Postprandial glucose, insulin, and glucagon-like peptide-1 responses of different equine breeds adapted to meals containing micronized maize

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ABSTRACT: The enteroinsular axis is a complex system that includes the release of incretin hormones from the gut to promote the absorption and utilization of glucose after a meal. The insulinogenic effect of incretin hormones such as glucagon-like peptide-1 (GLP-1) remains poorly characterized in the horse. The aim of this study was to compare postprandial glucose, insulin, and GLP-1 responses of different equine breeds adapted to twice-daily meals containing micronized maize. Four Standardbred horses, 4 mixed-breed ponies, and 4 Andalusian cross horses in moderate BCS (5.5 ± 0.2 out of 9) were fed meals at 0800 and 1600 h each day. The meals contained micronized maize (mixed with soaked soybean hulls and lucerne chaff), with the amount of maize gradually increased over 12 wk to reach a final quantity of 1.7 g/kg BW (1.1 g/kg BW starch) in each meal. Animals had ad libitum access to the same hay throughout. After 12 wk of acclimation, serial blood samples were collected from all animals over a 14-h period to measure concentrations of glucose, insulin, and GLP-1, with meals fed immediately after the 0 and 8 h samples. Glucose area under the curve (AUC) values were similar between breed groups (P = 0.41); however, ponies and Andalusian horses exhibited significantly higher insulin AUC values after both meals compared with Standardbred horses (both P < 0.005). Postprandial GLP-1 AUC values were also significantly higher in ponies and Andalusian horses compared with Standardbred horses (breed × time interaction; P < 0.001). Correlation analysis demonstrated a strong positive association between concentrations of insulin and GLP-1 over time (r = 0.752; P < 0.001). The increased insulin concentrations in ponies and Andalusian horses may partly reflect lower insulin sensitivity but could also be attributed to increased GLP-1 release. Given that hyperinsulinemia is a recognized risk factor for the development of laminitis in domestic equids, this study provides evidence that the enteroinsular axis warrants further investigation.

Keywords: equine, glucagon-like peptide-1, glucose, incretin, insulin, laminitis


INTRODUCTION

Hyperinsulinemia is a risk factor for the occurrence of laminitis in grazing equids (Treiber et al., 2006; Bailey et al., 2008; Carter et al., 2009). Factors that influence postprandial glucose and insulin concentrations include the type and amount of nonstructural carbohydrate (NSC; starch and water-soluble carbohydrate) consumed, the prececal digestibility of starch, and the rate of meal ingestion (Harris and Geor, 2009). When high-glycemic diets are regularly consumed, the downregulation of insulin receptors in target tissues can lead to a state of insulin resistance (Treiber et al., 2005). To adapt to decreased insulin sensitivity, postprandial insulin concentrations will increase in a self-perpetuating cycle (Frank and Tadros, 2014).

A potentiation of insulin responses when glucose is administered orally (as opposed to intravenously) is due to the release of incretin hormones from specialized enteroendocrine cells, which exert
insulinogenic effects on the pancreas (Hampton et al., 1986). Glucagon-like peptide-1 (GLP-1) is an incretin hormone that has been extensively investigated in human and rodent studies, but equine data are lacking (De Graaf-Roelfsema, 2014). Genetic factors influence glucose and insulin dynamics in equids (Treiber et al., 2006). Ponies and Andalusian horses produce significantly more insulin after consuming a glucose-containing meal when compared with Standardbred horses (Bamford et al., 2014). It has not yet been determined whether an incretin response is a driver for the difference in postprandial insulin responses observed between breeds.

This study assessed postprandial concentrations of glucose, insulin, and GLP-1 in horses and ponies after a 12-wk adaptation to twice-daily meals containing micronized maize. We hypothesized that ponies and Andalusian horses would demonstrate increased postprandial insulin concentrations compared with Standardbred horses and furthermore that there would be an associated increase in GLP-1 concentrations in these breeds.

MATERIALS AND METHODS

The study protocol was approved by the University of Melbourne Animal Ethics Committee (ID 1011918).

Animals and Diets

Four Standardbred horses (STB; 5 to 14 yr, 458 ± 17 kg, BCS 5.2 ± 0.2), 4 mixed-breed ponies (PON; 5 to 10 yr, 300 ± 19 kg, BCS 5.3 ± 0.3), and 4 Andalusian cross horses (AND; 4 to 11 yr, 509 ± 23 kg, BCS 5.7 ± 0.3) were enrolled in this 12-wk study. Animals were kept in large dirt paddocks with ad libitum access to hay (sourced from a single batch; Table 1) and fresh water throughout the study period. Meals were provided at 0800 h (AM meal) and 1600 h (PM meal) each day during the study period. To enable individual feeding at meal times, animals were moved to small separate pens along the perimeter of the paddock, with any meal refusals recorded. Each meal (Table 1) consisted of a base ration containing an equal mix (1.5 g/kg BW of each ingredient by dry weight) of soaked soy hull pellets (Maxisoy, Energreen Nutrition, Shailer Park, QLD, Australia) and lucerne chaff. A balanced vitamin and mineral supplement (60 mg/kg BW; Ranvet, East Botany, NSW, Australia) was added to the AM meal. Micronized maize (Micrmaize, Hygain, Officer, VIC, Australia) was mixed with the base ration in each meal to provide a glycemic and insulinemic stimulus. Animals were fed on a “per kilogram BW” basis, using the BW of each animal recorded on the first day of every week during the study. The ingredients for each meal were weighed and mixed individually to ensure the accurate provision of the study diets. The amount of grain added to each meal started at 0.7 g/kg BW and was gradually increased on a weekly basis over the 12-wk period to allow for digestive adaptation. The amount of micronized maize in each meal at wk 12 was 1.7 g/kg BW, providing 1.1 g/kg BW starch.

Morphometric Measurements

On the first day of wk 0 and 12, BW was measured using calibrated horse scales, and BCS was assessed by a single experienced observer using a 9-point scale (Henneke et al., 1983; Kohnke, 1992). Body weight at wk 12 was reported as the percentage change from wk 0 to account for the large difference in size between ponies and horses.

Sample Collection

After 12 wk of meal feeding, all animals underwent a serial blood sampling procedure over a 14-h period to assess the effect of the meals on glucose, insulin, and GLP-1 concentrations. Testing occurred over a 2-d period with 6 animals tested on each day in a randomized allocation. To replicate daily husbandry practices, animals remained in the dirt paddocks overnight with ad libitum access to hay and fresh water. On the day of testing, animals were moved to their individual pens, where they remained for the duration of the test. Meals were provided at the regular times of 0800 and 1600 h. Hay and fresh water were available to the animals at all times during the sampling period. Approximately 1 h before the AM meal, an intravenous catheter was placed in the left jugular vein of each animal under local anesthesia. A baseline

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Table 1. Proximate analysis of ration components (DM basis)¹

<table>
<thead>
<tr>
<th>Item</th>
<th>Hay</th>
<th>Base ration²</th>
<th>Micronized maize³</th>
</tr>
</thead>
<tbody>
<tr>
<td>DE, MJ/kg</td>
<td>7.1</td>
<td>9.5</td>
<td>16.3</td>
</tr>
<tr>
<td>CP, %</td>
<td>7.7</td>
<td>11.7</td>
<td>10.3</td>
</tr>
<tr>
<td>ADF, %</td>
<td>46.0</td>
<td>37.3</td>
<td>3.3</td>
</tr>
<tr>
<td>NDF, %</td>
<td>75.8</td>
<td>57.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Nonstructural carbohydrate; ⁴ %</td>
<td>9.2</td>
<td>13.1</td>
<td>73.4</td>
</tr>
<tr>
<td>Water-soluble carbohydrate, %</td>
<td>7.3</td>
<td>8.7</td>
<td>3.3</td>
</tr>
<tr>
<td>Starch, %</td>
<td>1.8</td>
<td>4.4</td>
<td>70.1</td>
</tr>
<tr>
<td>Fat, %</td>
<td>1.8</td>
<td>3.7</td>
<td>4.3</td>
</tr>
<tr>
<td>Ash, %</td>
<td>5.5</td>
<td>5.6</td>
<td>1.3</td>
</tr>
</tbody>
</table>

¹Analysis performed at Equi-Analytical Laboratories, Ithaca, NY.
²Equal mixture (by dry weight) of soy hull pellets (Maxisoy, Energreen Nutrition, Shailer Park, QLD, Australia) and lucerne chaff.
³Micrmaize, Hygain, Officer, VIC, Australia.
⁴Nonstructural carbohydrate = starch + water-soluble carbohydrate.
sample was drawn immediately before the AM meal, with serial blood samples drawn over the 14-h period. The PM meals were provided immediately after the 8 h blood sample. Samples (10 mL at each sampling time) were placed in tubes containing lithium heparin and kept on ice until centrifugation (1,000 × g for 10 min at 4°C). Plasma was harvested, and 1-mL aliquots were stored at −80°C until analysis.

**Plasma Analysis**

Glucose was measured using a hexokinase colorimetric assay (Cayman Chemical Co., Ann Arbor, MI), and insulin was measured using a RIA (Coat-A-Count, Siemens Diagnostics, Los Angeles, CA) previously validated for equine plasma (Tinworth et al., 2011). For samples in which insulin concentrations were above the range of the assay (389 mU/L), dilutions were performed using insulin-depleted plasma (Borer-Weir et al., 2012). Plasma concentrations of GLP-1 were determined using an ELISA (Merck Millipore, Darnstadt, Germany) previously validated for equine plasma (Chameroy et al., 2010a). Intra-assay CV were 0.8%, 3.8%, and 3.7%, and interassay CV were 0.9%, 5.8%, and 10.7% for glucose, insulin, and GLP-1, respectively.

**Data Analysis**

The area under the curve (AUC) for glucose, insulin, and GLP-1 was calculated using the nonoverlapping trapezoid method (GraphPad Prism, version 6.02, GraphPad Inc., La Jolla, CA). Baseline values for AUC calculations were defined as the 0-h sample for the AM meal and the 8-h sample for the PM meal. The AUC period for glucose and insulin was defined as the first 6 h after each meal. Concentrations of GLP-1 at the 13- and 14-h time points were not quantified because of the number of assay plates available; therefore, the AUC period for GLP-1 was defined as the first 4 h after each meal. An outlier that demonstrated exaggerated glucose and insulin responses (greater than 5 SD above the mean for both AM and PM meals) was identified within the PON group and was excluded from statistical analysis. Data were assessed for normality with the Shapiro-Wilk test and were analyzed using a mixed-model ANOVA (SPSS, version 22, IBM Corp., New York, NY). The model included the main effects of breed, time (AM meal vs. PM meal), and the interaction term (breed × time), with the random effect of individual animal. Significant main effects were compared in a pairwise manner using Tukey’s post hoc test when appropriate. Further assumptions of the model were checked using Levene’s test (homogeneity of variance) and Box’s test (homogeneity of covariance). Relationships between glucose, insulin, and GLP-1 were evaluated using Spearman’s rank-order correlation analysis of 20 individual time points from each animal over the 14-h sampling period. Data were reported as mean ± SEM, with significance defined as $P < 0.05$. 

![Figure 1. Plasma concentrations (mean ± SEM) of (A) glucose, (B) insulin, and (C) glucagon-like peptide-1 (GLP-1) in Standardbred horses (STB; n = 4), ponies (PON; n = 3), and Andalusian horses (AND; n = 4) over a 14-h period. Animals were adapted to twice-daily meals containing micronized maize over a 12-wk period. Each meal contained a base ration (soybean hulls and lucerne chaff) with 1.7 g/kg BW micronized maize (1.1 g/kg BW starch). The vertical dashed lines indicate the times of meal feeding at 0800 and 1600 h. Low–nonstructural carbohydrate hay was available throughout the sampling period.](image)
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**RESULTS**

**Animals**

All animals remained clinically healthy, and there were no meal refusals recorded on any occasion. Body weight increased in all individuals ($P < 0.001$), with no difference detected between STB (12.5% ± 1.1%), PON (15.6% ± 1.4%), and AND (14.1% ± 1.0%) groups ($P = 0.22$). Body condition score also increased in all individuals ($P < 0.001$), with no difference detected between wk 12 BCS in STB (6.9 ± 0.1), PON (7.1 ± 0.3), and AND (7.3 ± 0.3) groups ($P = 0.56$). On the day of blood sampling, meals were consumed in a similar amount of time by STB (16 ± 1 min), PON (15 ± 2 min), and AND (16 ± 2 min) groups ($P = 0.91$). Individual hay consumption (% BW, DM basis) over a 24-h period was not detectably different between STB (2.1% ± 0.1%), PON (2.3% ± 0.2%), and AND (2.1% ± 0.1%) groups ($P = 0.54$). Estimated group hay intake over the study period was consistent with the measured 24-h intake.

**Glucose and Insulin Responses**

Plasma concentrations of glucose and insulin over the 14-h sampling period are shown in Fig. 1, with AUC values shown in Table 2. For glucose, a significant effect of time was detected, with lower AUC glucose after the PM meal relative to the AM meal ($P = 0.037$). No effect of breed on glucose responses was detected ($P = 0.40$). For insulin, there was a significant effect of breed, with PON and AND demonstrating significantly larger AUC insulin compared with STB ($P = 0.002$ and $P = 0.005$, respectively). No effect of time (AM meal vs. PM meal) on insulin responses was detected ($P = 0.87$).

**GLP-1 Responses**

Plasma concentrations of GLP-1 over the 14-h sampling period are shown in Fig. 1, with AUC values shown in Table 2. For GLP-1, there was a significant breed × time interaction ($P < 0.001$). Values for AUC GLP-1 were higher in the PON group compared with STB during the AM meal ($P = 0.016$) and were higher in the PON and AND groups compared with STB during the PM meal ($P = 0.006$ and $P < 0.001$, respectively).

**Correlations**

Insulin concentrations were strongly correlated with GLP-1 concentrations over the sampling period ($r_s = 0.752; P < 0.001$). Weaker correlations existed between glucose and insulin ($r_s = 0.407; P < 0.001$) and between glucose and GLP-1 ($r_s = 0.271; P < 0.001$).

**Outlier Pony**

The outlier identified within the PON group demonstrated exaggerated postprandial concentrations of glucose (AM meal: peak 8.6 mmol/L, AUC glucose 12.9 mmol·h$^{-1}$·L$^{-1}$; PM meal: peak 10.4 mmol/L, AUC glucose 14.9 mmol·h$^{-1}$·L$^{-1}$), and insulin (AM meal: peak 387.5 mU/L, AUC insulin 1,454 mU·h$^{-1}$·L$^{-1}$; PM meal: peak 506.3 mU/L, AUC insulin 1,816 mU·h$^{-1}$·L$^{-1}$). However, postprandial concentrations of GLP-1 were not increased relative to the other ponies (AM meal: peak 11.8 pmol/L, AUC GLP-1 35.7 pmol·h$^{-1}$·L$^{-1}$; PM meal peak 28.9 pmol/L, AUC GLP-1 86.73 pmol·h$^{-1}$·L$^{-1}$). This pony did not demonstrate any clinical signs of laminitis despite the significant hyperinsulinemia observed.
DISCUSSION

The evidence presented in this study supports potential breed differences in the enteroinsular axis in horses and ponies adapted to eating twice-daily meals containing micronized maize. Despite similar postprandial glucose responses between breed groups, the ponies and Andalusian horses demonstrated significantly larger insulin responses compared with Standardbred horses. Postprandial GLP-1 concentrations were positively correlated with insulin concentrations, demonstrating an association between incretin hormones and hyperinsulinemia in ponies and Andalusian horses.

Prolonged hyperinsulinemia has been shown to cause acute laminitis in ponies and Standardbred horses under experimental conditions (Asplin et al., 2007; de Laat et al., 2010). Therefore, postprandial hyperinsulinemia may be an important risk factor for laminitis in predisposed individuals (Frank and Tadros, 2014). Several important observations have supported this link, including the hyperinsulinemic response of previously laminitic ponies to various forms of NSC (glucose, fructose, and inulin) compared with nonlaminitic ponies (Bailey et al., 2007; Borer et al., 2012) and the higher incidence of laminitis in grazing equids when pastures contain increased quantities of NSC (Menzies-Gow et al., 2010). Therefore, the purpose of this work was to examine a potential reason that some types of animals (grouped here by breed) exhibit a hyperinsulinemic response. A better understanding of incretin physiology may lead to new methods to control hyperinsulinemia in horses and ponies.

Micronized maize was selected as the source of supplementary NSC in the present study to ensure that starch underwent as much prececal digestion as possible (Vervuert et al., 2004). The quantity of grain in each meal was slowly increased to reduce the risk of grain overload and hindgut disturbances that could cause laminitis (Kronfeld and Harris, 2003). Although it would have been interesting, a preadaptation comparison experiment was not feasible. It was considered that feeding the final amount of grain without adaptation would place the ponies and Andalusians at undue risk of laminitis. There is little evidence to suggest an appropriate time frame to adapt horses and ponies to grain meals, but there is some evidence that horses may be slow to adapt (Dyer et al., 2009). Therefore, 12 wk was considered a suitably cautious time frame for the present study. The amount of grain in each meal during wk 12 provided 1.1 g/kg BW starch, a quantity previously shown to induce robust insulin responses (Vervuert et al., 2009). Although it may be common to feed larger quantities of grain to race horses (Crandell et al., 1999), it would be uncommon for ponies to receive more than this amount in general practice.

Differences in postprandial insulin concentrations between Standardbred, pony, and Andalusian breed groups have been reported when animals of moderate body condition consumed a single glucose-containing meal (Barnford et al., 2014). The present study found that differences of a similar magnitude were detected between the same breed groups after a period of dietary adaptation to meals containing starch. Despite adapting to the same meals, Standardbred horses secreted much less insulin to deal with the same glycemic load as ponies and Andalusian horses. This further emphasizes that studies of postprandial glucose and insulin responses to various types of NSC need to account for the breeds examined and that the application of a generic glycemic index in equine dietetics remains difficult (Harris and Geor, 2009).

Previous studies of Standardbred and light-breed horses have demonstrated reduced postprandial insulin concentrations after the second of 2 identical meals fed 8 h apart (Gordon and McKeever, 2005; Noble and Sillence, 2013); however, the present study did not observe this decrease. It is unclear why postprandial insulin responses were equivalent between meals in the present study, but this result could be due to the nature of a gradual adaptation to meal feeding or the provision of ad libitum hay throughout the study period.

The absorption and utilization of glucose from the mammalian intestinal tract is influenced by a complex enteroinsular axis, which has been scarcely studied in horses (De Graaf-Roelfsema, 2014). Two incretin hormones have been shown to exert insulinogenic effects in other species: GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). A previous study of horses confirmed the presence of an enteroinsular axis through the investigation of GIP, which is released from intestinal K cells (Duhlmeier et al., 2001). Higher glucose-to-insulin ratios were detected during an oral glucose tolerance test (associated with increased GIP concentrations) when compared with an equivalent intravenous glucose tolerance test. Shetland ponies were found to have greater glucose and insulin concentrations than large-breed horses; however, glucose-to-insulin ratios were similar, suggesting a comparable enteroinsular axis between these animals.

Glucagon-like peptide-1 was selected as the incretin hormone of interest in the present study. This incretin is released from intestinal L cells and acts to enhance postprandial glycemic control by increasing pancreatic β-cell responsiveness to glucose-stimulated insulin secretion (Holz et al., 1993). Glucagon-like peptide-1 has been extensively investigated in human and rodent studies; however, there has been little investigation of
GLP-1 in horses. One report confirmed an increase in GLP-1 concentrations during an oral sugar test but did not detect a difference between healthy and insulin-resistant groups (Chameroy et al., 2010b). Furthermore, 8 wk of overfeeding with sweet feed did not change GLP-1 concentrations in a group of obese, insulin resistant horses (Chameroy et al., 2011). In the present study, GLP-1 concentrations were found to be different between breed groups and were strongly correlated with insulin concentrations. Glucose concentrations were only weakly correlated with GLP-1, indicating that breed-related differences in GLP-1 may not be directly related to glycemic responses. These associations may be consistent with a potential role for incretin hormones in equine postprandial hyperinsulinemia.

One pony demonstrated extremely high glucose and insulin responses relative to the other individuals in the pony group. This pony did not eat faster or exhibit increased BCS relative to the other ponies, nor was it found to have an exaggerated insulin response when fed a glucose-containing meal in a previously reported study (Bamford et al., 2014). Because plasma glucose concentrations were also high, this pony might have had very efficient starch digestion and glucose absorption or decreased uptake of glucose by the liver. The GLP-1 response was similar to that of other ponies, so plasma glucose was presumably the main stimulus for insulin secretion. The rate of insulin clearance by the liver will also influence the degree of postprandial hyperinsulinemia. Therefore, the measurement of C-peptide concentrations in future studies may be indicated to assess the postprandial dynamics of insulin secretion and clearance (Toth et al., 2010).

The increased insulin and GLP-1 concentrations observed in the pony and Andalusian groups have implications for the feeding of high-glycemic meals to these (and similarly predisposed) breeds. Large amounts of NSC in the diet of these breeds may easily produce the degree of hyperinsulinemia that has been implicated in causing laminitis (de Laat et al., 2012). However, not all forms of NSC may have the same effect on GLP-1 (or indeed insulin). Because most cases of endocrinopathic laminitis are associated with grazing pasture, it would be best to assess insulin and GLP-1 responses under these conditions. It is difficult to feed controlled amounts of NSC as pasture; studies of glucose and insulin dynamics in horses have therefore relied on feeding measured amounts of NSC as grain, powdered, or pelleted formulations. Further studies are required to determine the effect of different types of NSC on incretin responses in horses.

An improved understanding of the factors that contribute to equine hyperinsulinemia is of critical importance in reducing the prevalence of laminitis in domestic horse populations. This study provides evidence that the enteroinsular axis warrants further investigation.


