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Glucagon, insulin, adrenocorticotropic hormone, and cortisol in response to carbohydrates and fasting in healthy neonatal foals

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Abstract

Background: The endocrine pancreas and hypothalamic-pituitary-adrenal axis (HPAA) are central to energy homeostasis, but information on their dynamics in response to energy challenges in healthy newborn foals is lacking.

Objectives: To evaluate glucagon, insulin, ACTH, and cortisol response to fasting and carbohydrate administration in healthy foals.

Animals: Twenty-two healthy Standardbred foals ≤4 days of age.

Methods: Foals were assigned to fasted (n = 6), IV glucose (IVGT; n = 5), PO glucose (OGT; n = 5), and PO lactose (OLT; n = 6) test groups. Blood samples were collected frequently for 210 minutes. Nursing was allowed from 180 to 210 minutes. Plasma glucagon, ACTH, serum insulin, and cortisol concentrations were measured using immunoassays.

Results: Plasma glucagon concentration decreased relative to baseline at 45, 90, and 180 minutes during the OLT (P = .03), but no differences occurred in other test groups. Nursing stimulated marked increases in plasma glucagon, serum insulin, and glucose concentrations in all test groups (P < .001). Plasma ACTH concentration increased relative to baseline at 180 minutes (P < .05) during fasting and OLT, but no differences occurred in other test groups. Serum cortisol concentration increased relative to baseline during OLT at 180 minutes (P = .04), but no differences occurred in other test groups. Nursing resulted in decreased plasma ACTH and serum cortisol concentrations in all test groups (P < .01).

Conclusions and Clinical Importance: The endocrine response to enterally and parenterally administered carbohydrates, including the major endocrine response to nursing, suggests that factors in milk other than carbohydrates are strong stimulators (directly or indirectly) of the endocrine pancreas and HPAA.

KEYWORDS
ACTH, cortisol, energy axis, glucagon, neonate

Abbreviations: DPP-4, dipeptidyl peptidase 4; GIP, gastric inhibitory polypeptide; GLP-1, glucagon-like peptide-1; HPAA, hypothalamic-pituitary-adrenal axis; IVGT, intravenous glucose test; OGT, oral glucose test; OLT, oral lactose test.

1 | INTRODUCTION

At birth, the neonatal foal undergoes many physiologic changes during transition from intrauterine to extrauterine life. In foals, the maturation of a number of endocrine systems occurs in late gestation and continues in the early postnatal period.[1-3] These adaptations are accompanied by changes in the hypothalamus-pituitary-adrenal axis (HPAA), energy metabolism, and cardiovascular system.[3-6] The endocrine pancreas must assume its glucoregulatory role after birth to maintain euglycemia,[7] as neonatal foals transition from intrauterine parenteral to extrauterine enteral nutrition.[8] The endocrine pancreas of the equine fetus is functionally mature before birth.[9,10] It can secrete insulin in response to glucose and amino acids, and is believed to be important in regulating fetal nutrient utilization and growth.[9] However, complete maturation of the endocrine system and associated energy metabolism in the neonate is delayed.[1,11-13] In addition, the neonatal foal has minimal hepatic fat stores, little glycogen, and little adipose tissue at birth.[2,3] Consequently, foals are highly dependent on milk intake and can rapidly develop hypoglycemia if caloric intake ceases. In these cases, nutrition must be supplied enterally or parenterally.

Although information exists regarding enteral and parenteral nutrition in critically ill neonatal foals, little is known about the endocrine pancreas and HPAA dynamic response to enteral and parenteral carbohydrates in healthy neonatal foals.[14] Developmental changes occur in both pancreatic alpha and beta cell function in foals during the first 10 days after parturition.[15] Additionally, the magnitude of the pancreatic endocrine response to various secretagogues varies between foals delivered spontaneously or by induction of labor at term.[4] In the equine fetus, blood cortisol concentrations increase before parturition and continue to increase immediately after birth.[5] Similar to other species, blood cortisol concentrations slowly decrease over a few days post-parturition and then stabilize, remaining similar to adult concentrations.[6,16] No information however exists regarding the HPAA response to enteral and parenteral carbohydrates or fasting in neonatal foals.

Human infants have different metabolic responses to breast milk and lactose-based formulas compared to syrup-based formulas.[17] People fed large carbohydrate and fat-rich meals have increased postprandial serum insulin concentrations compared to those fed small carbohydrate-rich meals.[18] Few studies have assessed the endocrine and gastrointestinal response to various types of nutrition in foals.[4,11,14,19] A recent study found that healthy neonatal foals have a functional enteroinsular axis that responds to enteral and parenteral carbohydrate administration and fasting, and the enteroinsular axis (insulin, glucose, glucose-dependent insulinotropic polypeptide [GIP], and glucagon-like peptide 1 [GLP-1]) was more responsive to milk and lactose compared to glucose administration.[14]

To our knowledge, glucagon, ACTH, and cortisol concentrations have not been assessed in healthy neonatal foals in response to enteral or parenteral carbohydrates and fasting. Insulin, however, was investigated in a recent study.[14] To better understand neonatal endocrine disturbances and energy axis regulation in the horse, we sought to evaluate pancreatic endocrine factors and their relationship to the HPAA in response to nursing in healthy newborn foals. We hypothesized that plasma glucagon concentrations would increase over time in fasted foals, but decrease in response to PO dextrose, IV dextrose, PO lactose, and nursing. Based on the concurrent increase in serum insulin concentrations in healthy foals[14] observed in response to nursing, we hypothesized that plasma ACTH and serum cortisol concentrations would increase in response to treatment interventions (ie, stress of prolonged fasting followed by vigorous nursing).

2 | MATERIALS AND METHODS

2.1 | Experimental design

Our study was performed using archived plasma and serum samples from a previous study investigating the enteroinsular axis in neonatal foals.[14] Experimental design was described previously.[14] Briefly, 22 Standardbred foals, 24 to 96 hours of age, and owned by a private breeding farm, were included in our study. Foals were considered healthy based on physical examination, normal CBC results, and serum immunoglobulin G (IgG) concentration >800 mg/dL. Sample collection and testing took place over 2 foaling seasons (2017 and 2018). Each foal was randomly assigned to a PO glucose tolerance test (OGT; n = 5), an IV glucose tolerance test (IVGT; n = 5), a PO lactose test (OLT; n = 6), or a fasted (n = 6) experimental group. Foals remained confined to stalls with their mares during sampling.

At 60 minutes before initiation of the experimental protocol, foals were muzzled and an IV catheter was placed in the jugular vein. Foals were manually restrained, and sedation was not employed. Foals were muzzled for 240 minutes (−60 to 180 minutes).

Foals that had an OGT were given a 50% dextrose solution (VetOne, MWI Animal Health, Boise, Idaho) PO at a dosage of 1000 mg/kg (n = 5) by syringe over 1 minute. Foals in the IVGT group were given a 50% dextrose solution (VetOne, MWI Animal Health, Boise, Idaho) IV at a dosage of 1000 mg/kg (n = 5) over 1 minute. Foals in the OLT group were given lactose at a dosage of 1000 mg/kg (Millipore Sigma, St. Louis, Missouri) as a 20% solution in water (n = 6) by nasogastric intubation over 1 minute. Foals in the fasted group (n = 6) remained muzzled and did not receive any treatments for the duration of the study (240 minutes).

Blood samples were collected at time 0 (before carbohydrate administration) and at 15, 45, 90, and 180 minutes. After 180 minutes, foals were unmuzzled and allowed to nurse from the mare ad libitum. Additional samples were collected in most foals at 210 minutes. Blood samples (8 mL) were placed in serum tubes and pre-chilled EDTA tubes containing aprotonin (GoldBio, St Louis, Missouri) and diprotin A (Bachem, Torrance, California). Aprotinin (500 kU/mL of blood) was added to inhibit protease-mediated degradation of peptide hormones and diprotin A (50 μmol/mL of blood) is a dipeptidyl peptidase-4 (DPP-4) protease inhibitor added to minimize degradation of GIP, GLP-1, and other peptides.[14] Blood samples were allowed to clot and centrifuged at 1000*g* for 10 minutes at 4°C. Plasma and serum samples were aliquoted and stored at −80°C until analysis.

TABLE 1 Median and interquartile range (IQR) data for blood hormone concentrations in neonatal foals based on treatment group. * indicates statistical significance ($P < .05$) from baseline

		0	15	45	90	180
Glucagon (pg/mL)	Fasted	181.7 (94.4-197.5)	122.3 (88.0-192.4)	120.6 (86.7-236.9)	163.3 (86.0-207.8)	147.4 (82.7-209.3)
	OLT	186 (106.3-236.4)	125.2 (89.8-252.4)	93.3 (76.9-174.8)	101.1 (78.4-129.5)*	99.8 (86.7-110.3)
	OGT	124 (82.2-139.0)	114 (94.4-177.3)	96.5 (75.0-143.7)	67.1 (55.6-85.9)	103.7 (74.7-158.2)
	IVGT	127.4 (59.4-173.1)	73.7 (53.4-164.2)	90.8 (57.6-251.1)	93.7 (53.6-119.8)	132.5 (60.5-210.8)
ACTH (pg/mL)	Fasted	21.3 (17.6-27.4)	26.1 (21.6-36.1)	33.6 (20.0-47.9)	27.2 (21.1-46.0)	30.4 (23.4-38.3)*
	OLT	32.4 (21.0-46.2)	35.9 (22.6-57.5)	34.4 (19.0-41.0)	31.9 (25.3-46.7)	44.4 (28.1-63.9)*
	OGT	37.2 (19.4-50.4)	35.3 (22.7-64.0)	41.5 (25.9-60.9)	28.2 (23.5-38.1)	35.0 (27.4-41.1)
	IVGT	36.2 (28.0-48.7)	28.3 (24.8-59.5)	24.9 (21.4-34.7)	41.2 (27.4-46.0)	46.3 (41.3-52.7)
Cortisol (μg/dL)	Fasted	0.48 (0.4-1.1)	0.9 (0.3-2.2)	1.4 (0.8-2.2)	1.1 (0.5-2.4)	1.2 (0.6-2.0)
	OLT	0.8 (0.2-1.2)	1.4 (0.5-1.8)	0.6 (0.6-0.8)	0.4 (0.0-0.8)	1.3 (0.8-2.6)*
	OGT	1.5 (0.5-2.1)	0.8 (0.5-2.0)	1.9 (1.1-2.3)	0.9 (0.6-2.8)	1.5 (1.0-2.6)
	IVGT	0.9 (0.6-2.3)	0.7 (0.5-1.3)	0.2 (0.2-0.4)	0.6 (0.3-0.7)	1.2 (0.8-1.7)
Glucose (mg/dL)	Fasted	139.5 (125.0-145.3)	130.5 (117.0-135.3)	114.5 (104.3-125.3)	111.0 (101.0-125.5)*	108.0 (83.8-122.0)*
	OLT	126.5 (119.8-161.5)	168.5 (151.0-194.0)	161.0 (154.0-218.8)*	143.5 (117.0-186.3)	118.0 (111.0-121.0)
	OGT	139.0 (122.5-155.5)	150.0 (139.5-163.0)	165.0 (147.0-188.5)	167.0 (135.0-204.0)	124.0 (96.0-142.5)
	IVGT	137.0 (105.5-154.0)	297.0 (153.0-315.0)	138.0 (135.5-145.8)	118.0 (98.0-137.0)	120.0 (113.0-142.0)
Insulin (μIU/mL)	Fasted	18.3 (10.4-53.3)	9.6 (5.7-20.4)	9.8 (8.5-20.1)	5.4 (3.4-10.6)	6.0 (3.5-7.6)
	OLT	11.6 (9.8-12.2)	46.1 (18.3-81.2)	26.9 (22.2-35.8)*	16.7 (10.0-26.9)	12.4 (8.4-13.3)
	OGT	6.9 (2.6-8.8)	16.8 (5.2-24.7)*	8.8 (4.6-16.7)	10.5 (6.8-15.3)	2.1 (1.0-16.2)
	IVGT	4.3 (0.9-14.3)	39.8 (17.4-63.2)	4.4 (2.6-8.2)	1.1 (0.2-2.2)	3.4 (1.2-8.9)

Abbreviations: IVGT, intravenous glucose test; OGT, PO glucose test; OLT, PO lactose test.

This study was approved by The Ohio State University Institutional Animal Care and Use Committee and adhered to the principles of humane treatment of animals in veterinary clinical investigations as stated by the American College of Veterinary Internal Medicine and National Institute of Health guidelines.

2.2 | Sample analysis

Plasma glucagon concentrations were measured using radioimmunoassay (EMD Millipore, St. Louis, Missouri), as described previously in equids.[20,21] The standard range and sensitivity of the assay are 25-400 and 18.45 pg/mL, respectively. Intra- and interassay coefficients of variation for glucagon were <7.7%. Serum cortisol concentrations were measured using a radioimmunoassay previously validated for use in horses.[22,23] Plasma ACTH concentrations were measured using a human-specific chemiluminescent assay previously validated for horses.[24] Serum insulin concentrations were measured using a human-specific ELISA (MP Biomedicals, Solon, Ohio), as described previously.[25]

2.3 | Data analysis

Data were tested for normality using the Shapiro-Wilk normality test and were not normally distributed, and therefore nonparametric methods were used. Descriptive statistics are reported as median and interquartile range (IQR). Results are reported as concentrations or as percentage change from baseline (time 0). Percentage change[14,26] was calculated as % change = $([T_X - T_{baseline}]/T_{baseline})\times 100$, where T = time point and X = time 0, 15, 45, 90, 180, or 210. All time points were compared using Friedman's test. Times before and after nursing (180 vs 210 minutes) were compared using the Wilcoxon signed rank test. Statistical analysis was performed using SPSS 26 (IBM, Armonk, New York) and Prism 8.0 (GraphPad Software Inc, San Diego, California). Statistical significance was set at $P < .05$.

3 | RESULTS

3.1 | Glucagon

No differences in plasma glucagon concentrations were observed from baseline to 180 minutes in the fasted, OGT, or IVGT groups (Table 1), but in the OLT group, plasma glucagon concentrations decreased from baseline to 45 minutes and remained low until 180 minutes ($P < .03$). When percentage change from baseline was calculated, no differences were observed in the fasted, OGT, or IVGT groups. A significant decrease in percentage change was observed from baseline to 90 minutes in the OLT group (Figure 1B; $P = .03$). Although changes in glucagon were observed in response to OLT, nursing resulted in a 3- to 4-fold increase in plasma glucagon concentrations (raw data and percentage changes) within 30 minutes in all groups after foals were allowed to nurse (Figure 2A; Table 2; $P < .001$).

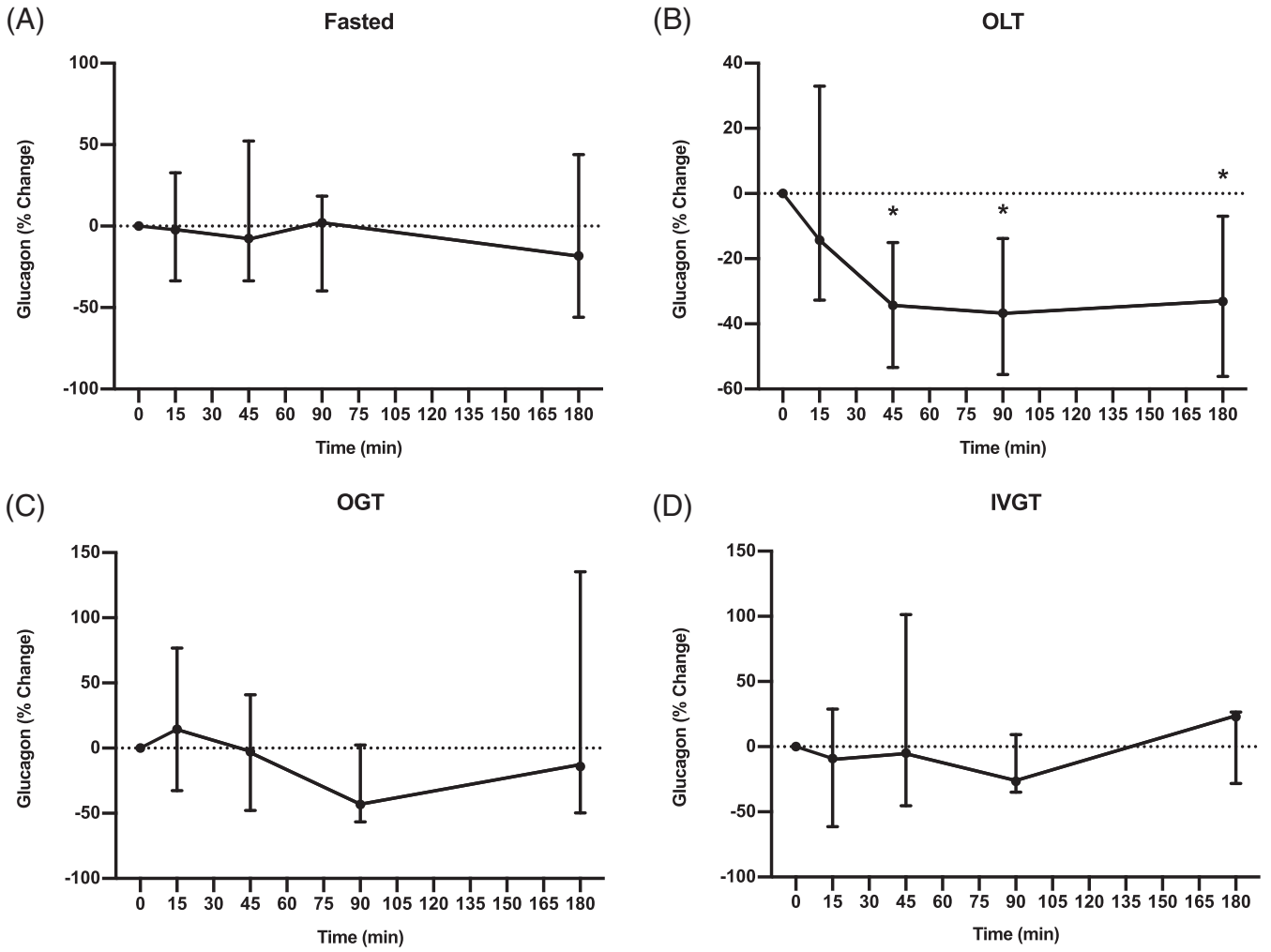

FIGURE 1 Glucagon concentrations expressed as percent change from baseline (time 0) in response to fasting (A), PO lactose (B), PO dextrose (C), and IV dextrose (D). Values expressed as medians with range. * indicates statistical significance ($P < .05$) from baseline. IVGT, IV glucose test; OGT, PO glucose test; OLT, PO lactose test

3.2 | Adrenocorticotropic hormone

No differences in plasma ACTH concentrations were observed in the fasting, OGT, or IVGT groups. However, plasma ACTH concentrations at 180 minutes were increased relative to baseline in the OLT group ($P = .008$). The fasting and OLT treatment groups had significant increases in percentage change at 180 minutes relative to baseline (Figures 3A,B; $P < .05$). No differences were observed in ACTH percentage change from baseline to 180 minutes in the OGT and IVGT groups. Nursing resulted in a decrease in plasma ACTH concentrations (raw data and percentage changes) in all groups (Figure 2B; Table 2; $P < .002$).

3.3 | Cortisol

No differences in serum cortisol concentrations were observed in the fasted, OLT, OGT, and IVGT groups from baseline to 180 minutes. When percentage change was calculated, no differences were observed from baseline to 180 minutes in the fasted, OGT, and IVGT groups. However, administration of lactose resulted in a significant increase in percentage change at 180 minutes relative to baseline (Figure 4B; $P = .04$). Nursing led to a significant decrease in serum cortisol concentrations (raw data and percentage changes) in all groups (Figure 2C; Table 2; $P < .001$).

3.4 | Insulin and glucose

Serum insulin and glucose concentrations (Table 1) have been reported previously.[14] Minimal to no change in serum glucose concentrations were observed in response to OGT, whereas minor increases were observed in response to OLT, and larger increases in serum glucose concentration were observed in response to IVGT. Fasted foals consistently exhibited decreases in serum glucose concentration from baseline, but foals maintained euglycemia throughout the 4-hour treatment period (Figure 5). Minimal to no changes were reported in serum insulin concentrations in response to OGT, minor increases in serum insulin concentration were observed in response to OLT, and

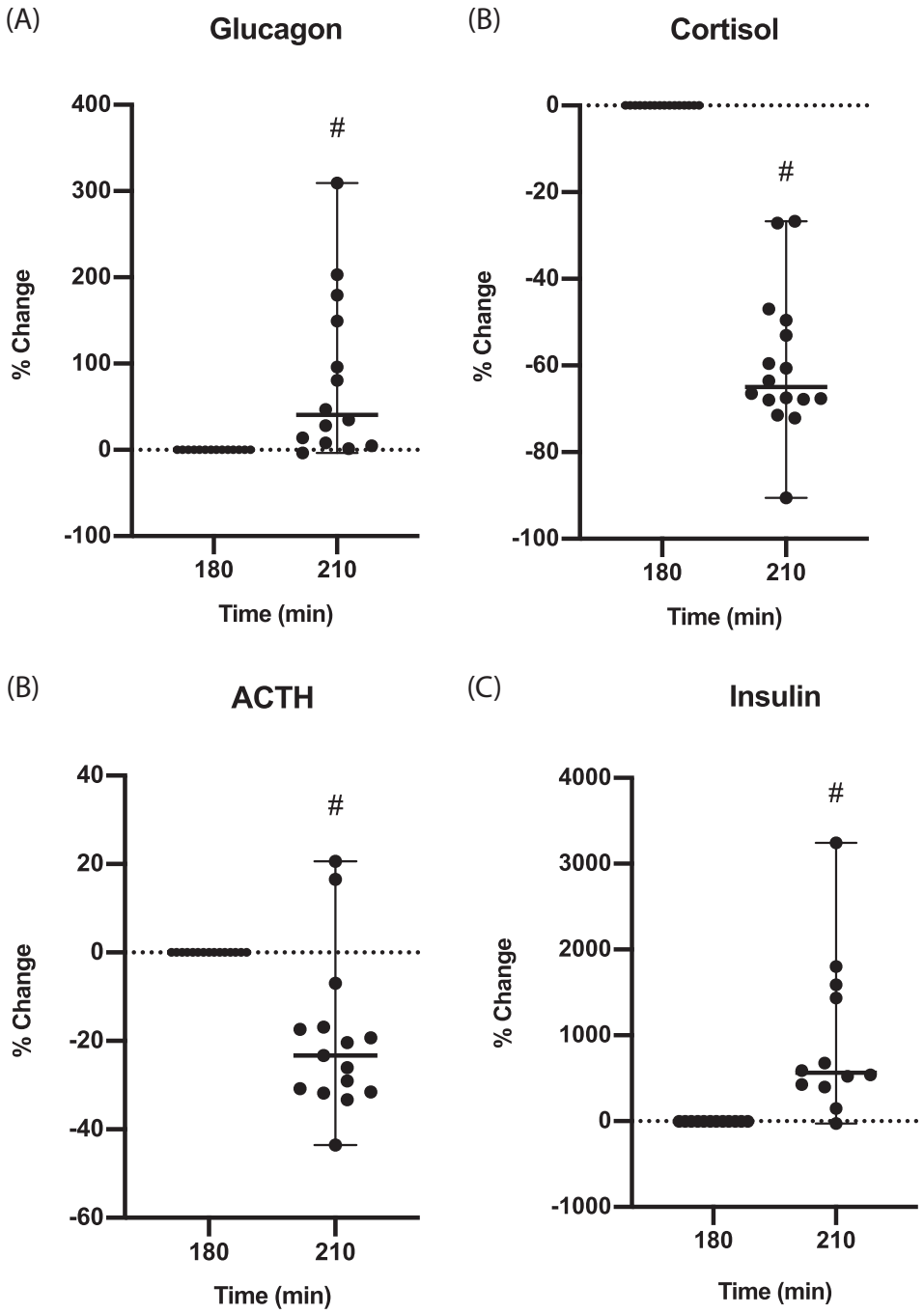

FIGURE 2 Glucagon (A), ACTH (B), cortisol (C), and insulin (D) concentrations expressed as percentage change from time point 180 in response to nursing. # indicates statistical significance ($P \leq .05$) from baseline

significant increases in serum insulin concentration were observed in response to IVGT (Figure 6).

4 | DISCUSSION

We showed that fasting and PO carbohydrate administration had minimal effects on glucagon secretion in healthy newborn foals. Nursing elicited a rapid and strong glucagon response, while suppressing ACTH and cortisol secretion. These findings were contrary to our hypothesis, in which an increase in plasma glucagon concentration was expected in response to fasting so as to maintain euglycemia by glycogenolysis and gluconeogenesis. However, based on previous data,[14] fasted foals were able to maintain euglycemia throughout the 180-minute time period. Based on this study, in which serum glucose concentrations did not change or decreased by ≤20% from baseline in fasted foals (ie, remained in the normoglycemic range), we suspect there was not a major hypoglycemic stimulus to induce glucagon secretion in response to fasting.

Although minor decreases in plasma glucagon concentration were observed in response to enterally administered lactose, nursing provoked a 3- to 4-fold increase in plasma glucagon concentrations within 30 minutes after nursing. This increase in plasma glucagon concentrations in response to nursing was unexpected and, to our

knowledge, not previously reported in foals or horses. This finding also is intriguing because it was recently shown that immediately after nursing, healthy foals exhibited hyperglycemia that was followed by hyperinsulinemia.[14] The fact that insulin and glucagon followed a similar secretion pattern after milk ingestion in newborn foals indicates that unique aspects of endocrine pancreas biology remain to be explored occur in the immediate neonatal period because our results are inconsistent with current understanding of these hormones. In human medicine, individuals with insulin resistance respond to meals with an increase in plasma glucagon concentration.[27,28] These observations suggest that neonatal foals secrete glucagon in response to milk in a fashion that may reflect gastrointestinal and islet maturation within the first days of life, and challenge our current understanding of the equine endocrine pancreas. The equine fetus has been shown to secrete glucagon in response to infusion of arginine,[29] and newborn pony foals are able to secrete insulin and glucagon in response to administration of arginine.[4] This glucagon response in equine neonates could reflect an adaptation to increased energy demands immediately after birth, considering that foals are born with minimal energy reserves compared to other species.[30] Human infants have positive correlations in glycemia and glucagon secretion after infusion of amino acids.[31] In addition, studies in sheep have shown increases in

TABLE 2 Median and interquartile range (IQR) data for blood hormone concentrations in response to nursing for all foals. * indicates statistical significance ($P < .05$) from time 180

	180	210
Glucagon (pg/mL)	171.1 (111.1-261.6)	442.8 (185.6-512.9)*
ACTH (pg/mL)	33.8 (24.9-48.7)	26.9 (20.6-36.5)*
Cortisol (μg/dL)	1.2 (0.8-2.0)	0.6 (0.4-0.9)*
Glucose (mg/dL)	118.5 (99.5-121.8)	221.5 (179.0-253.8)*
Insulin (μIU/mL)	7.7 (5.1-12.5)	50.8 (24.6-73.4)*

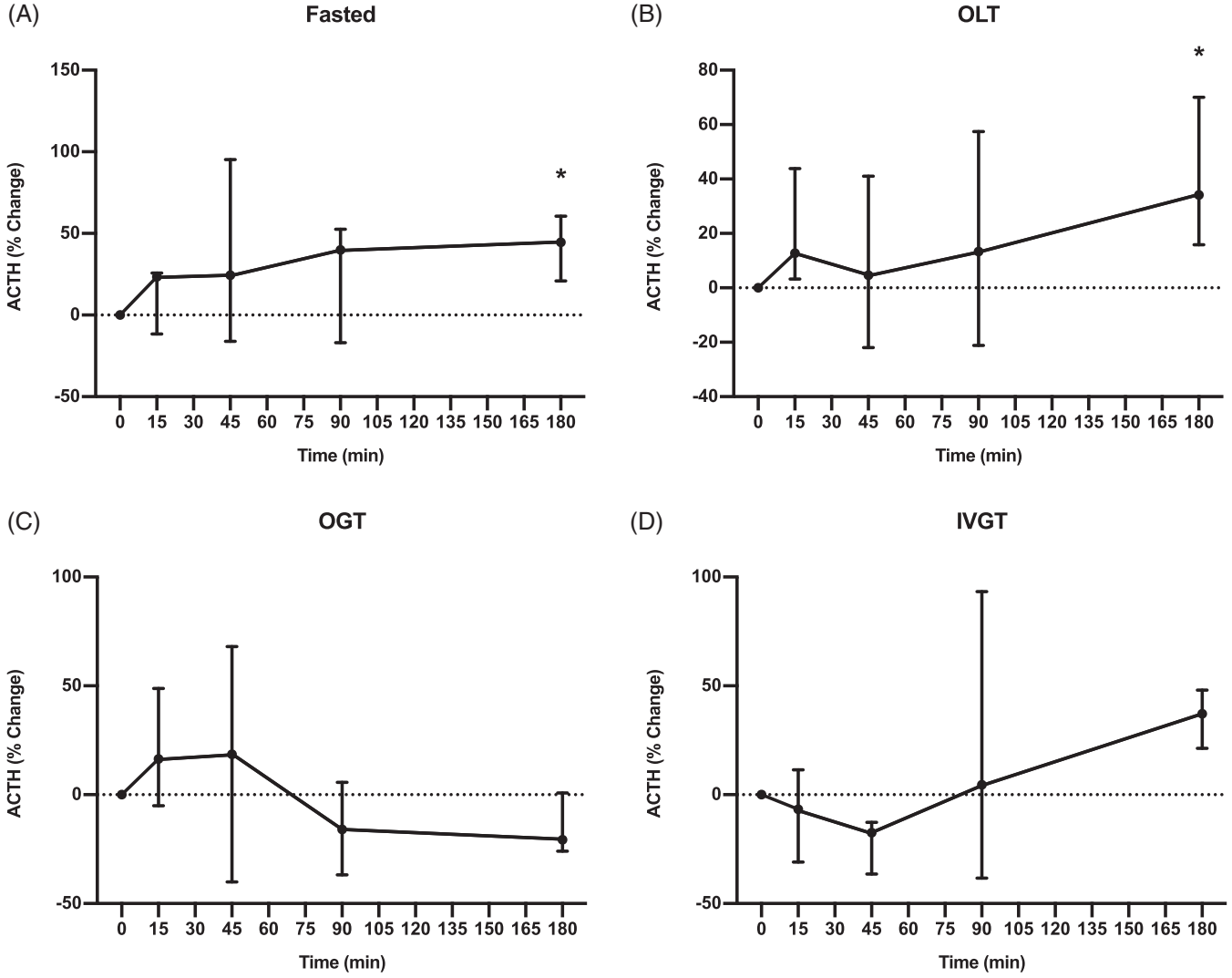

FIGURE 3 ACTH concentrations expressed as percent change from baseline (time 0) in response to fasting (A), PO lactose (B), PO dextrose (C), and IV dextrose (D). Values expressed as medians with ranges. * indicates statistical significance ($P < .05$) from baseline. IVGT, IV glucose test; OGT, PO glucose test; OLT, PO lactose test

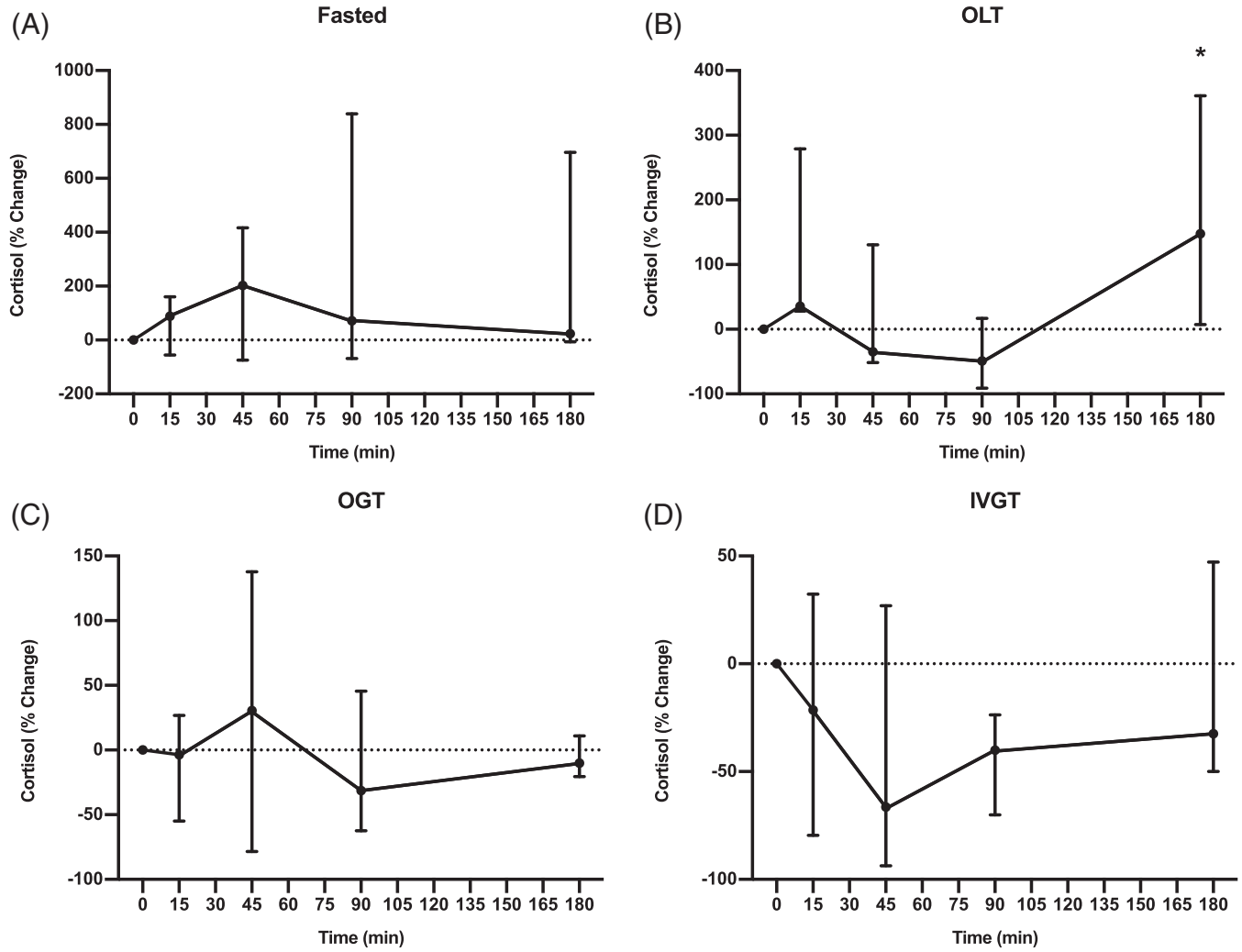

FIGURE 4 Serum cortisol concentrations expressed as percent change from baseline (time 0) in response to fasting (A), PO lactose (B), PO dextrose (C), and IV dextrose (D). Values expressed as medians with ranges. * indicates statistical significance ($P < .05$) from baseline. IVGT, IV glucose test; OGT, PO glucose test; OLT, PO lactose test

insulin and glucagon concentrations in response to infusion of fatty acids.[32] These studies suggest that this secretion pattern may reflect interactions among different endocrine systems, including incretins, glucocorticoids, growth hormone, catecholamines, and other factors yet to be determined.

Various studies have suggested that protein ingestion, resulting in increases in plasma concentrations of essential and nonessential amino acids, promotes insulin and glucagon secretion.[27,33,34] The literature also reports compensatory increases in energy intake after a period of caloric restriction, despite PO administration of glucose, as opposed to nonfasted individuals.[35] This finding suggests that proteins, fatty acids, or other components of mare's milk could have stimulated or enhanced glucagon secretion when the foals in our study nursed. Future investigations to further evaluate mechanisms involved in the hyperglucagonemic response to nursing observed in our study should include identifying which components of mare's milk have the ability to stimulate the endocrine pancreas, and assessment of the energy axis in the healthy foal in response to administration of various amino acids, such as L-arginine. Another potential explanation for

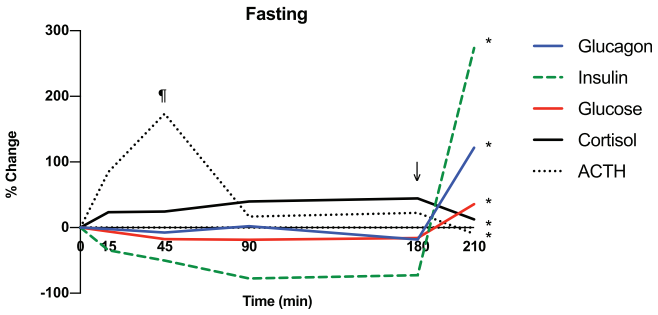

FIGURE 5 Glucagon, insulin, glucose, ACTH, and cortisol concentrations expressed as median percentage change from baseline in fasted foals. Insulin and glucose data previously reported.[14] ↓ indicates time point at which foals were allowed free-choice nursing. ¶ indicates statistical significance from baseline (time 0). * indicates statistical significance from time 180 ($P < .05$)

increased glucagon release is pancreatic sympathetic activation from hunger and stress, because glucagon secretion may occur from physiologic stress without a hypoglycemic stimulus.[36,37] Although

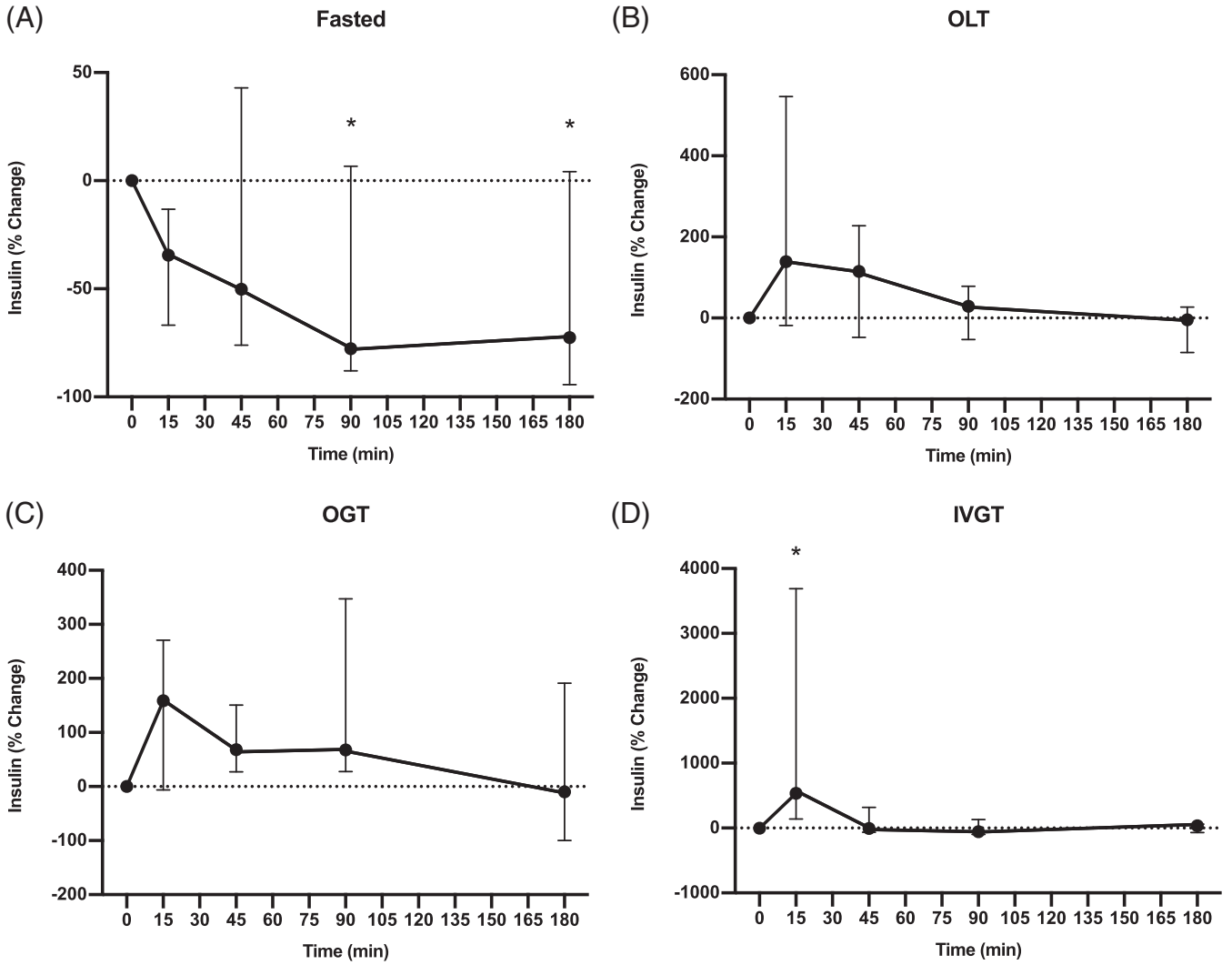

FIGURE 6 Serum insulin concentrations, reported previously,[25] expressed as percentage change from baseline (time 0) in response to fasting (A), PO lactose (B), PO dextrose (C), and IV dextrose (D). Values expressed as medians with ranges. * indicates statistical significance ($P < .05$) from baseline. IVGT, IV glucose test; OGT, PO glucose test; OLT, PO lactose test

catecholamines were not measured, elements of the HPAA (ACTH and cortisol) decreased in response to nursing in our study, making glucagon secretion in response to sympathetic activation less likely.

The increases in plasma ACTH concentrations at 180 minutes compared to baseline in fasted foals likely occurred because of hunger, stress of fasting, and inability to nurse while muzzled. In the lactose group, plasma ACTH concentrations also increased and also were associated with a significant rise in serum cortisol concentrations. The stress of passing the nasogastric tube could have contributed to ACTH and cortisol release in the OLT group. Additionally, as proposed previously,[14] lactose itself could have stimulatory effects on enteric cells, because the glucose and insulin response to lactose was higher than an equivalent dose of dextrose.

Contrary to our hypothesis, nursing provoked marked decreases in plasma ACTH and serum cortisol concentrations for all treatment groups. The decrease in ACTH and cortisol concentrations could be the result of a negative feedback mechanism in response to increased blood glucose concentrations.[14] Conceivably, it also could be the result of the ability to nurse ad libitum, because removal of the stressful stimulus (muzzling and inability to nurse) also eliminated the stimulus for ACTH and cortisol secretion. Although decreased secretion of ACTH and cortisol in response to nursing could be the result of a negative feedback loop, the observed decreases in these hormones remain confounding given the increased concentrations observed for insulin and glucagon. This secretion pattern has not been reported in the literature to our knowledge. Additional sampling would allow for prolonged observation of the secretion pattern for ACTH and cortisol and could provide additional information regarding the HPAA and endocrine pancreas in neonatal foals.

Although our study reports novel findings regarding the HPAA and endocrine pancreas in the neonatal foal, it is not without limitations. First, the number of foals per group was small, which increases the likelihood of type II statistical error, and could lead to misinterpretation of results. Although type II statistical error is possible,

significant and consistent increases still were observed after nursing, even with a small number of foals. Although our study was underpowered, more evident differences would have been expected with larger sample sizes. As such, the significant changes in hormone concentrations observed in response to dextrose, lactose, and nursing remain major findings. These results indicate that our knowledge on the biology of the endocrine pancreas in the equine neonate is limited, and extrapolation from other species or older equids may not apply, warranting additional studies to better understand these phenomena.

Second, although all foals were muzzled 60 minutes before the baseline time point (time 0), it was not recorded when the foals last suckled before muzzling. This could have predisposed to variability in hormone concentrations at the baseline time points. We expect this variation to be minimal based on the high frequency of nursing in this age group,[38] but to address this issue, the data was presented as percentage change from baseline. The wide age range of foals (24-96 hours) is another limitation of the study, because ACTH and cortisol concentrations have been shown to change dynamically over the first days of life in the neonatal foal.[5,12,39] The different methods of administration of PO dextrose (oral syringe) and PO lactose (nasogastric intubation) also could lead to variability in results, as discussed previously. However, nasogastric intubation was used for the lactose treatment group so as to administer a large quantity of solution (approximately 1 L).

Lastly, it has been suggested that ACTH[40,41] and cortisol[42] recovery decreases minimally with storage time and number of freeze-thaw cycles. Studies evaluating the stability of glucagon in humans suggest it is a very stable hormone, with minimal changes at room temperature for 3 days[43] and 5 days at 4°C.[44] The recovery of glucagon also has been shown to improve with the addition of EDTA/aprotinin[43,45] and DPP-IV inhibitors.[45] To our knowledge, no study has assessed the effect of long-term storage or freeze-thaw cycles on equine serum or plasma. In an ongoing study in our laboratory, foals fasted for a similar period of time also exhibited a decrease in plasma ACTH concentrations after nursing. The plasma ACTH concentrations were similar to the concentrations reported in the present study (HK, personal communication). Although long-term storage could have influenced our results, the effect of time and sample processing applied to all samples, likely influencing endocrine factors in a similar way, and therefore trends within foals should remain valid.

To our knowledge, ours is the first study to integrate factors of the HPAA and glucoregulatory components of the endocrine pancreas in healthy neonatal foals. Minor changes in theses hormones were observed with PO carbohydrate challenges, which were more evident in response to nursing. Our results and those of previous studies from our laboratory[14] support that the endocrine pancreas and HPAA are functional in newborn foals. The strong endocrine response to milk compared with other endocrine factors measured indicates that other yet to be elucidated endocrine stimulatory secretagogues are present in mare's milk, and their identification could enhance our understanding of energy metabolism, gastrointestinal physiology, and pancreatic biology in equids.

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CONFLICT OF INTEREST DECLARATION

Authors declared no potential conflicts of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by The Ohio State University IACUC.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

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