Seasonal variation in results of diagnostic tests for pituitary pars intermedia dysfunction in older, clinically normal geldings

Christopher M. Schreiber, DVM; Allison J. Stewart, BVSc, MS, DACVIM, DACVECC; Eddy Kwessi, PhD; Ellen N. Behrend, VMD, PhD, DACVIM; James C. Wright, DVM, PhD, DACVP; Robert J. Kemppainen, DVM, PhD; Katherine A. Busch, DVM

Objective—To determine whether seasonal variations exist in endogenous plasma ACTH, plasma α-melanocyte-stimulating hormone (α-MSH), serum cortisol, and serum insulin concentrations and in the results of a dexamethasone suppression test for older, clinically normal geldings in Alabama.

Design—Cohort study.

Animals—15 healthy mixed-bred geldings (median age, 14 years).

Procedures—Sample collection was repeated monthly for 12 months. Dexamethasone (0.04 mg/kg [0.02 mg/lb], IM) was administered and cortisol concentrations were determined at 15 and 19 hours. Radioimmunoassays were used to measure ACTH, α-MSH, cortisol, and insulin concentrations at each testing time. Hormone concentrations were compared between months via repeated-measures ANOVA and correlated with age within each month.

Results—A significant time effect was found between months for α-MSH and insulin concentrations. Endogenous cortisol and ACTH concentrations remained within existing reference ranges. Significant correlations were detected between age and ACTH concentration for several fall and winter months and between age and insulin concentration for September.

Conclusions and Clinical Relevance—Older horses have higher ACTH concentrations in several fall and winter months and higher insulin concentrations in September than do younger horses. Seasonally specific reference ranges are required for α-MSH and insulin concentrations, with significantly higher concentrations detected in the fall. Practitioners should be advised to submit samples only to local laboratories that can provide such reference ranges for their local geographic region. (J Am Vet Med Assoc 2012;241:241–248)

Abbreviations

Abbreviations

α-MSH α-Melanocyte–stimulating hormone
DST Dexamethasone suppression test
POMC Pro-opiomelanocortin
PPID Pituitary pars intermedia dysfunction

Pituitary pars intermedia dysfunction, also known as equine Cushing disease, is the most common endocrinopathy affecting older horses. Loss of dopaminergic inhibition by the hypothalamus results in hyperprolactinemia, hyperplasia, or a functional adenoma of the pituitary pars intermedia. As a result, production of POMC peptides increases with resultant excessive secretion of α-MSH and ACTH from the pituitary gland, which results in the commonly recog-
pared with their respective concentrations in spring. A third study conducted in Pennsylvania in clinically normal horses and ponies and those with PPID noted ACTH and α-MSH concentrations increasing after the end of June, peaking between the end of August and early to mid September, and beginning to decrease by October. The seasonal effects were more marked in the horses with PPID, but the overall pattern of seasonal regulation of the neuroendocrine axis was preserved. In contrast with the initial study, endogenous ACTH and α-MSH concentrations remained within reference limits (<35 pg/mL and <90 pmol/L, respectively) for all but 1 clinically normal horse sample for each hormone in late September and several pony samples between June and October. A study performed in Missouri describing a small number of clinically normal horses and those with equine metabolic syndrome found that ACTH concentrations were increased in August, September, and October, compared with other months, and that 4 of 7 clinically normal horses had ACTH concentrations >35 pg/mL in September. A seasonal pattern in insulin, cortisol, or glucose concentrations was not detected in clinically normal horses. All existing studies have occurred in the northern United States or England, and it is unknown whether seasonal variation in endocrine responses in clinically normal horses also occurs in the southeastern United States.

Veterinarians have been reluctant to test for PPID in the fall months on the basis of the increased likelihood of a false-positive test result. It is also unclear whether the seasonal variation in endocrine function is due to effects of temperature, photoperiod, or nutrition and whether such effects are likely to occur in all geographic locations. Winter months in Alabama are much milder than those in the northern United States, and less variation exists in the length of daylight hours between seasons at latitudes closer to the equator. Differences are also present in cool versus warm season pasture grasses, resulting in variable nonstructural carbohydrate consumption, which may influence insulin concentrations. It has been suggested that seasonal reference ranges be provided and that perhaps existing ranges are too high for winter months.

The purpose of the study reported here was to evaluate the variation in endogenous plasma ACTH and α-MSH concentrations, serum cortisol and insulin concentrations, and the cortisol response to a DST in association with month and age of clinically healthy older geldings in Alabama. Our hypothesis was that increased ACTH, α-MSH, cortisol, and insulin concentrations and failure of cortisol suppression after a DST would occur in the fall months but that the extent of seasonal deviations would be less dramatic than observed in the northern United States.

Materials and Methods

Animals—Fifteen geldings from the Auburn University Large Animal Teaching herd located in central Alabama (lat 32°36′35″N) were used. Breeds included Thoroughbred (n = 7), American Quarter Horse (4), Tennessee Walking Horse (1), Arabian (1), and cross-bred (2). The median age was 14 years (range, 7 to 26 years), and the mean ± SD age was 16 ± 5 years, with a mean body weight of 529.6 kg (1,165.1 lb; median, 532.4 kg [1,171.3 lb]; range, 465.9 to 590.9 kg [1,023 to 1,300 lb]) and mean body condition score of 5 of 9 (median, 5.9; range, 2.9 to 7.9). The geldings were maintained on free-choice coastal Bermuda grass hay and 1 kg of a 12% protein pelleted grain/d in small paddocks with limited amounts of fescue and coastal Bermuda grass. All horses were assessed to be healthy on the basis of history, physical examination, CBC, fibrinogen concentration, and serum biochemical profile. All horses were free from any clinical signs of PPID. No horse had received any exogenous glucocorticoid administration for 3 months prior to or during the study period. Horses were periodically dewormed and vaccinated but did not receive any medication within 2 weeks prior to each testing period. All aspects of the study were approved by the Auburn University Institutional Laboratory Animal Care and Use Committee.

Experimental design—Horses were caught in the field for blood collection and were not removed from their paddocks. No grain was fed for at least 8 hours prior to blood collection. Endogenous plasma ACTH and α-MSH and serum cortisol and insulin concentrations were measured from blood samples collected between 4:00 PM and 5:00 PM once per month on the Friday closest to the 15th of the month. Daylight duration and mean daily temperature were determined retrospectively from a website. After baseline blood collection, dexamethasone (0.04 mg/kg [0.02 mg/lb], IM) was administered. Blood samples were collected for measurement of serum cortisol concentration at 15 and 19 hours after injection.

For all samples, blood was collected via jugular venipuncture. Blood samples for serum cortisol and insulin concentration measurement were allowed to clot for 30 minutes at room temperature (22°C), and serum was collected after centrifugation and frozen at −20°C until analysis. Blood for endogenous plasma ACTH and α-MSH determination (0 hours only) was collected into cold EDTA tubes containing 0.5 mL of the protease inhibitor aprotonin (final concentration, 500 kallikrein inactivator U/mL of blood). Samples were transported on ice and centrifuged at 5,000 × g at 4°C for 5 minutes. The plasma was removed within 20 minutes after blood collection, placed in a plastic tube, and frozen at −20°C until assayed. Blood was collected at 15 and 19 hours after dexamethasone injection into plain serum clot evacuated tubes. Tubes were allowed to clot at room temperature, and serum was removed and frozen after centrifugation.

All hormone concentrations were measured in duplicate by use of batched frozen samples. Serum concentrations of cortisol were determined by a radioimmunoassay previously validated in horses. The sensitivity of the assay was 14.0 nmol/L. The inter- and intra-assay coefficients of variation were between 7.1% and 7.7% and 6.1% and 8.1%, respectively. Serum concentrations of insulin were determined by a radioimmunoassay previously validated in horses. The inter- and intra-assay coefficients of variation were 6.4% and 7.2%, respectively. Plasma endogenous ACTH concentration was measured with a sandwich immunoradiometric assay previously validated for use in horses. The sensitivity of the assay
was 1.0 pg/mL, and the inter- and intra-assay coefficients of variation were 9.2% and 4.5%, respectively, for values < 30 pg/mL. The α-MSH assay was previously validated for use with samples from dogs and cats. Serial dilution and assay of plasma samples from 4 horses gave displacement curves with slopes similar to that of the standard (P > 0.05). Assay sensitivity was 6 pg/mL; intra- and interassay coefficients of variation were < 8% and < 14%, respectively. Recovery was assessed by addition of 25, 50, and 100 pg of ACTH and α-MSH to separate 1-mL aliquots of pooled equine plasma. Following the respective assay, between 92% and 116% of the expected hormone concentration was measured in each sample, after correction for hormone content in the pooled plasma alone. Serum cortisol concentrations > 30 nmol/L at 15 and 19 hours, serum insulin concentrations > 30 µU/mL, plasma ACTH concentrations > 35 pg/mL, and α-MSH concentrations > 60 pg/mL were considered above the reference ranges for our laboratory.

Statistical analysis—Data are reported as mean ± SD and 95% confidence intervals and were graphically represented as median (25th to 75th percentile) in box plots. Residual plot analyses were performed to determine whether each hormone concentration was normally distributed. Nonnormal data (α-MSH, ACTH, and insulin concentrations) were transformed with statistical software after determination of the optimum transformation technique. Transformed data were normally distributed according to the Anderson-Darling transformation technique. Transformed data were normally distributed, equal variances, and sphericity. The Levene test was used to prove the assumption of equality of variances (P < 0.05). The Mauchly test indicated that the sphericity assumption was violated, and degrees of freedom were corrected by use of the Greenhouse-Geisser (and Huynh-Feldt) adjustment for nonsphericity. Because there was moderate correlation between variables, multivariate ANOVA was used to compare mean cortisol (0, 15, and 19 hours) and transformed α-MSH, ACTH, and insulin concentrations across months. When significant (P < 0.05) effects of month were identified, pairwise comparisons between months were made by use of Bonferroni post hoc analysis. Correlations between age and each hormone concentration were performed for each month by use of the Spearman correlation coefficient. Statistical analysis was performed with statistical software.

Results

Season—Endogenous α-MSH and insulin concentrations were significantly different across months, whereas no changes were detected throughout the year in basal endogenous ACTH and cortisol concentrations and cortisol concentrations after DST at 15 and 19 hours (Table 1). Plasma α-MSH concentrations in August, September, and October were significantly higher than in December through July (P < 0.001 to 0.007). Concentrations in August and September were significantly (P < 0.001) higher than in November. Concentrations in November were also significantly (P = 0.006) higher than in June (Figure 1). Serum insulin concentrations were significantly higher in November, compared with those in January (P = 0.037), February (P < 0.001), April (P = 0.019), May (P < 0.001), and July (P = 0.018); the difference between November and June was not significant (P = 0.052; Figure 2).

Of all hormones studied, the most obvious seasonal effect occurred in α-MSH concentrations in the fall months. Overall, endogenous plasma α-MSH concentrations were above the reference interval (ie, > 60 pg/mL) in 29 of 180 (16%) samples throughout the entire year; however, of 15 samples, 11 were outside the reference interval in both August and September, 5 were outside the reference interval in October, and 1 was outside the reference interval in both November and December. Between January and July, all horses had α-MSH concentrations < 60 pg/mL. Only 3 horses had α-MSH concentrations < 60 pg/mL for every month of the year. Overall, 12 of 15 horses would have had a false diagnosis of PPID on the basis of α-MSH concentrations during at least 1 month between August and December. Concentrations of α-MSH were lowest in June (13 ± 7 pg/mL) and highest in August (138 ± 86 pg/mL). Seasonal upper reference limits calculated as mean ± 2SD for α-MSH would be < 44.4 pg/mL between December and July; < 237 pg/mL for August, September, and October; and < 81 pg/mL for November.

Table 1—Seasonal variation in endogenous plasma ACTH, plasma α-MSH, serum insulin, and serum cortisol concentrations in 15 clinically normal geldings in Alabama with respect to photoperiod and temperature.

<table>
<thead>
<tr>
<th>Month</th>
<th>Daylight duration (h)</th>
<th>Mean daily temperature (°C)</th>
<th>ACTH hormone (pg/mL)</th>
<th>α-MSH (pg/mL)</th>
<th>Insulin (µU/mL)</th>
<th>Cortisol (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>13.3</td>
<td>27</td>
<td>13.7 ± 8.35 (9.52–18.0)</td>
<td>138 ± 86.1 (94.5–182)</td>
<td>15.4 ± 21.2 (4.6–26.14)</td>
<td>107 ± 26.0 (93.2–121)</td>
</tr>
<tr>
<td>September</td>
<td>12.4</td>
<td>22</td>
<td>11.6 ± 6.82 (8.27–15.0)</td>
<td>107 ± 63.4 (74.5–139)</td>
<td>32.5 ± 72.9 (6–69.3)</td>
<td>102 ± 40.9 (80.2–124)</td>
</tr>
<tr>
<td>October</td>
<td>11.5</td>
<td>14</td>
<td>14.3 ± 7.11 (10.5–17.9)</td>
<td>69.9 ± 40.0 (45.1–94.0)</td>
<td>17.8 ± 35.6 (6–97.2)</td>
<td>127 ± 80.0 (108–147)</td>
</tr>
<tr>
<td>November</td>
<td>10.5</td>
<td>14</td>
<td>10.5 ± 5.58 (7.72–13.4)</td>
<td>32.5 ± 23.6 (20.6–44.5)</td>
<td>48.7 ± 95.8 (0.24–97.2)</td>
<td>102 ± 31.7 (85.2–119)</td>
</tr>
<tr>
<td>December</td>
<td>10.0</td>
<td>13</td>
<td>9.06 ± 4.73 (6.67–11.5)</td>
<td>23.9 ± 15.2 (16.3–31.6)</td>
<td>8.60 ± 6.3 (5.38–11.8)</td>
<td>128 ± 22.1 (116–140)</td>
</tr>
<tr>
<td>January</td>
<td>10.2</td>
<td>15</td>
<td>9.69 ± 4.96 (7.36–12.0)</td>
<td>21.1 ± 13.0 (14.6–27.7)</td>
<td>6.07 ± 3.8 (4.3–8.0)</td>
<td>114 ± 24.9 (101–127)</td>
</tr>
<tr>
<td>February</td>
<td>11.0</td>
<td>0</td>
<td>7.94 ± 3.86 (6.05–9.79)</td>
<td>20.9 ± 12.1 (14.7–27.0)</td>
<td>4.38 ± 2.3 (3.2–5.5)</td>
<td>97.3 ± 21.4 (85.8–109)</td>
</tr>
<tr>
<td>March</td>
<td>12.0</td>
<td>12</td>
<td>7.94 ± 3.05 (6.40–9.48)</td>
<td>20.2 ± 11.9 (14.4–26.0)</td>
<td>11.0 ± 7.89 (7.00–15.0)</td>
<td>81.4 ± 36.0 (62.2–101)</td>
</tr>
<tr>
<td>April</td>
<td>12.9</td>
<td>17</td>
<td>8.08 ± 3.45 (3.63–9.83)</td>
<td>16.4 ± 10.7 (11.0–21.8)</td>
<td>5.86 ± 3.46 (4.1–7.52)</td>
<td>118 ± 42.8 (94.6–140)</td>
</tr>
<tr>
<td>May</td>
<td>13.9</td>
<td>18</td>
<td>9.12 ± 4.12 (7.04–11.2)</td>
<td>17.1 ± 11.0 (11.5–22.6)</td>
<td>4.33 ± 1.92 (3.38–5.30)</td>
<td>102 ± 34.7 (83.0–120)</td>
</tr>
<tr>
<td>June</td>
<td>14.3</td>
<td>23</td>
<td>7.51 ± 2.45 (6.28–8.73)</td>
<td>12.9 ± 7.33 (9.16–16.6)</td>
<td>12.5 ± 26.1 (0–25.8)</td>
<td>110 ± 35.1 (91.6–129)</td>
</tr>
<tr>
<td>July</td>
<td>14.1</td>
<td>26</td>
<td>8.88 ± 2.89 (7.21–10.1)</td>
<td>22.7 ± 19.2 (13.0–32.4)</td>
<td>5.07 ± 1.83 (4.14–5.99)</td>
<td>126 ± 23.6 (110–142)</td>
</tr>
</tbody>
</table>

Hormone concentrations are reported as mean ± SD (95% confidence interval).

* For each α-MSH and insulin, absence of a shared superscript letter indicates a significant (P < 0.05) difference between months.
Endogenous serum insulin concentrations were above the reference interval (ie, > 30 µU/mL) for 1 of 15 horses in June and August, 2 in September and October, and 3 in November. Between December and May, all horses had insulin concentrations < 30 µU/mL. Two horses had a single elevated insulin concentration (one in June [106 µU/mL] and another in November [35 µU/mL]). One horse had moderately elevated insulin concentrations from September through November (143 to 374 µU/mL), and 1 horse had mildly increased insulin concentrations from August through November (43 to 124 µU/mL). Twelve horses had normal insulin concentrations throughout the year. Concentrations of insulin were lowest in February (4.4 ± 2.3 µU/mL) and highest in November (49 ± 96 µU/mL; Table 1). From December to July, all horses had insulin concentrations < 21 µU/mL. Seasonal reference intervals for insulin were determined to be < 33 µU/mL between December and August, < 139 µU/mL for September and October, and < 241 µU/mL for November.

No horses had ACTH concentrations above the reference range at any time (n = 180 samples). Although no significant seasonal effect was detected, an increase in variability was detected between August and October (Figure 3). Between August and November, 3 horses had ACTH concentrations between 20 and 35 pg/mL (1 horse in September and October, 1 horse in August, October, and November, and 1 horse in August). From December to July, all horses had ACTH concentrations < 20 pg/mL. Seasonal reference intervals were determined to be < 27.9 pg/mL between August and October and < 16.6 pg/mL for November through July.

Endogenous cortisol concentrations were variable throughout the year, with no tendency toward a seasonal effect. Five elevated basal cortisol concentrations (> 180 nmol/L) were detected in different months and from different horses. Mean ± SD endogenous cortisol concentration for all samples (n = 180) was 110 ± 34.2 nmol/L, with a reference range calculated as 41.1 to 178 nmol/L.

No significant seasonal effect was detected in DST results at either 15 or 19 hours. Of the 180 DSTs performed, failure of cortisol to suppress and remain suppressed to < 30 nmol/L at 15 and 19 hours occurred only 3 times in the late summer and fall months. One horse had post–dexamethasone administration cortisol concentrations of 37 nmol/L at 15 hours and 33 nmol/L at 19 hours in September. A second horse had a post–dexamethasone administration cortisol concentration of 32 nmol/L at 19 hours in October.

Age—None of the hormone concentrations were highly correlated with age. However, a significant positive correlation was detected between age and endogenous ACTH concentration in September (r = 0.045), December (r = 0.027),
and January (P = 0.042) and between age and insulin concentration in October (P = 0.042). A negative correlation was found between age and basal cortisol concentration in September (P = 0.007). A positive, albeit nonsignificant, correlation was detected between age and endogenous ACTH concentration in October (P = 0.056), November (P = 0.064), and May (P = 0.067) and between age and endogenous α-MSH concentration in September (P = 0.058; Table 2).

**Discussion**

The results of the present study demonstrate seasonal changes in the plasma endogenous α-MSH and serum insulin concentrations in older, clinically normal geldings in Alabama. Significantly elevated α-MSH concentrations were observed in August, September, and October, compared with the remainder of the year. Insulin concentrations were significantly increased in November, compared with September between January and July. No significant changes in endogenous ACTH or cortisol concentrations or cortisol response to DST at 15 and 19 hours were detected between months. All basal cortisol and endogenous ACTH concentrations were within the reference ranges. The seasonal effect on the equine endocrine system is more marked in older horses, with a significant moderate positive correlation between age and endogenous ACTH concentration detected for several months between September and January and between age and insulin concentration in October. A positive, albeit nonsignificant, correlation was detected between age and α-MSH concentrations in September. However, there was a negative correlation between age and cortisol concentrations in September. Concentrations of endogenous α-MSH were 10.7-fold higher in August (peak), compared with June (trough), in the study horses in Alabama. The observed pattern of seasonal variation in plasma α-MSH does not appear to be affected by latitude because significantly greater α-MSH concentrations were reported by McFarlane et al.\(^2\) during September in ponies (11-fold) and in Standardbred horses (2-fold), compared with α-MSH concentration in spring. Whether the greater increase in α-MSH concentration observed in the ponies, compared with the horses, was because of geographic location, diet, daylight, type of housing, or metabolic differences between ponies and horses could not be determined.\(^10\)

Another study comparing horses and ponies by Beech et al.\(^13\) showed a 7.6-fold increase in clinically normal horses and a 22.3-fold increase in ponies between February and September. Our horses were older than those in the 2 previous studies,\(^10,11\) which may explain the more dramatic increases in α-MSH concentrations, given that we found a tendency toward increased α-MSH concentration with age in some months. Therefore, it appears that season, age, and type of equid can influence α-MSH concentrations. An effect of season on α-MSH concentrations has been described in sheep, hamsters, weasels, and humans.\(^23–25\) In feral Soay sheep in Scotland, changes in POMC-derived peptides have been suggested to play a role in metabolic preparation for winter.\(^24\) Similar elevations in POMC-derived peptides (ACTH and α-MSH) may occur in horses and ponies to metabolically prepare them for winter weather and a decrease in forage.\(^10\)

Measurement of plasma α-MSH is a promising diagnostic test for PPID for ambulatory care practitioners because of greater stability of the hormone in blood samples, compared with plasma ACTH.\(^10\) Additionally, the use of α-MSH rather than ACTH to detect PPID may be more physiologically appropriate because α-MSH is secreted primarily from the pars intermedia, whereas ACTH can also be a product of the pars distalis.\(^7\) Therefore, it is important, as indicated by the results of the present study, to determine reference intervals for clinically normal horses that account for physiologic autumal elevations.\(^7\) Although the degree of seasonal variation in endogenous α-MSH concentrations is far more marked than occurs with ACTH concentrations, the development of appropriate reference ranges for ACTH concentrations is also warranted.

Further study of seasonal variation in horses with PPID is also needed because horses with PPID have more extreme seasonal α-MSH concentration effects than do clinically normal horses.\(^11\) Contrary to recent anecdotal suggestions to avoid testing for PPID in fall, differentiation of clinically normal horses from horses with PPID via α-MSH concentrations appears to be more distinct in fall. A 97-fold increase in α-MSH concentration was found in horses with PPID between February and early September, resulting in a 70-fold difference between α-MSH concentrations in clinically normal horses versus horses with PPID in September.\(^11\) However, many of the horses classified as having PPID (on the basis of clinical signs) had α-MSH concentrations within the laboratory reference range between February and June and thus would have been falsely classified as clinically normal on the basis of α-MSH concentrations alone.\(^11\)

Two studies\(^4,11\) have detected seasonal variation in plasma ACTH concentrations in healthy ponies and horses in Pennsylvania, with the highest concentrations measured in September. In the initial study,\(^4\) 37 of 39 (95%) clinically normal horses would have been falsely classified as having PPID on the basis of endogenous ACTH concentrations when tested in September. How-

### Table 2—Correlations between age and endogenous plasma ACTH and α-MSH concentrations and serum cortisol and insulin concentrations in 15 clinically normal geldings in Alabama throughout the year.

<table>
<thead>
<tr>
<th>Month</th>
<th>ACTH</th>
<th>α-MSH</th>
<th>Cortisol</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>August</td>
<td>0.32 (0.23)</td>
<td>0.086 (0.81)</td>
<td>−0.34 (0.21)</td>
<td>0.35 (0.19)</td>
</tr>
<tr>
<td>September</td>
<td>0.94 (0.45)*</td>
<td>0.50 (0.058)</td>
<td>−0.66 (0.007)*</td>
<td>0.32 (0.24)</td>
</tr>
<tr>
<td>October</td>
<td>0.50 (0.056)</td>
<td>0.26 (0.31)</td>
<td>0.31 (0.26)</td>
<td>0.53 (0.042)*</td>
</tr>
<tr>
<td>November</td>
<td>0.49 (0.064)</td>
<td>0.26 (0.34)</td>
<td>0.31 (0.26)</td>
<td>0.22 (0.44)</td>
</tr>
<tr>
<td>December</td>
<td>0.57 (0.027)*</td>
<td>0.24 (0.38)</td>
<td>−0.17 (0.54)</td>
<td>0.27 (0.33)</td>
</tr>
<tr>
<td>January</td>
<td>0.53 (0.042)*</td>
<td>0.022 (0.94)</td>
<td>0.11 (0.69)</td>
<td>0.34 (0.22)</td>
</tr>
<tr>
<td>February</td>
<td>0.30 (0.27)</td>
<td>0.059 (0.84)</td>
<td>−0.16 (0.56)</td>
<td>0.18 (0.52)</td>
</tr>
<tr>
<td>March</td>
<td>0.30 (0.27)</td>
<td>0.19 (0.49)</td>
<td>0.046 (0.57)</td>
<td>0.18 (0.52)</td>
</tr>
<tr>
<td>April</td>
<td>0.13 (0.65)</td>
<td>0.091 (0.75)</td>
<td>0.0018 (0.99)</td>
<td>0.43 (0.11)</td>
</tr>
<tr>
<td>May</td>
<td>0.48 (0.067)</td>
<td>0.35 (0.20)</td>
<td>−0.14 (0.61)</td>
<td>−0.16 (0.56)</td>
</tr>
<tr>
<td>June</td>
<td>0.41 (0.12)</td>
<td>0.089 (0.75)</td>
<td>0.22 (0.43)</td>
<td>−0.26 (0.35)</td>
</tr>
<tr>
<td>July</td>
<td>0.28 (0.51)</td>
<td>0.36 (0.18)</td>
<td>−0.42 (0.12)</td>
<td>−0.21 (0.44)</td>
</tr>
</tbody>
</table>

*Spearman correlation coefficient (P value) shown.

\(\alpha\) P value is < 0.05.
ever, in a second study, only 1 horse had an ACTH concentration above their reference range, occurring in September. Given that the first study used horses of a variety of ages and found a correlation between age and ACTH but the second study was limited to clinically normal horses < 11 years of age, age differences may explain the conflicting results. In a recent report from England, ACTH concentrations were significantly higher in September and December, compared with March and June, in aged clinically normal horses and horses with PPID. That study provided a cutoff value to differentiate clinically normal horses from horses with PPID of < 40 to 50 pg/mL in March through June and < 80 to 100 pg/mL in September through December. In the present study, no horse had ACTH concentrations above our reference range in any month. Therefore, the risk of falsely diagnosing PPID in a clinically normal horse on the basis of endogenous ACTH concentrations for horses in our geographic region at any time of the year by means of existing reference ranges is minimal. However, it is obvious from results of the present study and other reports that current laboratory reference cutoff values for clinically normal horses should be lowered from approximately December until June or July (depending on the location) and increased in the late summer and fall. This will ultimately improve the accuracy of the test, increasing the overall specificity of testing and thus decreasing the risk of false-positive diagnosis of PPID in fall and also potentially increasing the sensitivity of testing, which may, more importantly, allow for earlier treatment of horses with PPID.

The horses in our study also had access to a limited amount of pasture containing tall fescue grass that was most likely infected with the endophyte Neotyphodium coenophialum. Ergopeptine alkaloids are responsible for most of the toxicities associated with tall fescue. Concentrations of ergot alkaloids can increase with drought stress and application of nitrogen fertilizer. Ergopeptines are dopamine D2-receptor agonists (similar to pergolide) and could conceivably have a protective effect on reducing ACTH concentrations, possibly explaining why our horses had lower concentrations of ACTH than reported in other studies. However, this is unlikely because the amount of pasture available to our horses was limited, with coastal Bermuda grass hay composing most of their diet. In the present study, only 3 of 180 DST results were outside the reference interval. Two of 15 horses (1 each in September and October) had cortisol concentrations that just failed to suppress normally at 19 hours after DST, and 1 of these horses also failed to suppress at 15 hours in August. Thus, a mean of only 1 of 15 horses had an abnormal DST result in each of the months between August and October in the present study. These 2 horses did develop moderate suppression of cortisol concentrations in response to the DST, but the post-DST cortisol concentrations were just above the cutoff values for clinically normal horses. In comparison, 10 of 39 (26%) horses had abnormal DST results in September versus January in a study by Donaldson et al. Obtaining a false-negative diagnosis for PPID appears to be more likely in Pennsylvania than in Alabama when the DST is used.

In the present study, there was a significant increase in insulin concentrations in November. Four horses had insulin concentrations above our laboratory reference range in at least 1 month between late summer and fall, and a single horse had an elevated insulin concentration in June. Elevated endogenous insulin concentrations and insulin resistance are common in horses with PPID and a hallmark of equine metabolic syndrome, but the findings on effects of season on insulin concentrations in horses have not been consistent between studies. An increase in insulin concentrations in June, compared with December, in a study conducted in England was attributed to grazing of summer pasture with a higher concentration of nonstructural carbohydrates such as fructans. Nutritional factors are unlikely to account for findings of the present study, considering that the horses consumed the same grass hay throughout the year, with minimal pasture availability. The elevated insulin concentrations were observed in only a subset of horses, none of which had any physical characteristics of equine metabolic syndrome such as obesity or regional adiposity. One horse had insulin concentrations of 268, 143, and 374 µU/mL in September, October, and November, respectively, with concentrations between 3 and 20 µU/mL in all months except fall. The reason 4 of 15 horses had elevated insulin concentrations in fall is unknown. These 4 horses all had α-MSH concentrations within the 60th percentile in September, but there were 5 other horses with similarly high α-MSH concentrations in the fall and normal insulin concentrations. In contrast to the present study, Beech et al. observed minimal seasonal effects on insulin concentrations in clinically normal horses and horses and ponies with PPID, although numbers in each group were small. Further evaluation of stringent indicators of insulin sensitivity needs to be performed in both healthy and insulin-resistant horses to determine the exact role of photoperiod, ambient temperature, and forage types.

Insulin concentrations are affected by external factors such as stress, forage, and time after feeding and can change rapidly throughout the day. Previous recommendations were to withhold grain and pasture and continue to feed free-choice hay prior to measuring insulin concentrations. It was considered that the effects of food withholding may induce stress, elevate cortisol and insulin concentrations, and affect results of endocrine testing. Several studies have reported insulin concentrations when food hasn’t been withheld. Current recommendations are to measure insulin concentrations after 6 hours of food withholding.

Considering that there was a moderate positive correlation in the fall and winter months between age and both ACTH and insulin concentrations and a negative correlation between age and cortisol concentrations, it appears that the seasonal influence on ACTH and insulin concentrations is more likely to occur in older horses. Similar to our findings, age was positively correlated with ACTH concentrations in 1 study, but a significant effect of age on ACTH concentrations was not detected in another study. In agreement with the present study, there appears to be minimal influence of age on α-MSH concentrations. In contrast to findings...
of the present study, age has previously been found to be positively correlated with endogenous cortisol and response to DST. The reason for these differences is unknown. It is also unclear why cortisol concentrations were significantly lower in the present study in older horses in September, whereas ACTH concentrations were significantly higher in several fall and winter months. Age-related changes in the hypothalamic-pituitary-adrenal axis have also been found in other mammalian species, and further investigation is warranted in geriatric horses. Preliminary findings show a seasonal influence on the frequency of detection of histopathological lesions in the equine pituitary gland of older horses.

There are several limitations to the present study. Necropsies were not performed; therefore, no histologic confirmation was made of the absence of pituitary hyperplasia. We chose to study older horses because this is the population in which PPID testing occurs; without necropsy, we could not rule out the presence of subclinical pituitary hyperplasia. However, no horse had clinical signs of PPID, and no abnormalities were detected in results of CBC and biochemical analysis. Further, feed analysis was not performed, eliminating the capability to relate endogenous insulin concentrations to nonstructural carbohydrate concentrations in the hay and in the limited pasture that was available to the horses throughout the year.

The small sample size may have affected our ability to obtain statistical significance for several variables at various months, as many of our data had P values between 0.05 and 0.07. If a larger number of horses had been available for study, statistical significance may have been reached.

We speculate that seasonal elevations of ACTH and α-MSH concentrations in individual older horses may be an early predictor of pituitary hyperplasia and perhaps a warning that subclinical PPID is occurring. As geriatric equine medicine becomes increasingly important, diagnosis of subclinical PPID and preemptive treatment prior to the development of devastating complications such as laminitis are needed. Season-specific reference ranges should be determined for each laboratory, and practitioners should be advised to submit samples only to local laboratories that can provide such reference ranges for their local geographic region.

References

From this month’s AJVR

Effects of syringe type and storage conditions on results of equine blood gas and acid-base analysis

Sarah A. Kennedy et al

Objective—To determine effects of syringe type and storage conditions on blood gas and acid-base values for equine blood samples.

Sample—Blood samples obtained from 8 healthy horses.

Procedures—Heparinized jugular venous blood was equilibrated via a tonometer at 37°C with 12% O2 and 5% CO2. Aliquots (3 mL) of tonometer-equilibrated blood were collected in random order by use of a glass syringe (GS), general-purpose polypropylene syringe (GPPS), or polypropylene syringe designed for blood gas analysis (PSBGA) and stored in ice water (0°C) or at room temperature (22°C) for 0, 5, 15, 30, 60, or 120 minutes. Blood pH was measured, and blood gas analysis was performed; data were analyzed by use of multivariable regression analysis.

Results—Blood P02 remained constant for the reference method (GS stored at 0°C) but decreased linearly at a rate of 7.3 mm Hg/h when stored in a GS at 22°C. In contrast, P02 increased when blood was stored at 0°C in a GPPS and PSBGA or at 22°C in a GPPS; however, P02 did not change when blood was stored at 22°C in a PSBGA. Calculated values for plasma concentration of HCO3 and total CO2 concentration remained constant in the 3 syringe types when blood was stored at 22°C for 2 hours but increased when blood was stored in a GS or GPPS at 0°C.

Conclusions and Clinical Relevance—Blood samples for blood gas and acid-base analysis should be collected into a GS and stored at 0°C or collected into a PSBGA and stored at room temperature. (Am J Vet Res 2012;73:979–987)