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Blood Glucose and Insulin Concentrations after Octreotide Administration in Horses With Insulin Dysregulation

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Background: Octreotide is a somatostatin analog that suppresses insulin secretion.

Hypothesis: We hypothesized that octreotide would suppress insulin concentrations in horses and that normal (N) horses and those with insulin dysregulation (ID) would differ significantly in their plasma glucose and insulin responses to administration of octreotide.

Animals: Twelve horses, N = 5, ID = 7.

Methods: Prospective study. An oral sugar test was performed to assign horses to N and ID groups. Octreotide (1.0 μg/kg IV) was then administered, and blood was collected at 0, 5, 10, 15, 20, 25, 30, 45, 60, 75, and 90 minute, and 2, 3, 4, 6, 8, 12, and 24 hour for measurement of glucose and insulin concentrations. Area under the curve (AUC) values were calculated.

Results: Mean AUC values for glucose and insulin did not differ between normal (n = 5) and ID (n = 7) groups after octreotide injection. Significant time ($P < .001$) effects were detected for glucose and insulin concentrations. A group × time interaction ($P = .091$) was detected for insulin concentrations after administration of octreotide, but the group ($P = .33$) effect was not significant.

Conclusions and Clinical Importance: Octreotide suppresses insulin secretion, resulting in hyperglycemia, and then concentrations increase above baseline as glycemic control is restored. Our hypothesis that octreotide causes insulin concentrations to decrease in horses was supported, but differences between N and ID groups did not reach statistical significance when blood glucose and insulin responses were compared. The utility of an octreotide response test remains to be determined.

Key words: Equine metabolic syndrome; Insulin resistance; Laminitis; Pancreas; Somatostatin.

Somatostatin (SST) plays an important role in glucose homeostasis and is secreted by delta cells located next to alpha- and beta-cells within the Islets of Langerhans.[1] Glucose, amino acids, and glucagon-like peptide-1 stimulate secretion of this hormone, and SST binds to its receptors on Islet cells to inhibit secretion of insulin, glucagon, and pancreatic polypeptide. Somatostatin acts as a buffering hormone and prevents excess secretion of glucagon and insulin, so that insulin secretion decreases as blood glucose concentrations decrease, and glucagon secretion decreases when hyperglycemia develops.[2] Paracrine signaling occurs through binding of SST to receptors on alpha- and beta-cells, and 5 different SST receptor subtypes have been cloned.[3,4] Somatostatin analogs such as octreotide were developed for the treatment of acromegaly and adult Cushing's disease in humans.[5,6] There are only 2 published studies of octreotide use in horses to date which examined its effects on gastric pH in ponies and testicular function in pony stallions.[7,8] Somatostatin solution administered intravenously as a continuous rate infusion suppresses insulin secretion in horses.[9] To our knowledge, effects of octreotide on blood glucose and insulin concentrations have not been previously investigated in horses.

Somatostatin analogs are primarily used for the treatment of acromegaly in humans, and octreotide suppresses growth hormone secretion from the pituitary gland.[10] The elimination half-life of octreotide is approximately 1.5 hour after intravenous or subcutaneous administration in humans, and octreotide is 20 times more potent than SST when suppressing growth hormone secretion.[11] The pharmacokinetics of octreotide have not been evaluated in horses, but the half-life of octreotide acetate solution was 12.6, 0.567, and 0.66 hour in rats, rabbits, and dogs, respectively, when administered via single subcutaneous injection at dosages of 20, 0.025, and 0.022 mg/kg, respectively.[12]

Abbreviations:

AUC	area under the curve
CV	coefficient of variation
ID	insulin dysregulation
ORT	octreotide response test
OST	oral sugar test
SST	somatostatin
SSTR	somatostatin receptor

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Serum insulin concentrations decreased in response to subcutaneous injection of 50 μg SMS 201-995 (octreotide) in healthy human subjects, with subsequent increase in blood glucose concentrations.[13] Octreotide also lowered plasma glucagon concentrations and blunted the nocturnal rise in thyroid-stimulating hormone concentrations, but did not impact thyroid hormone or cortisol concentrations. These findings provide evidence that SST analogs affect multiple endocrine systems and this should be considered when interpreting results of studies performed in horses.

Insulin dysregulation (ID) is detected in horses and ponies, and evidence provided by experimental models establishes hyperinsulinemia as a cause of laminitis.[14,15] Equine metabolic syndrome (EMS) includes ID as well as increased adiposity, hypertriglyceridemia, and altered adipokine concentrations, and affected horses, ponies, and donkeys have a higher risk of developing laminitis.[16] Obesity, EMS, and endocrinopathic laminitis are growing concerns for the equine population, and additional research is required to better understand the mechanisms of hyperinsulinemia in horses, and to enable early diagnosis.

The role of SST in the development of hyperinsulinemia in equids warrants investigation and study of the effects of SST analogs such as octreotide on blood glucose and insulin concentrations is warranted. We therefore hypothesized that octreotide would suppress insulin concentrations in horses and that normal (N) horses and those with ID would differ significantly in their plasma glucose and insulin responses to octreotide. An additional goal of this study was to begin the development of a new diagnostic test for ID in horses.

Materials and Methods

Animals

Twelve horses from a university teaching and research farm were evaluated: 9 Morgan horses, 2 Hanoverians, and 1 Appaloosa.

Experimental Design

Octreotide was administered to horses between February and June 2015. Horses were tested for ID by performing the oral sugar test (OST) and assigned to N or ID groups on the basis of test results. Octreotide was administered to 12 horses (7 ID, 5 N); 2 horses (1 ID, 1 N) were tested in February 2015, 2 horses (1 ID, 1 N) in early May 2015, 4 horses (3 ID, 1 N) in late May 2015, and 4 horses (2 ID, 2 N) in June 2015. Time intervals between performing the OST and administering octreotide ranged from 5 to 14 days for 11 horses. One mare with a history of ID was last tested 3.5 months before octreotide administration. Horses received an intravenous injection of octreotide, and blood was collected for measurement of plasma glucose and insulin concentrations over 24 hour. The Institutional Care and Use Committee of the University of Massachusetts at Amherst approved the study protocol.

Oral Sugar Test

Feed was withheld overnight. Horses were fed 1 flake of hay at 20:00 the night before, and no additional feed was provided until the test was completed the next morning, between 09:30 and 11:00. Water was withheld during the procedure. The OST procedure described by Schuver et al.[17] was performed, but only a single blood sample was collected 75 minute after sugar administration to detect ID. Corn syrup[a] was administered orally with 60-mL catheter-tip syringes at a dose of 0.15 mL/kg body weight. Blood (20 mL) was then collected into tubes containing EDTA at 75 minute after administration of corn syrup. A positive insulin result was defined by a plasma insulin concentration >45 μU/mL at the same time point, in accordance with Equine Endocrinology Group recommendations.[b]

Octreotide Injection

Feed was withheld again overnight before octreotide was administered. Horses received 1 flake of hay at 20:00 the night before, and then, feed was withheld until the regular feeding times at 16:00 on the day of testing and 07:00 the following day. Horses received 1 flake of first cutting mixed grass hay weighing approximately 1.0–1.5 kg at these time points. An intravenous catheter was inserted into the jugular vein the morning of testing between 07:00 and 08:00. A pre-injection blood sample was collected, and then octreotide (1.0 μg/kg)[c] was administered intravenously via the catheter at time 0. Blood (10 mL) was collected through the IV catheter at 5, 10, 15, 20, 25, 30, 45, 60, 75, 90, 120, 180, 240, 360, and 480 minute. The catheter was removed after the 480 minute blood collection, and additional blood samples were collected at 720 minute and 24 hour postinjection via jugular venipuncture with an 18-gauge 1.5-inch needle. Rectal temperature, heart rate, and respiratory rate were monitored at the same time that blood samples were collected.

Plasma Glucose and Insulin Concentrations

Blood was transferred to tubes containing EDTA that were placed in a chilled container with ice within 2 minute of collection, and centrifugation was performed within 4 hours of collection. Plasma samples were stored at −20°C. Frozen plasma samples were packaged with ice packs and sent via overnight mail to the Animal Health Diagnostic Center at Cornell University[d] for measurement of plasma glucose and insulin concentrations.

Data Analysis

Glucose and insulin data for samples collected at 12 and 24 hours were excluded from analyses because stress associated with venipuncture might have impacted results, and horses were fed after 12 hour. Area under the curve (AUC) (0–480 minute) values for glucose and insulin were calculated by the trapezoidal method. Normality was assessed by examining plotted results and performing Shapiro–Wilk tests, and all data were normally distributed. Analysis of variance analysis (ANOVA) for repeated measures was performed for glucose and insulin concentrations (0–480 minute) to examine the main effects of group and time, and the group × time interaction. Time of year that testing was performed (February, May, or June) was also examined. The effect of Student's *t*-tests was performed to compare age, body weight, body condition score, AUC glucose, and AUC insulin between N and ID groups, and the Bonferroni correction for multiple comparisons was applied when mean values at individual time points were compared. The Pearson correlation coefficient was calculated to compare plasma insulin concentrations at 75 minute during the OST with insulin concentrations detected 240 minute after injection of octreotide. A data analysis and statistical software program[e] was used to perform these analyses, and statistical significance was defined at a value of $P < .05$.

Results

Signalment data are displayed in Table 1. Groups did not differ with respect to age, body weight, or body condition score. No adverse events were observed when OSTs were performed. Horses were allocated to groups on the basis of OST results, and mean ± SD insulin concentration at 75 minute was 30.3 ± 3.9 μU/mL for the N group (n = 5) and 58.7 ± 5.8 μU/mL for the ID group (n = 7); there was a significant difference between groups ($P < .001$). Insulin concentrations at 75 minute ranged from 26.2 to 36.2 (median, 29.6) μU/mL for the N group and from 52.9 to 70.0 (median, 56.6) μU/mL for the ID group.

There were no alterations in behavior or physical examination variables detected after injection of octreotide. Time of year of testing (February, May, June) did not appear to affect results. Mean ± SD basal (pre-injection) plasma glucose concentrations were 87 ± 13 mg/dL and 92 ± 13 mg/dL for N and ID groups, respectively, and groups did not differ significantly ($P = .55$). Plasma glucose concentrations increased in response to octreotide injection and then returned to baseline (Fig 1). Mean AUCg for the ID group (n = 7) was 53,053 ± 8,787 mg/dL·min compared to 48,206 ± 5,038 mg/dL·min for the N group (n = 5), and groups did not differ significantly ($P = .30$). Repeated measures ANOVA revealed a significant time effect ($P < .001$) for glucose concentrations, but group ($P = .47$) and group × time ($P = .17$) effects were not significant. When groups were compared at individual time points by *t*-tests, the difference in mean glucose concentrations between N (92 ± 7 mg/dL) and ID (116 ± 27 mg/dL) groups approached statistical significance at 240 minute ($P = .081$).

Mean ± SD basal (pre-injection) plasma insulin concentrations were 13 ± 7 μU/mL and 17 ± 8 μU/mL for N and ID groups, respectively, and groups did not differ significantly ($P = .353$). Plasma insulin concentrations initially decreased in response to octreotide injection and then increased (Fig 2). Mean AUCi for the ID group was 8,738 ± 3,133 μU/mL·min compared to 6,221 ± 3,123 μU/mL·min for the N group, and groups did not differ significantly ($P = .20$). There was a significant time ($P < .001$) effect and the group × time interaction approached statistical significance ($P = .091$) for insulin concentrations, but the group ($P = .33$) effect was not significant. The difference in mean insulin concentrations between N (14 ± 5 μU/mL) and ID (24 ± 10 μU/mL) groups approached statistical significance at 240 minute ($P = .072$). Plasma insulin concentrations measured 240 minute after injection of octreotide were positively correlated ($r = 0.62$; $P = .030$; n = 12) with insulin concentrations measured at 75 minute when the OST was performed.

Table 1. Signalment and morphometric data for 12 horses.

	N (n = 5)	ID (n = 7)	*P*
Age (years)	13 ± 8	19 ± 5	.14
Sex	2G; 3M	3G; 4M	
Breeds of horse	3 Morg 1 Hanov 1 App	6 Morg 1 Hanov	
BWT (kg)	544 ± 79	538 ± 46	.86
BCS	7.0 ± 0.6	6.9 ± 0.5	.83
OST Ins (μU/mL)	30.3 ± 3.9	58.7 ± 5.8	<.001

App, Appaloosa; BCS, body condition score; BWT, body weight; Hanov, Hanoverian; Morg, Morgan horse; OST Ins, oral sugar test insulin concentration; ID, insulin dysregulation.

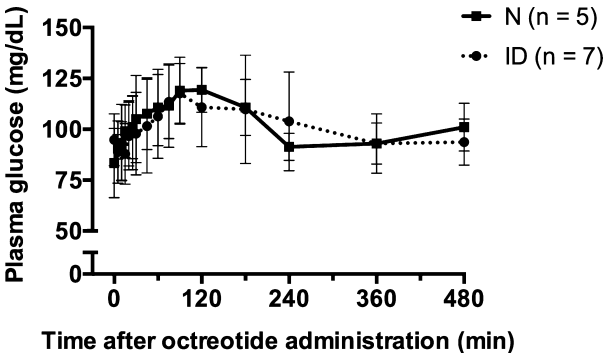

Fig 1. Mean ± SD plasma glucose concentrations measured after injection of octreotide (1.0 μg/kg IV) in 12 horses assigned to normal (N; n = 5; solid line) and insulin dysregulation (ID; n = 7; dashed line) groups on the basis of oral sugar test insulin values.

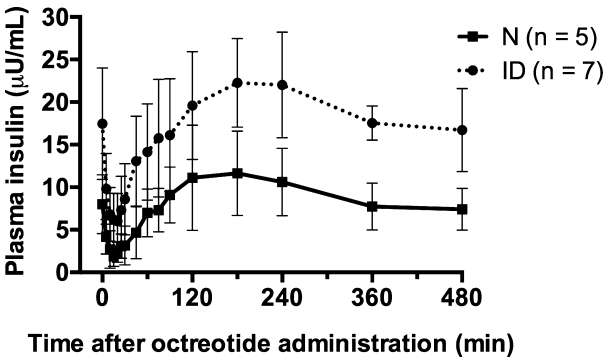

Fig 2. Mean ± SD plasma insulin concentrations measured after injection of octreotide (1.0 μg/kg IV) in 12 horses assigned to normal (N; n = 5; solid line) and insulin dysregulation (ID; n = 7; dashed line) groups on the basis of oral sugar test insulin values.

Discussion

Insulin concentrations decreased and hyperglycemia developed within minutes of octreotide being injected intravenously and our hypothesis was supported. Once the effects of octreotide waned, insulin concentrations increased above baseline and blood glucose concentrations decreased. Plasma insulin concentrations at the 75-minute time point of the OST and 240 minute after octreotide injection were positively correlated, but other differences in glucose and insulin responses between N and ID groups did not reach statistical significance.

Additional studies could now be performed to develop an octreotide response test for diagnosing ID in horses.

Horses were allocated to groups on the basis of OST results, and an insulin concentration >45 μU/mL at 75 minute was used to define ID. The OST is easily performed in the field and assesses the increase in blood insulin concentrations that occurs after ingestion of sugars. Recent reports suggest that the 60 μU/mL insulin cutoff value proposed when the OST was first introduced is too low and a lower cutoff value should be selected. In this study, we selected the lower cutoff value of 45 μU/mL recommended by the Equine Endocrinology Group[b] and 7 affected horses were identified, with insulin concentrations ranging from 52.9 to 70.0 μU/mL at 75 minute. Although these were positive results, it should be noted that insulin results fell close to the cutoff value, horses were only mildly affected, and the 45 μU/mL threshold for detecting ID has not been rigorously evaluated. Nevertheless, a positive correlation was detected between OST insulin values at 75 minute and insulin concentrations measured at 240 minute. The observation that horses included in this study were only mildly affected is noteworthy because diagnostic tests should ideally detect early/mild disease, and this is particularly important when considering ID because hyperinsulinemia is associated with the development of laminitis in horses.

The method used to assess insulin status and define groups should also be considered when examining results of this study because insulin concentrations were only measured at a single time point during the OST. Ideally, multiple blood samples would have been collected across a longer time period so that area under the insulin curve values could have been calculated for the OST. Additional diagnostic tests might also have been performed to further characterize the insulin abnormalities detected in horses included in this study. For example, tissue insulin resistance might have been measured more directly by performing the insulin tolerance test, frequently sampled intravenous glucose tolerance test, or euglycemic-hyperinsulinemic clamp procedure.[18]

Octreotide was administered at a dosage of 1.0 μg/kg body weight, which is equivalent to 500 μg (0.5 mg) for a 500-kg horse. This dosage was selected because administration of SST solution to horses as a 500 μg intravenous bolus injection, followed by a continuous rate infusion of 500 μg SST solution per hour for 330 minute, suppressed insulin secretion.[9] A low dose of octreotide was used because of the cost of the drug. Higher octreotide dosages have been selected in other studies, including to ponies as single subcutaneous injections at dosages of 0.1, 0.5, 1.0, and 5.0 μg/kg or 100 mg octreotide twice daily via subcutaneous injection to Shetland pony stallions that ranged in weight from 150 to 180 kg for 10 days, and this is equivalent to dosages of 0.55 to 0.67 mg/kg (556–667 μg/kg).[7,8]

There were no adverse events detected after octreotide administration in our study or the 2 previous reports, and this suggests that octreotide has a wide margin of safety in Equidae. However, dose escalation studies are required to determine the safety of octreotide in horses with normal insulin status and those with ID. It must be determined whether the hyperinsulinemia that develops after the effects of octreotide on insulin secretion decreases places horses and ponies with ID at risk of developing laminitis. No clinical signs of laminitis were observed in horses receiving octreotide in the study reported here and in the 2 previous studies, but horses with severe ID were not evaluated and these animals are likely to be more susceptible to developing laminitis.

Somatostatin analogs, including octreotide and pasireotide, have been used for the medical management of acromegaly in the cat[19,20] and insulinoma in the dog.[21] Diabetes mellitus is a major concern in acromegalic cats and SST analogs lower the dose of exogenous insulin required to manage hyperglycemia in these patients. Octreotide and pasireotide exert this effect on glucose homeostasis in acromegalic cats by inhibiting growth hormone secretion from the pars distalis and lowering insulin-like growth factor-1 concentrations. These drugs differ in their relative affinity for different SST receptors, and pasireotide is emerging as the better treatment for acromegaly in the cat.[20] It is also possible that SST analogs alter secretion of glucagon and insulin from alpha- and beta-cells, when used as treatments for feline hypersomatotropism, but insulin secretion has not been measured in cats. Major differences exist in the glucose and insulin abnormalities occurring in cats compared with horses, with low insulin concentrations relative to glucose concentrations detected in feline diabetes mellitus,[20] compared to hyperinsulinemia in the horse. These species differences are important because the ability of octreotide to suppress insulin secretion may be advantageous when managing ID in horses. New formulations of octreotide must be developed to lengthen the time that insulin secretion is suppressed after injection of octreotide, but this class of drugs warrants further investigation as strategies are developed for medically managing ID to prevent laminitis in severely affected horses.

One goal of this study was to begin the process of developing a new diagnostic test for ID in horses. It would be both time-consuming and expensive to collect multiple blood samples and plot glucose and insulin curves in order to calculate AUC values for every horse or pony screened for ID in clinical practice. Differences in glucose and insulin concentrations between groups approached statistical significance at 240 minute, and this was when insulin concentrations were above baseline while the horse regained glycemic control. A simpler test protocol might be developed in the future if the veterinarian administers octreotide and returns 4 hours later to collect a blood sample. If octreotide is confirmed to have a wide margin of safety in the horse, owners might be able to administer the drug via subcutaneous injection.

Limitations of this study included the low number of horses evaluated for each breed of horse and the selection of a single octreotide dosage level. Additional studies are required to investigate blood glucose and insulin

responses to octreotide in larger groups of horses, and different breeds of horse should be evaluated. The majority of animals in this study were Morgan horses, and horses of other breeds affected by EMS should be examined in the future. It is also important for octreotide to be administered at different dosage levels so that the optimal dose can be selected for development of a diagnostic test.

We conclude that intravenous injection of the SST analog octreotide causes plasma insulin concentrations to decrease in the horse and this is followed by an increase in blood glucose concentrations. Plasma insulin concentrations then increase above the pre-injection concentration as the effects of octreotide on insulin secretion wane and the horse regains glycemic control. An octreotide response test for detecting ID in horses might be developed in the future.

Footnotes

[a] Karo Light Corn Syrup, ACH Food Companies, Inc, Memphis, TN

[b] Equine Endocrinology Group 2016 Recommendations on diagnosis and management of equine metabolic syndrome; http://sites.tufts.edu/equineendogroup/

[c] Sandostatin®, Novartis Pharmaceuticals Corporation, East Hanover, NJ

[d] Animal Health Diagnostic Laboratory at Cornell University, Ithaca, NY

[e] Prism, version 6, GraphPad Software Inc, San Diego, CA.

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Conflict of Interest Declaration: Authors declare no conflict of interest.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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