. Furthermore, although it has been shown that hyperinsulinemia predicts nonsurvival in PPID ponies [12], it is less clear whether the converse is also true, that is, whether elevated ACTH concentrations and/or PPID influence the probability of hyperinsulinemia and laminitis. Such information would be valuable.

2.2. Sample population

The ponies included 21 mares and 16 geldings, all unrelated. Breeds were assorted, with a predominance of Welsh Mountain, Arabian, Shetland, Miniature Horse, Australian Pony types, and derivatives. The mean (±standard error) age, BW, and wither height were 14.8 ± 0.7 yr, 210 ± 13 kg, and 107 ± 3.8 cm, respectively. The mean BCS on a scale of 1 to 9 [13] was 7, and the mean cresty neck score (CNS) from 1 to 5 [14] was 3.8. Although laminitis history was unknown, 13 ponies had radiographic evidence of previous laminitis, of which 10 would be classed as insulin-dysregulated according to the 2-h OGT cutoff value of >85 μIU/mL [10].

2.3. Animal management

For a minimum of 3 wk before the diet challenge period (DCP), the ponies were kept in groups in yards and fed 0.9% BW lucerne hay (as fed) twice daily, plus a vitamin/mineral supplement (Equilibrium mineral mix, Loganholme, Australia) mixed with 200 g of lucerne chaff. Routine treatments included Hendra virus vaccination, dental and anthelmintic treatment, and hoof trimming. Mares were only enrolled if not pregnant. All ponies received a thorough health assessment, including the measurement of blood biochemistry, hematology, and basal ACTH concentrations, and a veterinary clinical examination establishing baseline health status. During the experiment, the ponies were stabled individually on sawdust substrate and allowed outside into a large pen in pairs for 2 h daily. As stable availability was limited, the study was conducted using 8 groups over 12 mo.

2.4. Prestudy procedures

The OGT was repeated within 5 d of the DCP to obtain baseline data on metabolic status for each pony. The ponies were subjected to a laminitis examination to determine that they were not currently laminitic (Supplementary Item 1). This examination was filmed, and the recordings were examined by 2 laminitis experts (C.C. Pollitt and D.M. Walsh), who were blinded to the project stage (before, during, or after the DCP) and to each other's score. The assessors determined a laminitis score using a 12-point scale (Supplementary Item 1) designed for this project and based on the Obel grading system [15] (Supplementary Item 2). Digital pulse was an important part of laminitis diagnosis [16], but as the assessors were not present to palpate the pulse, the managing veterinarian (A.D. Meier) allocated this score. In addition to the laminitis evaluation, radiographs were taken of both front feet including the views: lateromedial, dorso 65° proximal-palmarodistal oblique, and dorso 0° palmar.

2.5. Diet challenge period

The total daily DCP allocation of mixed-feed was divided into 3 equal portions given at 8 AM, 12 PM, and 4 PM to