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Effect of dietary carbohydrates and time of year on ACTH and cortisol concentrations in adult and aged horses

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ABSTRACT

Diagnosis of equine pituitary pars intermedia dysfunction (PPID) remains a challenge as multiple factors (stress, exercise, and time of year) influence ACTH and cortisol concentrations. To assess endocrine status in a study designed to evaluate the effects of age and diet on glucose and insulin dynamics, we performed thyrotropin-releasing hormone (TRH) stimulation tests and overnight dexamethasone suppression tests in March, May, August, and October on 16 healthy Thoroughbred and Standardbred mares and geldings. Horses were grouped by age: adult (mean ± SD; 8.8 ± 2.9 yr; n = 8) and aged (20.6 ± 2.1 yr; n = 8). None of the horses showed clinical signs (hypertrichosis, regional adiposity, skeletal muscle atrophy, lethargy) of pituitary pars intermedia dysfunction. Horses were randomly assigned to groups of 4, blocked for age, and fed grass hay plus 4 isocaloric concentrate diets (control, starch-rich, fiber-rich, and sugar-rich) using a balanced Latin square design. Data were analyzed using a multivariable linear mixed regression model. Baseline ACTH was significantly higher in aged horses (mean ± standard error of the mean; 60.0 ± 10.7 pg/mL) adapted to the starch-rich diet compared to adult horses (15.7 ± 12.0 pg/mL) on the same diet ($P = 0.017$). After controlling for age and diet, baseline ACTH concentrations were significantly increased in October (57.7 ± 7.1 pg/mL) compared to March (13.2 ± 7.1 pg/mL; $P < 0.001$), May (12.4 ± 7.1 pg/mL; $P < 0.001$), and August (24.2 ± 7.1 pg/mL; $P < 0.001$), whereas post-TRH ACTH was higher in August (376.6 ± 57.6 pg/mL) and October (370.9 ± 57.5 pg/mL) compared to March (101.9 ± 57.3 pg/mL; $P < 0.001$) and May (74.5 ± 57.1 pg/mL; $P < 0.001$). Aged horses had significantly higher post-dexamethasone cortisol on the starch-rich diet (0.6 ± 0.1 μg/dL) compared to the sugar-rich diet (0.2 ± 0.1 μg/dL; $P = 0.021$). Post-dexamethasone cortisol was significantly higher in October (0.6 ± 0.1 μg/dL) compared to March (0.3 ± 0.1 μg/dL; $P = 0.005$), May (0.2 ± 0.1 μg/dL; $P < 0.001$), and August (0.3 ± 0.1 μg/dL; $P = 0.004$). Breed did not influence ACTH or cortisol measurements. In conclusion, in addition to age and time of year, diet is a potential confounder as animals on a starch-rich diet may be incorrectly diagnosed with pituitary pars intermedia dysfunction.

1. Introduction

Pituitary pars intermedia dysfunction (PPID) is the most common endocrine disorder of older horses, yet definitive diagnosis remains a challenge. Measurement of plasma ACTH concentrations is a commonly used diagnostic test;

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Table 1
Key nutrients for each dietary (grass hay + concentrate) profile on a dry matter basis (Dairy One, Ithaca, NY).

Diet	% Crude protein	% NDF[a]	% Starch	% Sugar	% NSC[b]
Control	12.4	54.8	5.1	9.4	14.4
Starch	11.1	48.1	15.7	8.8	24.5
Fiber	11.0	55.4	2.9	9.7	12.5
Sugar	10.7	50.3	4.0	18.7	22.7

[a] NDF (neutral detergent fiber) = hemicellulose, cellulose, and lignin.
[b] NSC (nonstructural carbohydrate) = starch + water soluble carbohydrates (monosaccharides, disaccharides, and polysaccharides).

however, ACTH concentrations are influenced by multiple factors such as stress [1], feeding status (fasted vs fed) [2,3], exercise [4,5], and time of year [6–9]. Therefore, several dynamic tests have been proposed to evaluate endocrine responses in older horses. Measurement of ACTH concentration at baseline (>35 pg/mL (Animal Health Diagnostic Center at Cornell University)) and at 10 min (>110 pg/mL (Animal Health Diagnostic Center at Cornell University)), following administration of thyrotropin-releasing hormone (TRH), is widely used for the diagnosis of PPID. In cases where clinicians suspect early PPID the TRH stimulation test is recommended [10]. However, from July to October, clinically normal horses demonstrate increased ACTH concentrations following administration of TRH [7,8,11]. Furthermore, there is variability in ACTH laboratory reference ranges complicating interpretation of the test results. Adequate reference ranges, especially in clinically normal animals, need to be established.

Similar to the TRH stimulation test, cortisol measurement at baseline (>6 μg/dL; Animal Health Diagnostic Center at Cornell University) and following an overnight dexamethasone suppression test (>1 μg/dL; Animal Health Diagnostic Center at Cornell University), suffers from variability due to the time of year [6,11]. The overnight dexamethasone suppression test also looks less favorable due to concerns about the development of laminitis following corticosteroid administration although evidence is limited [12,13] and that a minimal number of PPID animals may demonstrate hyperadrenocorticism. Similar to ACTH concentrations, it is recommended that cortisol concentrations are interpreted with caution and in conjunction with the animal's clinical signs.

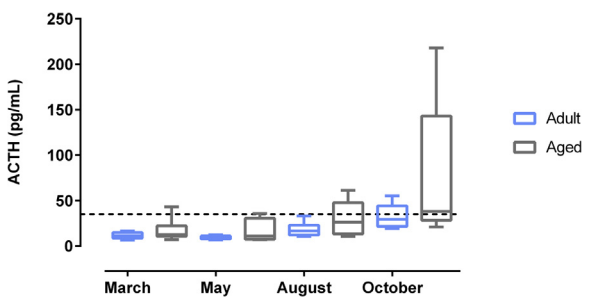

Fig. 1. Box and whisker plots of clinical laboratory baseline ACTH concentrations in adult and aged horses at different times of the year. The solid horizontal line represents the median, box indicates interquartile range, and bars indicate the range of values. The dashed horizontal line represents the laboratory cutoff (35 pg/mL) for diagnosis of pituitary pars intermedia dysfunction.

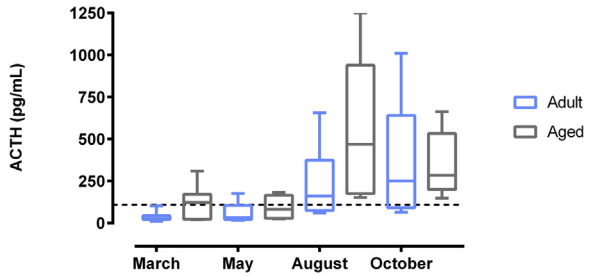

Fig. 2. Box and whisker plots of clinical laboratory ACTH concentrations, at 10 min, following administration of TRH in adult and aged horses at different times of the year. The solid horizontal line represents the median, box indicates interquartile range, and bars indicate the range of values. The dashed horizontal line represents the laboratory cutoff (110 pg/mL) for diagnosis of pituitary pars intermedia dysfunction.

As part of a study to evaluate the effects of dietary carbohydrates on glucose and insulin dynamics in healthy adult and aged horses, we performed TRH stimulation tests and overnight dexamethasone suppression tests in a group of 16 healthy horses consuming 4 different diets at 4 different times of the year. Our data demonstrate that ACTH and cortisol concentrations in these horses vary due to both diet and time of year. To the authors' knowledge, although a small sample size, this is the first study to demonstrate an effect of diet on ACTH and cortisol concentrations as well as TRH stimulation and dexamethasone suppression tests.

2. Materials and methods

Data presented in this manuscript were collected as part of a study designed to evaluate the effects of age and dietary adaptation to diets with varying carbohydrate composition [14]. The study was conducted from February to October. Endocrine testing was performed in March, May, August, and October. All methods were approved by the Institutional Animal Care and Use Committee (IACUC) at Michigan State University.

2.1. Horses and groups

Sixteen healthy Thoroughbred (TB) and Standardbred (STB) mares and geldings were divided into 2 groups by age: adult (5–13 yr; 8.8 ± 2.9 yr; n = 9; 4 TB mares, 1 STB mare, 3 TB geldings, 1 STB gelding) and aged (18–24 yr; 20.6 ± 2.1 yr; n = 9; 3 TB mares, 6 STB mares). One horse had to be replaced in each age group due to failure to eat the diet

Table 2
Least squares means estimates and pairwise significant differences ($P \leq 0.05$) for ACTH concentrations (pg/mL) from a TRH stimulation test after controlling for diet, time of year, and breed.

Time point	Adult		Aged		*P* value
	Least squares means	95% confidence interval	Least squares means	95% confidence interval	
Baseline	14.6	(−1.09–30.1)	39.6	(24.4–54.9)	0.04
Post-TRH	141.0	(10.4–271.0)	321.0	(197.2–446.0)	0.06

TRH, thyrotropin-releasing hormone.