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Equine Endocrinology

Corticosteroid Administration Enhances the Glycemic, Insulinemic, and Incretin Responses to a High-Protein Mixed Meal in Adult Horses

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ABSTRACT

Background: Corticosteroids are used routinely in horses and induce insulin dysregulation (ID). Nutrition is important for ID management and includes low nonstructural carbohydrate (NSC) diets and, often, high-protein ration balancers (RB). Insulin and incretin secretion increase after high-protein meals; corticosteroids may influence these effects.

Hypothesis: A high-protein mixed meal will induce hyperinsulinemia and increased concentrations of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1) in horses with ID; dexamethasone (DEX) will amplify this effect.

Animals: Five horses with naturally occurring ID.

Methods: Horses underwent an IV glucose tolerance test and a feed challenge test (FCT; 1 kg RB). Tests were repeated after DEX administration (0.08 mg/kg PO q24h, 7 days). Insulin, glucose, and incretin dynamics were compared pre- and post-DEX.

Results: Corticosteroids exacerbated post-prandial hyperinsulinemia and hyperglycemia after a high-protein meal. The FCT area under the curve for insulin (AUC_{INS}) after DEX was significantly higher than baseline ($558 \pm 182\,\mu IU/mL \times min$ vs. $257 \pm 93.9\,\mu IU/mL \times min$; $p = 0.03$). The maximum concentration of GIP (C_{maxGIP}) after DEX (381 ± 70.6 pg/mL) was significantly higher than baseline (262 ± 13.7 pg/mL; $p = 0.013$). The AUC for GLP-1 ($AUC_{GLP\text{-}1}$; 31.1 ± 15.2 vs. 50 ± 20.2 pg/mL; $p = 0.19$) and the C_{max} of GLP-1 ($C_{maxGLP\text{-}1}$; 39.1 ± 25.3 vs. 29.6 ± 12.2 pg/mL; $p = 0.32$) did not differ between DEX and baseline.

Conclusions and Clinical Importance: Metabolic responses to a high-protein mixed meal were exacerbated by corticosteroids. Horses receiving corticosteroids had larger GIP responses, which may enhance post-prandial hyperinsulinemia.

1 | Introduction

Corticosteroids are commonly used in horses and exacerbate insulin dysregulation (ID) [1–3]. Uncontrolled ID increases the risk of hyperinsulinemia-associated laminitis (HAL). Given the prevalence of ID associated with equine metabolic syndrome (EMS), the likelihood of encountering an EMS-affected individual that may require corticosteroid treatment is likely

Abbreviations: ACTH, adrenocorticotropic hormone; AIR_g, acute insulin response to glucose; AUC, area under the curve; DEX, dexamethasone; DI, disposition index; EMS, equine metabolic syndrome; FCT, feed challenge test; FSIGTT, frequently sampled insulin-modified intravenous glucose tolerance test; GLP-1, glucagon-like peptide-1; GIP, glucose-dependent insulinotropic peptide; HAL, hyperinsulinemia-associated laminitis; ID, insulin dysregulation; mTOR, mechanistic target of rapamycin; NSC, nonstructural carbohydrate; RB, ration balancer; S_g, glucose effectiveness; SI, insulin sensitivity.

high, leading to concerning overlap of these two causes of ID in horses.

Effective ID treatment includes dietary management [4], 2 principal goals of which are minimizing post-prandial hyperinsulinemia and encouraging weight loss, where appropriate. Both are usually achievable by minimizing the amount of dietary nonstructural carbohydrates (NSC). Current recommendations include offering low-NSC grass hay in combination with commercially available ration balancers (RB) to supplement protein, vitamins, and minerals that are often deficient in grasses [4, 5].

The incretin effect describes the situation when PO glucose elicits a larger insulinemic response than IV glucose. Glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) mediate this effect. Glucagon-like peptide 1 stimulates insulin secretion in response to enteral carbohydrates, decreases gastric emptying, and promotes satiety [6]. Glucose-dependent insulinotropic polypeptide has an amino acid-dependent effect on insulin secretion [7]. This effect occurs indirectly through the release of glucagon from α-cells after stimulation by GIP upon amino acid ingestion. Glucagon then stimulates insulin release from β-cells by signaling through the glucagon receptor [7].

In humans, dietary protein content correlates with systemic insulin resistance [8, 9]. Glucose is the primary stimulus for insulin synthesis and secretion, but arginine and other amino acids (the concentrations of which are often increased post-prandially) also play a role. Previous work in horses has demonstrated a 9-fold larger insulinemic response after a high-protein meal in horses with ID when compared with normal horses, but this observation has not been identified consistently in all studies involving high-protein meal feeding in horses [10, 11]. Because long-term, high-protein diet consumption in humans can contribute to insulin resistance and short-term, high-protein diet consumption in horses with ID can promote increased post-prandial insulinemic responses, the effects of high-protein feeding in horses (both short- and long-term) deserve more scrutiny. Our aims were to evaluate the effects of corticosteroid administration on insulin, glucose, and incretin dynamics after consumption of a commercially available high-protein mixed meal in horses with naturally occurring ID. We hypothesized that high-protein mixed meals would induce hyperinsulinemia and increase concentrations of GIP and GLP-1 in horses; dexamethasone (DEX) administration would amplify this effect.

2 | Materials and Methods

2.1 | Animals

Five light-breed horses 5–16 years of age were enrolled. All horses had ID (insulin sensitivity [SI] $< 1.0 \times 10^{-4}/\text{min} \times (\text{pmol/L})^{-1}$) but were otherwise healthy based on history and physical examination. All horses had normal basal plasma adrenocorticotropic hormone (ACTH) concentrations (Animal Health Diagnostic Center, Cornell University College of Veterinary Medicine, Ithaca NY). The horses received *ad libitum* water and grass hay during the study, except when fasted for ID testing. All horses were monitored daily for complications (e.g., laminitis, thrombophlebitis) during the study and were housed and managed according to an approved animal use protocol (The Ohio State University IACUC; protocol 2022A00000012).

2.2 | Sample Collection

Horses arrived to The Ohio State University Galbreath Equine Center on Day 1 and were acclimated on Days 1–4. Body weights were obtained with a scale on Day 1. All horses were muzzled for 6 h before testing. On Days 5 and 6, a frequently-sampled insulin-modified IV glucose tolerance test (FSIGTT; FSIGTT1) and a feed challenge test (FCT; FCT1) were performed, respectively, to determine baseline results for outcomes of interest related to systemic insulin and glucose dynamics; all testing was initiated between the hours of 7–8 a.m. On Day 5, a 14 ga 5.25″ catheter was placed in the left jugular vein for blood collection. A second catheter was similarly placed in the right jugular vein and used for administration of dextrose and insulin. The FSIGTT then was performed as previously described [12]. Baseline blood samples (from the IV catheter after a minimum collection of 10 mL of discarded blood) were obtained at 10, 5, and 1 min before the administration of dextrose. One dose of 50% dextrose (150 mg/kg) was administered IV as a bolus at Time 0, followed by 0.1 IU/kg regular insulin IV (Humulin-R U-100) 20 min later. Blood was collected at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 19, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min after dextrose administration. Blood glucose concentrations were measured stall-side at each time point using a handheld glucometer previously evaluated for use in horses (AlphaTrak2) [13] immediately after sample collection. The right IV catheter was removed after insulin administration. The left IV catheter remained in place overnight and was irrigated with heparinized saline q6h. Horses then were fasted again as for the FSIGTT in preparation for the FCT.

On Day 6, blood was collected at Time 0 before offering a 1 kg high-protein RB meal (minimum 32% crude protein, maximum 13% NSC; GRO 'N WIN RB, BUCKEYE Nutrition, MARS Horsecare, Dalton OH). The horses were given 10 min to consume the meal, with any feed remaining after this time point collected and weighed; muzzles were replaced after consumption of the meal. Blood samples then were collected from the IV catheter as described above at the following time points: 30, 60, 90, 120, 180, and 240 min after meal consumption. Blood glucose concentration was measured stall-side at each time point (including baseline [Time 0]) as for the FSIGTT. Muzzles were removed at the 120-min time point, after which all horses had *ad libitum* access to grass hay. Plasma and serum samples were processed immediately after completion of each FSIGTT and FCT at both study time points. All blood samples were centrifuged at 3000 rpm for 15 min and stored at −80°C until further testing. On Days 7–15, all horses received dexamethasone (DEX; 0.08 mg/kg PO q24h) to exacerbate systemic ID. On Days 14 and 15, FSIGTT (FSIGTT2) and FCT (FCT2) testing was repeated.

2.3 | Insulin and Glucose Measurements

Blood glucose concentrations were measured and recorded at every blood collection time point during FCT and FSIGTT testing. Plasma insulin concentration was measured using

an insulin ELISA designed for humans (07M-60102, MP Biomedicals, Solon, OH); this assay has been validated for use in horses (inter- and intra-assay coefficient of variation [CV] < 10%, limit of detection [LOD] 0.75 μIU/mL) [14]. Plasma insulin concentration was measured at every time point during the FSIGTT and at the 0–240 min time points for the FCT. Minimal model parameters (MINMOD Millennium) were calculated from the glucose and insulin concentration data generated during the FSIGTT; these parameters included the acute insulin response to glucose (AIR_g), glucose effectiveness (S_g), SI, and disposition index (DI). Table S1 contains these parameters for each of the horses and definitions. Areas under the curves (AUC) for insulin and glucose concentrations were calculated using the trapezoidal method from the data generated during the FCT, and peak concentration (Cmax) and time of peak concentration (Tmax) for insulin and glucose were recorded.

2.4 | Incretin Hormone Measurements

Serum GLP-1 and GIP concentrations were measured using multi-species ELISAs previously validated for use in horses (EZGLP1T-36K [interassay CV, 1.6%; LOD, 0.95 pM] and EZHGIP-54K [interassay CV, 9.1%; LOD, 1.83 pM], Millipore Sigma, Burlington, MA) [14, 15]; serum GLP-1 and GIP concentrations were measured at the 0–240 min time points for the FCT. Areas under the curves for GLP-1 and GIP concentrations were calculated from these data using the trapezoidal method.

2.5 | Statistical Analysis

Statistical analyses were performed using commercially available software (GraphPad Prism v 9.4). Normality was determined using the Shapiro–Wilk and Kolmogorov–Smirnov tests. AUC (time 0–240 min) for glucose (AUC_{GLC}), insulin (AUC_{INS}), GLP-1 ($AUC_{GLP\text{-}1}$), and GIP (AUC_{GIP}) concentrations were calculated from the FCT data using the trapezoidal method. Basal concentration, maximum concentration (C_{max}), and time to achieve maximum concentration (T_{max}) also were recorded based on visual inspection of the data for each FCT. A paired *t*-test (or Wilcoxon matched pairs signed rank test for nonparametric data) was performed to compare parameters derived from the FCT (AUC for GLP-1, GIP, insulin, and glucose; C_{max} for insulin and glucose; and, T_{max} for insulin and glucose) and the FSIGTT (AIRg, Sg, SI, and DI) at baseline and after DEX administration (FCT1 vs. FCT2; FSIGTT1 vs. FSIGTT2). Normally distributed data are reported as mean ± SD, and nonnormally distributed data are reported as median [25%–75% interquartile range]. Statistical significance was accepted at $p \leq 0.05$.

3 | Results

3.1 | Animals

Five horses were included (3 geldings and 2 mares), all of which completed the study protocol without complication. Three breeds were represented: American Quarter Horse ($n = 2$), Thoroughbred ($n = 2$), and Standardbred ($n = 1$). Endogenous plasma ACTH concentration was within reference ranges for all horses, and all were physically and behaviorally normal throughout the study. Mean body weight was 561.6 ± 71.1 kg and mean age was 9 ± 3.9 years.

3.2 | Insulin and Glucose Dynamics

3.2.1 | FSIGTT

The FSIGTT parameter data (including results for each individual horse) are presented in Table S1. No statistically significant difference was found in AIR_g, DI, or SI between baseline and DEX conditions ($p = 0.98$, $p = 0.14$, and $p = 0.19$, respectively). Insulin sensitivity was lower after DEX than at baseline (0.034 [0.03–0.08] vs. 0.62 [0.02–0.92]); all 5 horses had SI $< 1.0 \times 10^{-4}$/min × (pmol/L)$^{-1}$ at baseline, and four of the five horses had lower SI after DEX administration (see Figure 1).

3.2.2 | FCT

All horses consumed > 95% of the RB at both time points (baseline and DEX). The AUC_{INS} was significantly increased after DEX administration compared with baseline (558 ± 182 mIU/L × min vs. 257 ± 93.9 mIU/L × min; $p = 0.03$), but AUC_{GLC} was not (111 mg/dL × min [81.7–235] vs. 89.3 mg/dL × min [64–94.2], $p = 0.13$; see Figure 2). Basal insulin concentration (131 ± 73.7 mIU/L vs. 22.8 ± 14.1 mIU/L; $p = 0.04$) and C_{maxINS} (330 ± 107 mIU/L vs. 117 ± 34.6 mIU/L; $p = 0.01$) were both significantly increased after DEX administration compared with baseline; no difference was found in T_{maxINS} ($p > 0.99$; Figure 3). Basal glucose concentration was not significantly different after DEX administration (133 mg/dL [125–209] vs. 108 mg/dL [107–119], $p = 0.06$), but C_{maxGLC} (215 ± 56.5 mg/dL vs. 145 ± 11.3 mg/dL, $p = 0.03$) was significantly increased; no difference was found in T_{maxGLC} ($p = 0.25$; Figure 3). Plasma insulin concentration data during both FCTs for each of the five individual horses are presented in Figure 4.

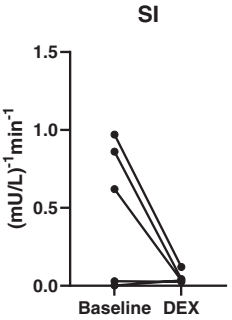

FIGURE 1 | Insulin sensitivity of five adult light breed horses before ("Baseline") and after administration of dexamethasone ("DEX"). Insulin sensitivity was derived from the insulin and glucose concentrations measured during a frequently sampled insulin-modified IV glucose tolerance test using Minimal Model kinetics. The same five horses are represented under Baseline and DEX conditions; four of the five horses' SI was lower after 7 days of dexamethasone (0.08 mg/kg by mouth q24h). DEX, dexamethasone; SI, insulin sensitivity.

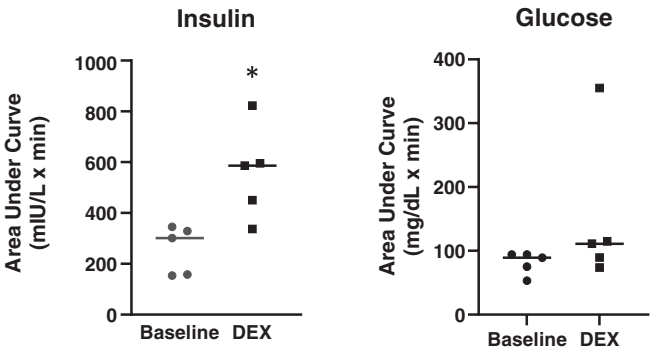

FIGURE 2 | Areas under the curves for [insulin] (panel on left) and [glucose] (panel on right) measured in adult horses after consumption of a high-protein meal before ("Baseline") and after administration of dexamethasone ("DEX"). The same five horses are represented under Baseline and DEX conditions in both panels. The post-prandial AUC_{INS} was significantly increased after administration of DEX. AUC, area under the curve; ins, insulin; glc, glucose.

3.3 | Incretin Hormones

The C_{maxGIP} during DEX (381 ± 70.6 pg/mL) was significantly higher than at baseline (262 ± 13.7 pg/mL; $p = 0.01$). The $AUC_{GLP\text{-}1}$ (31.1 ± 15.2 vs. 50 ± 20.2 pg/mL; $p = 0.19$) and $C_{maxGLP\text{-}1}$ (39.1 ± 25.3 vs. 29.6 ± 12.2 pg/mL; $p = 0.32$) did not differ between DEX and baseline (Figure 5).

4 | Discussion

Our results indicate that horses receiving corticosteroids have significantly higher glycemic and insulinemic responses to a high-protein mixed meal than observed in the same horses at baseline. These results are consistent with a previous study, which reported a 9-fold larger insulinemic response in horses with naturally occurring ID when compared with normal horses after consumption of a high-protein meal (31% crude protein, 4 g/kg) [10]. The results of our study also indicate that horses receiving corticosteroids have significantly increased serum GIP responses to this meal than at baseline. Given that this same response was not observed for GLP-1, these data suggest a more important role for GIP in promoting the incretin effect in horses receiving dexamethasone. Corticosteroid administration suppresses GLP-1 secretion in other species [16]. In humans, glucocorticoid-induced IR results in decreased insulinotropic properties for both GLP-1 and GIP [17]. In our study, the GIP response to the meal was enhanced by DEX, suggesting that corticosteroids do not suppress GIP secretion in horses.

Diets for EMS/ID patients often are optimized based on their NSC content, with the target nutritional composition including < 10% NSC [18]. Grass hay containing > 10% NSC still can be used in some circumstances, but methods to decrease the NSC content (including soaking protocols) may render this forage safer for consumption by these individuals [19, 20]. However, soaking forage to decrease its NSC content will also indiscriminately leach out other water-soluble components, such as trace minerals and vitamins, which may result in nutritional deficiencies over time. Thus, RBs often are fed to supplement minerals, vitamins, and protein, because most grass forages are also relatively low in crude protein [4]. Dietary protein requirements for adult horses in light work are estimated to be 0.76 kg/day with 33 g/day of lysine [21]. No known difference in dietary protein requirement exists between horses with ID and metabolically healthy horses. Protein deficiency is uncommon in adult horses (even those on forage-only diets), but specific amino acids may be deficient based on the composition of an individual horse's

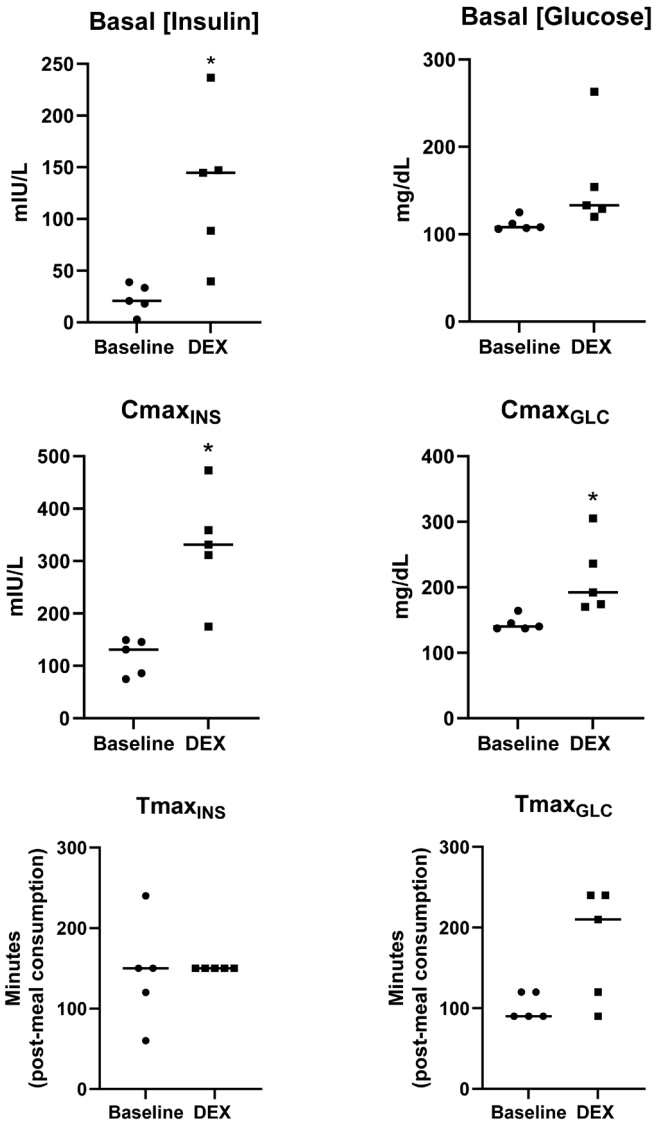

FIGURE 3 | Parameters of insulin and glucose dynamics measured after consumption of a high-protein meal in five adult horses at baseline and following administration of dexamethasone ("DEX"). Basal insulin and glucose concentrations (top 2 panels), maximum post-prandial insulin and glucose concentration (middle 2 panels), and time to maximum post-prandial insulin and glucose concentration (bottom 2 panels) are displayed, within insulin parameters on the left and glucose parameters on the right. Basal [insulin] was significantly increased after DEX administration ($p = 0.04$), as were post-prandial peak [insulin] and [glucose] ($p = 0.01$ and $p = 0.03$, respectively). Basal [glucose] and the time to peak [insulin] and [glucose] was not significantly different between baseline and DEX conditions ($p = 0.06$ and $p = 0.25$, respectively). C_{max}, peak post-prandial concentration; glc, glucose; ID, insulin dysregulated; ins, insulin; T_{max}, time to peak post-prandial concentration.

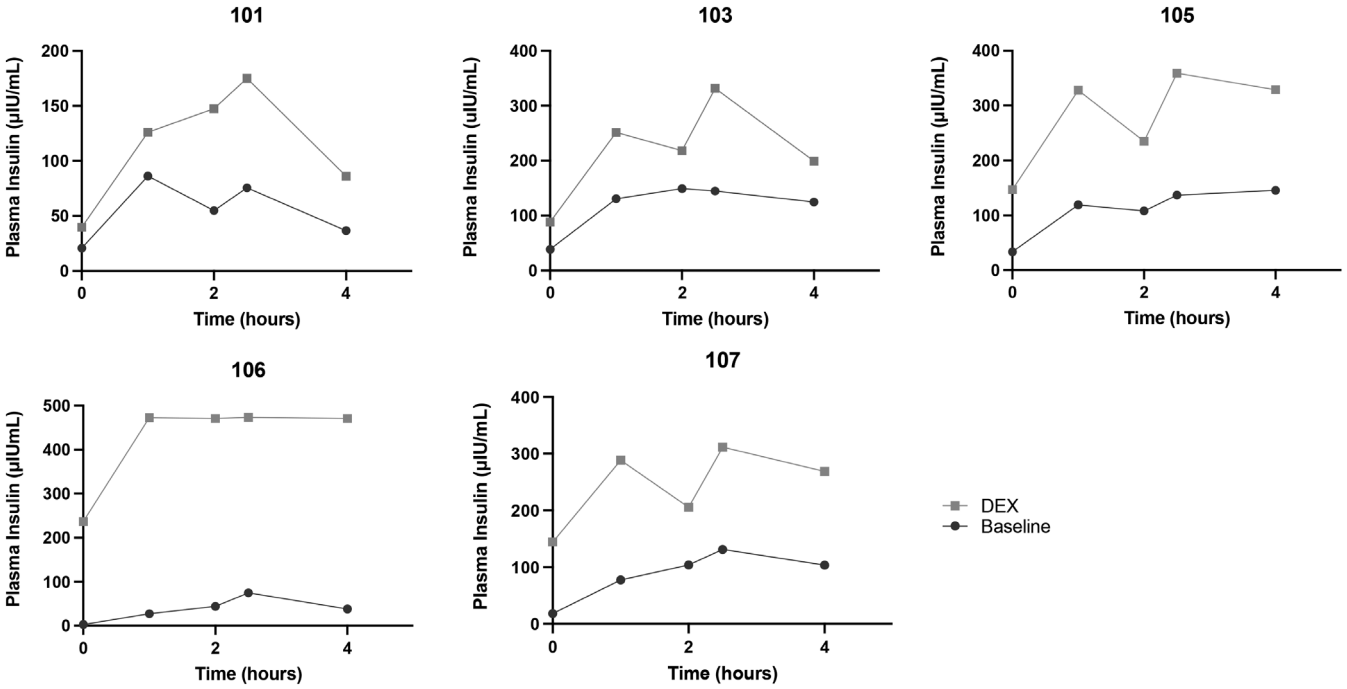

FIGURE 4 | Plasma [insulin] over time for five individual horses after consumption of a meal of a high-protein ration balancer at baseline (circles) and after dexamethasone ("DEX") administration (squares). Each panel represents the same horse tested before and after DEX administration; in all cases, plasma [insulin] was significantly increased post-prandially, and this was exacerbated with DEX administration. In fact, all but one horse (101) experienced plasma insulin concentrations > 200 mIU/L after consumption of a high-protein meal when receiving DEX, which is a degree of hyperinsulinemia that has been associated with enhanced risk of laminitis.

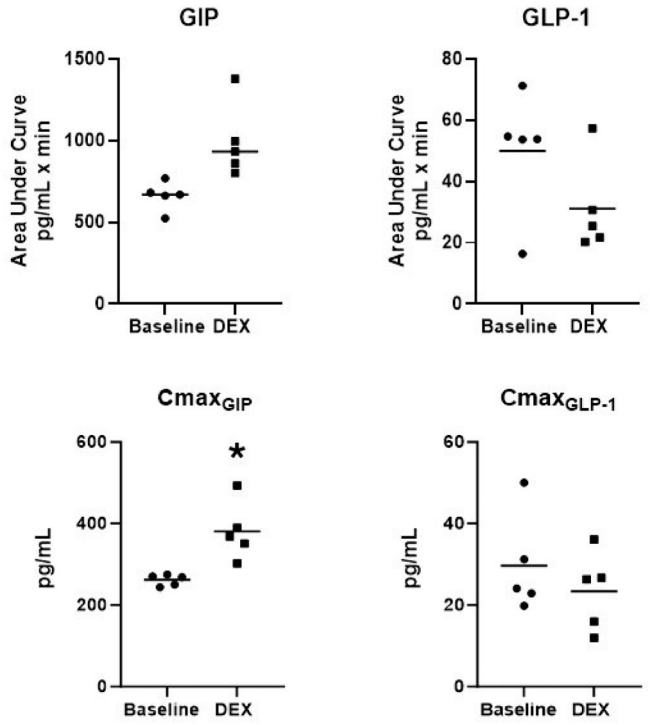

FIGURE 5 | Areas under the curves and maximum concentrations for [GIP] (panels on left) and [GLP-1] (panels on right) measured in adult horses after consumption of a high-protein meal before ("Baseline") and after administration of dexamethasone ("DEX"). The same five horses are represented under Baseline and DEX conditions in both panels. C_{maxGIP} was significantly increased after DEX administration. AUC, area under the curve; GIP, glucose-dependent insulinotropic peptide; GLP-1, glucagon-like peptide 1.

diet. Empirically addressing this potential problem using high-protein RBs in horses being managed for ID historically has been considered a safe, low-risk nutritional strategy. Our results however suggest that this benefit of high-protein RBs should not be assumed, because post-prandial hyperglycemia and hyperinsulinemia were both worsened after consumption of a meal of 1 of these products (fed according to the label). Importantly, the post-prandial plasma insulin concentrations observed were often within a range reported to increase the risk of HAL (> 200 mIU/L) for a prolonged period of time after meal consumption [22]. Based on our results, high-protein mixed meal feeding should be carefully considered in horses receiving corticosteroids.

Our study had some limitations. One is the mixed nature of the RB meal used, which contained many other nutrients in addition to crude protein (including a NSC content of 13%). Whereas the RB used in our study contained 320 g of protein per kilogram, it also contained 130 g of NSC per kilogram, indicating that post-prandial hyperinsulinemia could not be attributed solely to the crude protein content of the ration. This distinction is important because our results do not implicate dietary protein as the only driver of post-prandial hyperinsulinemia. However, they do support critical evaluation of high-protein RBs (which may receive less scrutiny than other dietary components, given that they are relatively low in NSC and fed in small quantities) for the nutritional management of ID in horses, because post-prandial hyperinsulinemia after a RB meal can be substantial and prolonged. Another limitation is the baseline metabolic status of the study horses. Although normal seasonally adjusted ACTH concentration was an inclusion criterion, the presence or absence of ID (by any measure) initially was not. All 5 horses

had SI values $< 1.0 \times 10^{-4}/\text{min} \times (\text{pmol/L})^{-1}$ at baseline, supportive of ID (systemic insulin resistance) before administration of DEX, although the breeds and phenotypes of the horses did not suggest any predisposition.

In conclusion, high-protein RBs should be fed carefully to corticosteroid-treated horses, particularly those with established ID. Based on our results and those of others, RBs can be associated with exacerbated post-prandial hyperinsulinemia. Incretin hormones, specifically GIP, may play a role in this post-prandial hyperinsulinema. Based on our study, in horses with naturally occurring ID receiving corticosteroids, high-protein mixed meal feeding is associated with enhanced glycemic, insulinemic, and incretin responses. To minimize metabolic complications, the ID status and diet of horses warrant evaluation before corticosteroid treatment.

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Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

All horses were housed and managed according to a protocol approved by The Ohio State University Institutional Animal Care and Use Committee (IACUC), protocol 2022A00000012, in accordance with the guidelines outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Authors declare human ethics approval was not needed.

Conflicts of Interest

The Authors declare no conflicts of interest.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.