Use of fecal glucocorticoid and salivary cortisol concentrations as a measure of well-being of New York City carriage horses

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OBJECTIVE

To use noninvasive approaches to assess stress in New York City (NYC) carriage horses during the course of their daily routine to determine whether use of these horses affected their well-being.

DESIGN

Prospective case control study.

ANIMALS

13 (5 mares and 8 geldings) stabled working NYC carriage horses and 5 pastured (nonworking) NYC carriage horses (1 mare and 4 geldings).

PROCEDURES

Samples for determination of fecal glucocorticoid and salivary cortisol concentrations were collected on 3 successive days from 10, 8, and 9 working carriage horses during rest (time 1), preparation for work (time 2), and return to the stable (time 3) and at 1 hour after work (time 4). Infrared thermography (IRT) measurements were made to determine maximum temperature of the medial canthus at each time point. Fecal samples were also collected from 5 pastured carriage horses for determination of glucocorticoid concentrations.

RESULTS

No difference was found in mean ± SE fecal glucocorticoid concentrations between pastured (22.1 ± 9.8 ng/g) and working (19.5 ± 4.2 ng/g) carriage horses. A significant difference was found in salivary cortisol concentrations of working carriage horses between time 3 (0.96 ± 0.06 ng/mL) and time 4 (0.77 ± 0.07 ng/mL). The IRT measurement at time 2 (35.5 ± 0.64°C [95.9 ± 1.2°F]) was significantly lower than that at time 3 (36.2 ± 0.64°C [97.1 ± 1.2°F]). No other differences in IRT measurements were found.

CONCLUSIONS AND CLINICAL RELEVANCE

These working NYC carriage horses did not have physiologic responses indicative of a negative welfare status. (*J Am Vet Med Assoc* 2017;250:316–321)

Recent scrutiny of the living and working conditions of NYC carriage horses has brought into question the well-being of these animals. Although a contentious issue, no objective data have been produced to determine the physiologic state of these horses, nor their response to their daily activities.

One of the favored means by which to quantify stress is by measuring glucocorticoid concentrations. The main physiologic role of glucocorticoids is to modulate metabolism, but they are produced in greater quantities during stress.[1] Acute increases in glucocorticoid concentrations in response to stress is adaptive, allowing the animal to respond to its environment; however, chronic or repeated stressors can lead to detrimental effects including immunosuppression and tissue atrophy.[2,3]

ABBREVIATIONS

IRT Infrared thermography
NYC New York City

Although glucocorticoid concentrations are traditionally measured in blood, measurements of the concentrations of glucocorticoid and its metabolites have been made from saliva[4–6] and fecal[7–10] samples as a means to assess adrenal gland activity. The advantage of these noninvasive approaches to obtaining samples is to facilitate frequent sample collection and measure adrenal gland function without provoking a stress response, a noted disadvantage of blood sample collection. Glucocorticoid concentrations in saliva closely mirrors those in plasma, with a minor time lag in salivary glucocorticoid concentrations that smooths the pulsatility of glucocorticoid secretion patterns but still provides detection of acute adrenal gland activity. The particular advantage of glucocorticoid measurements from fecal samples is that they reflect general adrenal gland activity over a longer period (ie, 24 to 48 hours), providing a better profile of day-to-day adrenal gland activity.[11,12]

In addition to affecting adrenal gland production of glucocorticoids, stress can also stimulate the sympathetic nervous system and, as a result, increase body temperature.[13] An increase in body temperature

is accurately reflected in eye temperature. To assess chronic and acute stress responses, eye temperature is determined noninvasively by use of IRT.[13,14]

The purposes of the study reported here were to determine whether there are differences in fecal glucocorticoid concentrations between working and pastured NYC carriage horses, measure changes in salivary cortisol concentrations and IRT measurements of the medial canthus throughout the workday over a series of days in working NYC carriage horses, and determine whether there was a relationship between measured adrenal gland activity and body temperature changes in working NYC carriage horses. The already described noninvasive sample collection approaches were used to measure the stress response throughout the day of working NYC carriage horses and to compare their results with those obtained from their counterparts on pasture. It is necessary to use ≥ 1 assessment of stress, as adrenal gland activity can be stimulated by nonstressful events such as exercise and excitement.

Materials and Methods

Animals

All aspects of the study were approved by the Western University of Health Sciences Institutional Animal Care and Use Committee. Thirteen horses (5 mares and 8 geldings) were selected from a population of 70 day-shift NYC carriage horses stabled at Clinton Park Stables, NY. The mean ± SD age of subjects was 11.6 ± 2.7 years. The subjects' previous experience in service ranged from 4 months to 11 years, with a mean ± SD of 5.5 ± 2.9 years. All horses were privately owned, and owners provided consent for their participation in the study. Horses were housed in individual stalls approximating 2.4 × 3.0 m, with 1 horse residing in a 3.0 × 3.0-m stall. Stalls were constructed with solid walls to the level of the horse's shoulders, and bars above that allowed for visual and auditory contact with conspecifics. Stall flooring consisted of thick rubber mats deeply bedded with straw. Horses were fed grain throughout the workday (grain-filled pails were carried on the carriages), and timothy hay was provided nearly ad libitum while in the stable. Five additional NYC carriage horses (1 mare and 4 geldings) on pasture were included in the study. Pastured horses were on furlough, in accordance with the city code, and had no workload assigned to them. The mean ± SD age of these horses was 10 ± 2.2 years. Fecal samples were collected from these subjects while on pasture at a farm in Denver, Pa. These horses were housed indoors overnight and turned out in a 4-acre pasture where sample collection took place. Indoor housing was provided in a large banked barn formerly used to house cattle.

Study design

Sample collection on days 1, 2, and 3 involved 10, 8, and 9 working horses, respectively. All day-shift horses (n = 70) were deemed eligible by investigators for inclusion in the study. The investigators selected a convenience sample of horses by starting at the front end of the stable and enrolling all eligible horses as they walked the aisles until the desired number of subjects was reached. Samples were collected from horses that were working on any of the 3 days within the sample collection period. Four sample collection time points occurred on collection days. Time 1 occurred from 6:00 AM to 8:00 AM (all reported times are Eastern Standard Time). Subjects were at rest in their stalls prior to work or the arrival of carriage drivers. Time 2 occurred after horses were just harnessed and hitched to carriages in preparation for work. The horses then traveled approximately 1.5 km by hoof to Central Park where they stood until hired. When hired, they circumnavigated the interior of Central Park on trips of approximately 1.6 to 3.2 km in distance (short and long fees applied). Trips around the park could have occurred multiple times in a workday. The horses traveled approximately 1.6 km by hoof back to the stable at the conclusion of their workday. Time 3 occurred immediately after being placed in their stalls at the conclusion of the workday (2:20 PM to 7:40 PM). Time 4 occurred 1 hour after horses were returned to their stall. The sample collection regimens were designed to prevent any modification in the subjects' daily work routines. As such, the interval between time 2 and time 4 varied across individual subjects. All study participants were working the day shift, which entailed leaving the stable no earlier than 8:00 AM on day 1 and 9:00 AM on days 2 and 3. All subjects returned to the stable at no more than 9 hours after leaving the stable.

Additionally, fecal samples were collected during a 1-day period from 5 NYC carriage horses while they were turned out on pasture. A morning and evening fecal sample were collected from each of these subjects.

Sample collection

The primary collection period occurred during 3 consecutive days in NYC, from August 3 to 5, 2014. An additional collection period occurred August 1, 2014, in Denver, Pa. Fresh fecal samples were collected directly from the bedding at time 1 from all subjects in NYC. Additional fecal samples were collected from the 5 pastured horses in Pennsylvania. As these horses were turned loose on pasture, fresh fecal samples were collected from the ground immediately after each horse defecated. Samples were collected from the pastured horses at 2 time points. These included a morning sample (after 7:00 AM) and evening sample (after 4:00 PM). Exact collection time was dictated by the individual horse's defecation. Sample collection for pastured horses was limited to fecal collection only. No other modes of testing were explored in this population. All fecal samples were stored on ice during the collection period and then transferred to a freezer and stored at –20°C until processed for hormone analysis.

Saliva samples were collected as per the manufacturer's protocol at all 4 time points (times 1 through 4) from the NYC carriage horses by use of commercially available swabs.[a] The 125-mm-long swab allowed the handler to grip one end of the swab while inserting the other end into the horse's mouth at the level of the maxillary third premolar tooth. The sample collection period ranged from 30 seconds to 1 minute. The swab was then folded in half lengthwise and inserted into a storage tube.[b] Saliva samples were stored on ice and immediately transferred to a freezer and stored at –20°C until hormone extraction was performed.

Static images of both eyes were captured by use of an IRT camera.[c] Images were obtained at an angle of approximately 90° and a distance of approximately 0.75 m, consistent with previously published studies.[13,15] A minimum of 2 images were captured of the left and right eye for each collection time point. Images were collected at all 4 time points prior to collection of saliva samples. Ambient temperature and humidity were recorded as images were obtained.

Sample processing

Fecal sample processing and extraction followed previously described methods with minor modifications.[16,17] Briefly, feces were freeze-dried within a month of collection and crushed to powder. Samples (n = 48) were weighed (0.25 ± 0.007 g) and vortexed twice at 20-minute durations in 90% ethanol (10 mL). Supernatants were evaporated until dry, resuspended in PBS (1 mL), and stored frozen (–20°C) until analysis (at 96 days). Saliva was extracted from the oral swab samples within 52 days of collection by centrifugation (15 minutes, 4,303 × *g* at 2°C).[d] Samples were stored on ice between centrifugation and hormone analysis (< 2 hours).

Endocrine analysis

Feces were analyzed for glucocorticoid metabolites via a group-specific monoclonal, double antibody cortisol enzyme immunoassay.[e] Serially diluted fecal extracts displaced labeled antigen similarly to the standard (r^2 = 0.99; P = 0.009). The extract (0.005 mL) was added in duplicate to the assay. The standards of the assays (n = 2) were within the reported range by the manufacturer, and intra-assay coefficient of variation was 3.5 ± 2.7%. Cortisol concentration was quantified from saliva samples by use of a commercially available enzyme immunoassay.[f] Displacement of labeled antigen in serially diluted saliva samples was similar to the standard (r^2 = 0.99; P = 0.01). Saliva samples were added (0.025 mL) in duplicate to assay. The interassay coefficients of variation for 2 internal controls (n = 4 assays) were 4.5% (mean binding, 17.0%) and 20.6% (mean binding, 58.3%), and the intra-assay coefficient of variation was 2.5 ± 2.3%.

IRT

Images were uploaded to a software program[c] to extract the maximum temperature of the medial canthus for each image. Recorded temperatures were averaged to obtain a mean maximum temperature of the medial canthus for each eye.

Statistical analysis

Morning and evening fecal glucocorticoid concentrations of pastured horses were comparable with the Wilcoxon signed rank test. To determine whether fecal glucocorticoid concentrations differed between pastured and working NYC carriage horses, morning samples from the pastured horses were compared with time 1 samples of working NYC carriage horses by use of a Mann-Whitney rank sum test. No differences were noted in salivary cortisol concentrations among the 3 collection days (Kruskal-Wallis 1-way ANOVA; P = 0.792); therefore, data for all time periods during the collection days were combined for further analysis. To determine whether salivary cortisol concentrations and IRT eye temperatures differed from time 1 through time 4, Friedman repeated-measures ANOVA on ranks with a post hoc Tukey test for pairwise comparisons were run on respective results. Correlation between salivary cortisol concentrations and IRT eye temperatures was determined via Pearson product moment correlation. All data are presented as mean ± SE unless otherwise noted, and tests were run by use of statistical software[18,g] with α set at 0.05. Correlation between IRT and ambient temperature was determined by Spearman rank correlation.

Results

The fecal glucocorticoid concentrations of the pastured horses in the morning (mean ± SE, 22.1 ± 9.8 ng/g) did not differ (P = 0.125) from evening fecal glucocorticoid concentrations (27.7 ± 7.2 ng/g). There was no difference (P = 0.955) in fecal glucocorticoid concentrations between pastured horses (22.1 ± 9.8 ng/g) and working NYC carriage horses (19.5 ± 4.2 ng/g) in the morning (time 1). Median (range) morning (time 1) fecal glucocorticoid concentrations were 25.5 ng/g (12.1 to 35.6 ng/g) and 19.4 ng/g (12.7 to 29.0 ng/g) for pastured horses and working horses, respectively.

Salivary cortisol concentrations in working horses were significantly (P = 0.03) different among time points **(Figure 1)**. Salivary cortisol concentrations at time 3 (0.99 ± 0.06 ng/mL) were significantly (P < 0.05) greater than those at time 4 (0.77 ± 0.07 ng/mL). However, all other time point comparisons were not different (P > 0.05).

A significant (P = 0.02) difference was found among IRT measurements in working horses among time points, with time 2 temperatures (35.5 ± 0.64°C [95.9 ± 1.2°F]) significantly (P < 0.05) lower than those at time 3 (36.2 ± 0.64°C [97.1 ± 1.2°F]); however, no other difference was found among the other time points **(Figure 2)**. Mean ambient temperature recorded was 26.1 ± 4.5°C (79.0 ± 4.11°F). There was a significant (r = 0.35; P = < 0.001) positive correlation between ambient and IRT temperatures.

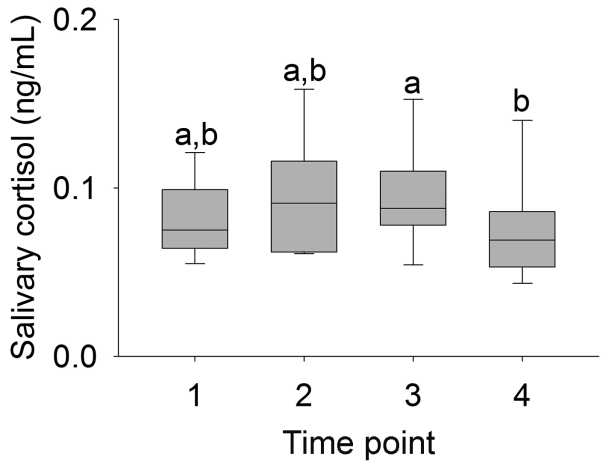

Figure 1—Box-and-whisker plots of salivary cortisol concentrations for working NYC carriage horses across 4 time points. The lower and upper limits of the box represent the 25th and 75th percentiles, the horizontal line within the box represents the median value, and whiskers indicate 10th and 90th percentiles. Differences ($P < 0.05$; Friedman repeated-measures ANOVA on ranks) among the sampled time points are noted with different letters (a and b).

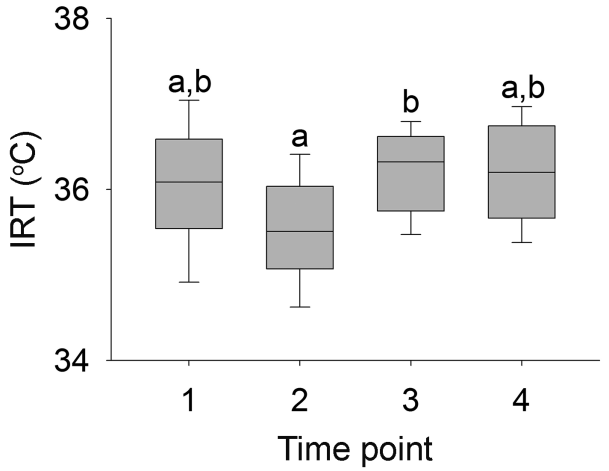

Figure 2—Box-and-whisker plots of IRT findings from imaging the medial canthus for working NYC carriage horses across 4 time points. *See* Figure 1 for key.

Paired IRT measurements and salivary cortisol concentrations at the various time points were not significantly ($r = 0.04$; $P = 0.66$) correlated.

Discussion

Results of the present study indicated that adrenal gland activity did not differ between pastured and working NYC carriage horses, and the daily activities of working NYC carriage horses did not evoke a maladaptive stress response. These results provide some insight into the well-being of NYC carriage horses and suggest that these animals do not perceive their environment or daily activities as a threat.

Transport to and stabling for 5 weeks at a rural pasture in Pennsylvania is part of the annual activity for NYC carriage horses. Because the horses on pasture are of the same population and live in a similar climate but under a less rigorous daily routine than counterparts in NYC, they served as the best proxy to evaluate stress in NYC carriage horses at work. Morning and evening fecal samples from the pastured horses did not reveal significant differences in glucocorticoid concentrations, suggesting that both time points are equally likely to capture general glucocorticoid values. Although this differs from previously described diurnal patterns of serum cortisol concentration in horses,[19] it was not surprising given the pooling effect of excreted steroids in the gut of large mammals.[20] However, to exclude the possibility of diurnal adrenal gland activity, only glucocorticoid concentrations in fecal samples collected in the morning were compared between pastured and working NYC carriage horses. If chronic stress were a factor, greater glucocorticoid concentrations would be expected in the working NYC carriage horses, compared with the pastured NYC carriage horses; however, no difference was found. This observation suggested that the adrenocortical activity in carriage horses between leisure versus working daily routine was not different.

In the present study, salivary cortisol concentrations were used as a proxy for serum concentrations to measure acute stress responses in working carriage horses without the perturbation caused by repeated venipuncture. The only increase in salivary cortisol concentrations in working horses occurred at time 3, which corresponded to when the horses returned to their stalls at the conclusion of their workday. Although an increase in glucocorticoids can be elicited by stressors, glucocorticoids are hormones that also increase during periods of increased metabolic demand (eg, exercise). The mild increase in salivary cortisol concentration may have been the result of the 1.6-km jog back to the stable, rather than a deleterious stressor. Within 1 hour of time 3 (time 4), salivary cortisol concentrations had decreased significantly. When acute adrenal gland activity is stimulated in horses, it can take up to 3 hours for salivary cortisol concentrations to return to baseline.[6] Additionally in the present study, the magnitude of increase from baseline (time 1) to time 3 was not suggestive of stress. For example, transport of horses elicited upwards of a 6-fold increase in salivary cortisol concentrations,[21] whereas the difference between time 1 and time 3 was 1.22-fold. Furthermore, the lack of a discernable diurnal pattern of adrenocortical activity (eg, greater measurement values in the morning than at other time periods) is also a possible adaptation to routine management.[19] Therefore, although an increase in salivary cortisol concentrations was measured at the end of the workday in the horses of the present study, the increase appeared adaptive and related more to meeting the energy demands of the 1.6-km trot and routine daily activities rather than a response to deleterious events.[22]

In the present study, time 2 corresponded to the period immediately after horses were harnessed outside their stalls on the second floor of the stable before being walked down the ramp to the ground floor to be hitched to their carriage either in the stable or in the street in front of the stable. If it were expected that horses would anticipate an aversive experience, the IRT measurements at time 2 would be the highest of the day, but results indicated they were instead the lowest. The NYC carriage horses are carefully screened before selection for this work. A compliant is a desirable and a sought-after trait. In a study conducted with horses at a riding school, Yarnell et al[13] demonstrated that both compliant and noncompliant groups of horses had physiologic changes in response to an aversive procedure (ie, sham clipping of hair). The study highlighted that behavioral response alone cannot be used to evaluate animal management procedures. The compliant nature of urban carriage horses does not provide a solitary explanation for their lack of physiologic response to their impending workday. Importantly, by the variables measured in this study, these horses demonstrated no aversion to the workday.

Although a correlation between salivary cortisol concentrations and IRT measurements across time points previously has been observed in horses,[13] findings in the present study did not demonstrate this association. Given the minimal to nonexistent changes in glucocorticoid concentration and IRT measurements observed in the NYC carriage horses of the present study, a strong correlation between the values was not expected. It should be noted that a change in the hypothalamic-pituitary-adrenal axis or sympathetic shift is not mutually associated with a stressful stimulus or negative psychological response.[23] These alterations are also closely tied to maintenance of homeostasis and metabolic processes.

The study presented here demonstrated the value of objective, noninvasive tools to evaluate the welfare status of specific equine populations. The NYC carriage horses investigated in the present study did not substantiate any physiologic responses that would be indicative of a negative welfare status. Evidence collected indicated no significant perturbation in the state of well-being of this population of horses.

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This text was not reviewed by the sponsor prior to submission.

Footnotes

a. SalivaBio children's swabs, Salimetrics Inc, State College, Pa.
b. Swab storage tube, Salimetrics Inc, State College, Pa.
c. FLIR-T6210, FLIR Systems Inc, Wilsonville, Ore.
d. Allegra X-14R centrifuge, Beckman-Coulter Inc, Brea, Calif.
e. Double antibody monoclonal cortisol enzyme immunoassay, Arbor Assays, Ann Arbor, Mich.
f. Single antibody polyclonal cortisol enzyme immunoassay, Salimetrics Inc, State College, Pa.
g. SigmaPlot, version 12.0, Systat Software Inc, San Jose, Calif.

References

1. Charmandari E, Constantine T, Chrousos G. Endocrinology of the stress response. *Annu Rev Physiol* 2005;67:259–284.
2. Möstl E, Palme R. Hormones as indicators of stress. *Domest Anim Endocrinol* 2002;23:67–74.
3. Lane J. Can non-invasive glucocorticoid measures be used as reliable indicators of stress in animals? *Anim Welf* 2006;15:331–342.
4. Kirschbaum C, Hellhammer DH. Salivary cortisol. In: Fink G, ed. *Encyclopedia of stress*. Vol 3. Oxord: Academic Press, 2000;405–409.
5. Perogamvros I, Keevil BG, Ray DW, et al. Salivary cortisone is a potential biomarker for serum free cortisol. *J Clin Endocrinol Metab* 2010;95:4951–4958.
6. Peeters M, Sulon J, Beckers JF, et al. Comparison between blood serum and salivary cortisol concentrations in horses using an adrenocorticotropic hormone challenge. *Equine Vet J* 2011;43:487–493.
7. Merl S, Scherzer S, Palme R, et al. Pain causes increased concentrations of glucocorticoid metabolites in horse feces. *J Equine Vet Sci* 2000;20:586–590.
8. Palme R. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. *Anim Welf* 2012;21:331–337.
9. Schulman M, Becker A, Ganswindt S, et al. The effect of consignment to broodmare sales on physiological stress measured by faecal glucocorticoid metabolites in pregnant Thoroughbred mares. *BMC Vet Res* 2014;10:25.
10. Fureix C, Benhajali H, Henry S, et al. Plasma cortisol and faecal cortisol metabolites concentrations in stereotypic and non-stereotypic horses: do stereotypic horses cope better with poor environmental conditions? *BMC Vet Res* 2013;9:3.
11. Touma C, Palme R. Measuring fecal glucocorticoid metabolites in mammals and birds: the importance of validation. *Ann N Y Acad Sci* 2005;1046:54–74.
12. Schmidt A, Biau S, Mostl E, et al. Changes in cortisol release and heart rate variability in sport horses during long-distance road transport. *Domest Anim Endocrinol* 2010;38:179–189.
13. Yarnell K, Hall C, Billett E. An assessment of the aversive nature of an animal management procedure (clipping) using behavioral and physiological measures. *Physiol Behav* 2013;118:32–39.
14. Stewart M, Webster J, Schaefer A, et al. Infrared thermography as a non-invasive tool to study animal welfare. *Anim Welf* 2005;14:319–325.
15. Valera M, Bartolomé E, Sánchez MJ, et al. Changes in eye temperature and stress assessment in horses during show jumping competitions. *J Equine Vet Sci* 2012;32:827–830.
16. Wasser SK, Monfort SL, Southers J, et al. Excretion rates and metabolites of oestradiol and progesterone in baboon (*Papio cynocephalus cynocephalus*). *J Reprod Fertil* 1994;101:213–220.
17. Scarlata CD, Elias BA, Godwin JR, et al. Characterizing gonadal and adrenal activity by fecal steroid analyses in pygmy rabbits (*Brachylagus idahoensis*). *Gen Comp Endocrinol* 2011;171:373–380.
18. *SigmaPlot user's guide: version 12*. San Jose, Calif: Systat Software Inc, 2010.
19. Irvine CH, Alexander SL. Factors affecting the circadian rhythm in plasma cortisol concentrations in the horse. *Domest Anim Endocrinol* 1994;11:227–238.
20. Millspaugh JJ, Washburn BE. Use of fecal glucocorticoid metabolite measures in conservation biology research: considerations for application and interpretation. *Gen Comp Endocrinol* 2004;138:189–199.

21. Schmidt A, Hodl S, Mostl E, et al. Cortisol release, heart rate, and heart rate variability in transport-naive horses during repeated road transport. *Domest Anim Endocrinol* 2010;39:205–213.
22. Peeters M, Closson C, Beckers J-F, et al. Rider and horse salivary cortisol levels during competition and impact on performance. *J Equine Vet Sci* 2013;33:155–160.
23. Mormède P, Andanson S, Auperin B, et al. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. *Physiol Behav* 2007;92:317–339.

From this month's *AJVR*

Influence of respiratory tract disease and mode of inhalation on detectability of budesonide in equine urine and plasma

Ann Kristin Barton et al

OBJECTIVE
To evaluate the influence of respiratory tract disease (ie, recurrent airway obstruction [RAO]) and mode of inhalation on detectability of inhaled budesonide in equine plasma and urine samples.

ANIMALS
16 horses (8 healthy control horses and 8 horses affected by RAO, as determined by results of clinical examination, blood gas analysis, bronchoscopy, and cytologic examination of bronchoalveolar lavage fluid).

PROCEDURES
4 horses of each group inhaled budesonide (3 μg/kg) twice daily for 10 days while at rest, and the remaining 4 horses of each group inhaled budesonide during lunging exercise. Plasma and urine samples were obtained 4 to 96 hours after inhalation and evaluated for budesonide and, in urine samples, the metabolites 6β-hydroxybudesonide and 16α-hydroxyprednisolone.

RESULTS
Detected concentrations of budesonide were significantly higher at all time points for RAO horses, compared with concentrations for the control horses. All samples of RAO horses contained budesonide concentrations above the limit of detection at 96 hours after inhalation, whereas this was found for only 2 control horses. Detected concentrations of budesonide were higher, but not significantly so, at all time points in horses that inhaled budesonide during exercise, compared with concentrations for inhalation at rest.

CONCLUSIONS AND CLINICAL RELEVANCE
Results of this study indicated that the time interval between inhalation of a glucocorticoid and participation in sporting events should be increased when inhalation treatment is administered during exercise to horses affected by respiratory tract disease. (*Am J Vet Res* 2017;78:244–250)

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