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An exploratory study of competition scores and salivary cortisol concentrations in Warmblood horses

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ABSTRACT

The main objective of this explorative study was to describe the relationship between competition scores and salivary cortisol concentrations in young horses during dressage and showjumping competitions. The study also investigated whether the diurnal rhythm of salivary cortisol concentrations was affected by competition over consecutive days compared with the home environment. Saliva samples were collected from 126 dressage horses and showjumpers in their home environment and at 3 different events. The relationship between scores given by judges at the competition and cortisol concentrations at the event was assessed. The results demonstrated that competition scores correlated positively to baseline cortisol concentrations at one of 3 events ($r = 0.53$, $P < 0.001$). Salivary cortisol concentrations followed a diurnal rhythm with the highest concentrations measured in the morning and the lowest in the evening, both at home and in the competition environment ($P < 0.05$). Salivary cortisol concentrations were greater during the competitions than at home ($P < 0.05$) except at one event where showjumpers did not increase between home and competition. Dressage horses had the highest baseline cortisol concentrations at competition, and exercise caused cortisol concentrations to increase in both showjumpers and dressage horses ($P < 0.001$). In conclusion, the diurnal rhythm in salivary cortisol concentrations was maintained in the novel environment. Dressage horses demonstrated greater baseline cortisol concentrations at competition than showjumpers, suggesting that they may perceive the novel environment as more stressful. Furthermore, there was no consistent relationship between baseline salivary cortisol concentrations and competition scores across the events.

1. Introduction

Increased cortisol levels are widely used as a physiological stress indicator in animals [1,2]. Cortisol increases in horses in response to both physical and psychological stressors such as transport [3–5], exercise [6–9], and competition [10,11]. During exercise, an increase in hypothalamic–pituitary–adrenal (HPA)-axis activity (resulting in eg, cortisol secretion) stimulates substrate mobilization by enhancing gluconeogenesis, protein catabolism, and the mobilization of free fatty acids [12,13]. The cortisol response to exercise increases with intensity and duration and decreases after repetitive training and with the competition experience of the horse [6,7,14–16]. Little is known however, about the relation between HPA-axis activity and competition performance in horses. Quantifications of cortisol in saliva can be used as a noninvasive estimate of plasma cortisol, as these have been shown to correlate well across the diurnal rhythm in humans [17] and horses [18,19].

In humans, there is some evidence for a relationship between performance in sport competitions and HPA-axis

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activity. A positive correlation was found between postexercise salivary cortisol concentrations and performance during weightlifting competitions [20], and an anticipatory rise in salivary cortisol before competition was associated with better performance in Judo athletes [21] and rowers [22]. In elite female athletes, baseline cortisol concentrations were greater than in nonelite athletes [23].

In horses, a previous report has shown a positive association between postexercise salivary cortisol concentrations and placing in a showjumping competition in an unfamiliar environment [24]. However, the relationship between baseline cortisol, exercise-induced cortisol release, and competition scores in an unfamiliar environment has not yet been studied in horses. Discipline-related differences in cortisol response to exercise have been identified in one previous study: a greater plasma cortisol response was measured in dressage horses compared with showjumpers when introduced to a new environment and after the competition [10]. In addition, the level of competition experience has been found to decrease cortisol concentrations in response to exercise at competitions in both dressage horses [10] and showjumpers [10,11].

Horses kept in a familiar environment accustomed to management, and exercise routines have shown a constant diurnal rhythm in plasma and salivary cortisol concentrations, with the highest concentrations measured in the morning and a decrease throughout the day (eg, [25–28]). There is some evidence that the diurnal rhythm can be disturbed by removing the horse from its familiar environment, or other acute or chronic physical and/or psychological stressors [1,29]. To our knowledge, there is no published literature concerning the effect of consecutive days at competition in an unfamiliar environment on the diurnal rhythm of salivary cortisol concentrations in horses.

The main objective of this explorative study was to investigate the relationship between salivary cortisol concentrations and competition scores in dressage horses and showjumpers. We further aimed to investigate whether the diurnal rhythm of salivary cortisol concentrations was affected by consecutive days of competition compared with the home environment.

2. Materials and methods

The study was in agreement with the 'guidelines for ethical treatment of animals in applied animal behavior and welfare research' from the ethics board of the International Society of Applied Ethology (www.applied-ethology.org).

The data presented in this article were collected on-site at 3 large competition events in Denmark as well as in the various home environments of the horses and should be considered an exploratory study. The 3 events were: (1) the Danish National Stallion Show for 3-yr-old stallions (n = 23) held jointly with the National Championship for 5-yr-old Danish Warmblood horses (n = 21) in 2013 (SH13), (2) the Danish National Stallion Show for 3-yr-old stallions (n = 30) in 2014 (SH14), and (3) the Young Horse Championship (n = 56) in 2013 (YHC). All participants in the 3 events were offered the chance to participate in the study, and horses were included if both the trainer and the owner signed an agreement form. At each event, the horses were assigned to a specific competition according to age.

Table 1
Distribution of sex, discipline, and age in horses at 3 separate events: the Danish National Stallion Show and National Championship for 5-yr-old Danish Warmblood horses in 2013 (SH13), the Danish National Stallion Show in 2014 (SH14), and the Young Horse Championship in 2014 (YHC) and in the home environment.

Event	Item	SH13	SH14	YHC
Sex	Stallion	23	30	19
	Mare	7	0	12
	Gelding	10	0	25
	Total	40	30	56
Discipline	Dressage	22	24	34
	Jumping	18	6	22
Age	3 yr	19	30	0
	4 yr	0	0	22
	5 yr	21	0	15
	6 yr	0	0	17
	Range	3–5	3	4–6
	Mean age of dressage horses	4.1	3	4.8
	Mean age of showjumpers	4.0	3	5.1
Day of collection	Home	39	22	56
	Event day 1	40	30	56
	Event day 2	40	30	0
	Event day 3	40	30	0
	Event day 4	0	24	0
Time of collection at home	Morning 06:00–08:00	Yes	None	Yes
	Midday 11:00–13:00	Yes	Yes	Yes
	Evening 17:00–19:00	Yes	None	Yes
	Pre-exercise	Yes	None	None
	Postexercise 10 min	Yes	None	None
Time of collection at event	Morning 06:00–08:00	Yes	None	Yes
	Midday 11:00–13:00	Yes	Yes	Yes
	Evening 17:00–19:00	Yes	None	Yes
	Pre-exercise	Yes	Yes	Yes
	Postexercise 10 min	Yes	Yes	Yes

2.1. Description of events, horses, and procedures

All horses were Warmblood riding horses (Hanoverian, Oldenburger, Holstein, Danish, or Dutch Warmblood), bred for either dressage or showjumping. Details on age distribution, sex, and discipline at the different events are presented in Table 1. All horses were trained and ridden by professional riders. In the 7 different home environments, the horses were stabled in individual pens and trained daily.

2.1.1. Danish National Stallion Show 2013 (SH13)

Stallions that qualified for the Danish National Stallion Show and horses that qualified for the National Championship for 5-yr-old Danish Warmblood horses in March 2013 were included in the study (n = 40; Table 1). All included horses were sampled 3 times per day (as described in Section 2.2), both at home and on Days 1, 2, and 3 of the event. Furthermore, pre-exercise and postexercise samples were collected at home and on Days 1 and 2 of the event. For the 3-yr-old stallions, home exercise on the day of sampling involved approx. Fifteen min of lunging divided equally by trot and canter. Event exercise consisted of approximately 10 min of free exercise in the show arena on Day 1, and 15 min of lunging divided equally by trot and

canter with side reins on Day 2. The 3-yr-old stallions were regarded as either dressage horses or showjumpers based on their pedigree, but the event exercise did not differ between the disciplines. The 5-yr-old horses were ridden and their exercise at competition consisted of approximately 25 min of warm up and 10 min of either showjumping or dressage, and home exercise was set up to equal the effort at competition.

2.1.2. Danish National Stallion Show 2014 (SH14)

Stallions that qualified for the Danish National Stallion Show in March 2014 were included (n = 30) (Table 1). Saliva samples were collected at midday both at home and at the event on Days 1, 2, 3, and 4. Saliva samples were also collected postexercise on Day 2 of the event. Exercise consisted of 15 min of lunging divided equally by trot and canter with side reins. The stallions were regarded as either dressage horses or showjumpers based on their pedigree, but the event exercise did not differ between the disciplines.

2.1.3. Young Horse Championship (YHC)

Horses that qualified for the Danish Young Horse Championship 2013 for 4-, 5- and 6-yr-old horses were included (n = 56; Table 1). Saliva samples were collected 3 times per day (as described in Section 2.2), at home and on Day 1 of the event. Saliva samples were also collected postexercise at the event, and the exercise consisted of approximately 35 min of warm up and 10 min of competition in either dressage or showjumping. In dressage, the level of difficulty—and thus the expected physical demand—of the competition increased with age; in showjumping 4-yr-old horses jumped ten 110 cm high obstacles, 5-yr-old horses jumped fourteen 120 cm high obstacles, and 6-yr-old horses jumped fifteen 130 cm high obstacles.

2.2. Saliva sampling method and analysis

Saliva was collected with synthetic swabs (Salivette, Nümbrecht-Rommelsdorf, Germany). The swabs were placed under and over the tongue with the help of an arterial clamp for approximately 1 min, until they were soaked with saliva. The swab was then placed in a polypropylene tube and stored at −18°C until analysis.

Sampling of saliva was performed in the morning (06:00–08:00 h), at midday (11:00–13:00 h), in the evening (17:00–19:00 h), and 0–1 h before (pre-exercise) and 10 min after exercise (postexercise) both at home and at the events. However, the number of samples (morning, midday, or evening) and days (at home and Day 1, 2, 3, or 4 of the events) where saliva was collected, differed between the events (Table 1) due to practical reasons. Saliva was sampled either before feeding or a minimum of 1 h after feeding and at least 2 h after exercise when the horses were resting in their stables. The morning or midday samples were used as pre-exercise samples if the horses were exercised within 1 h after either the morning or midday sample. If the morning or midday samples could not be used as pre-exercise samples, a separate pre-exercise sample was collected approximately 1 h before the exercise. Postexercise samples were collected in the stable area 10 min after the horses left the exercise arena. At all the events, the same 3 persons performed the sampling procedure.

All obtained saliva samples (after a 1:10 dilution with assay buffer) were analyzed using a cortisol enzyme immunoassay (for details see Palme and Möstl [30]) without extraction, validated for equine saliva [27,31]. Values should be interpreted as cortisol immunoreactivity, because the antiserum cross-reacts with cortisone and some cortisone metabolites. The intra-assay coefficient of variation was 5.0%, the inter-assay variation was 6.7%, and the minimum detectable concentration was 0.1 ng/mL.

2.2.1. Cortisol levels

Baseline salivary cortisol concentrations refer to the daily average salivary cortisol concentrations both at home and for the events SH13 and YHC (ie, the average of the morning, midday, and evening samples), and the midday salivary cortisol concentrations for event SH14, since only midday samples were obtained at this event. The exercise-induced increase in saliva cortisol concentrations (EXDIF) was calculated as the difference between pre-exercise and postexercise saliva cortisol concentrations. At SH13, EXDIF was measured on two different competition days (Day 1 and 2) and the EXDIF mean was used for the correlation analysis. At SH14, EXDIF was only determined on one of the competition days (Day 2). For the event YHC, the EXDIF values relate directly to the exercise that resulted in the respective competition score, the score was given on the same day as the EXDIF was measured. In the discussion, the EXDIF is further expressed as the percent increase relative to the pre-exercise values to compare the levels found in different studies.

2.3. Competition scores

In both dressage and showjumping, the performance of each horse was evaluated by competition scores given by judges. The 3-yr-old stallions in SH13 and SH14 were evaluated for the movement and conformation in 10 parameters; each parameter was scored from 1 to 10 with a total maximum score of 100. If the total score was below 70, the stallions were not approved for breeding, and the exact scores were not released and were also unavailable for this study. Seven stallions at SH13 and 9 stallions at SH14 were not approved for breeding and they were given a score of 69 in the analysis. The performance score for the remaining stallions was the summation of scores given across 3 days at the event (maximum: 100). For the 5-yr-old horses in SH13 and the 4-, 5- and 6-yr-old horses in YHC, scores were given from 1 to 10 with a total maximum of 100. In dressage, the scores were based on walk, trot, canter, capacity, and rideability, whereas in showjumpers, the scores were based on canter, technique, capacity, and rideability. Since the competition scores at SH13 and SH14 were a summation of scores across days and the scores for individual days were not released, the EXDIF values do not reflect the total amount of exercise that resulted in these competition scores.

2.4. Data analysis and statistics

Except when mentioned specifically, the statistical analyses were performed using the SigmaPlot statistics package, version 13.0 (Systat Software Inc, Chicago, USA). Normality of data was assessed using the Shapiro-Wilk test, and variance homogeneity was assessed via the Brown-Forsythe test. Post hoc testing was performed using a pairwise comparison procedure (Holm-Sidak test).

Due to the skewed distribution on age, sex, and discipline across events, we were unable to perform a multivariable analysis including all these factors. Therefore, the obtained data were analyzed in a number of separate analyses as described below. Effects were considered to be significant at $P < 0.05$ and showing a tendency at $0.05 < P < 0.10$. Values are presented as the mean ± the standard error of the mean.

Spearman's rank correlation coefficient was used to test for correlations between competition scores and cortisol concentrations (both baseline at event and EXDIF) at each separate event. *P*-values <0.05 were considered to be significant.

2.4.1. SH13 (N = 40)

Due to the distribution of age and sex (eg, all 3-yr-old horses were stallions), the data were initially analyzed separately per age class. Thus, data from the nineteen 3-yr-old stallions (discipline; 13 dressage horses, 6 showjumpers) were analyzed in a 1-way ANOVA test for the effect of discipline on baseline cortisol concentration and EXDIF. Data were logarithm transformed to fit normality.

Data from the 21 5-yr-old horses (4 stallions, 10 geldings, 7 mares; discipline; 11 dressage horses, 10 showjumpers) were analyzed in a 2-way ANOVA test for the effect of sex and discipline on baseline cortisol concentration and EXDIF. Data were logarithm transformed to fit normality.

Furthermore, the effects of age and 'time of the day' (morning, midday, evening) on baseline cortisol levels, and the effects of age and environment (home and event Day 1, 2, and 3) on baseline cortisol levels were analyzed in a 2-way ANOVA for repeated measures to account for repeated sampling. Data were logarithm transformed to fit normality.

2.4.2. SH14 (N = 30)

Data from the thirty 3-yr-old stallions (discipline; 24 dressage horses, 6 showjumpers) were analyzed in a 1-way ANOVA test for the effect of discipline on baseline cortisol concentration and EXDIF. Data were logarithm transformed to fit normality.

The effect of discipline and environment (home and event Day 1, 2, 3, 4) on baseline cortisol levels were analyzed in a 2-way ANOVA for repeated measures to account for repeated sampling. Data were logarithm transformed to fit normality.

2.4.3. YHC (N = 56)

Due to the limited number of horses, the data from the 56 horses (age; 22 4-yr-old-, 15 5-yr-old, 17 6-yr-old; sex; 12 mares, 25 geldings, 19 stallions; discipline; 33 dressage horses, 23 showjumpers) could not be analyzed in a 3-way ANOVA test with all the interactions. Therefore the main effects of age, sex, discipline, and time of day on baseline cortisol as well as interactions between these factors were analyzed in SAS (Statistical Analysis System, version 9.3) with a proc mixed procedure including age, sex, discipline, and time of day as well as all 2-way interactions as fixed factors and horse as a random factor. Differences between home and event and interactions between sex, age, and discipline were analyzed using the mean values from home and event with the same model except that time of day was omitted.

3. Results

3.1. The effect of sex and age on salivary cortisol concentrations at the events

For 5-yr-old horses at SH13 and horses at YHC, we did not find any effect of sex on baseline cortisol concentrations (SH13: stallions 1.21 ± 0.21, mares 2.08 ± 0.32, geldings 1.84 ± 0.22 ng/mL, $P = 0.13$, and YHC: stallions 0.67 ± 0.18, mares 0.70 ± 0.17, geldings 0.86 ± 0.10 ng/mL, $P = 0.56$) nor on EXDIF (SH13: stallions 2.20 ± 0.41, mares 2.11 ± 0.50, geldings 1.73 ± 0.33 ng/mL, $P = 0.83$, and YHC: stallions 1.78 ± 0.36, mares 2.25 ± 0.52, geldings 1.81 ± 0.31 ng/mL, $P = 0.72$).

Similarly, in SH13 and YHC, there was no significant effect of age on baseline cortisol concentrations (SH13: 3-yr-old 2.16 ± 0.41, 5-yr-old 2.05 ± 0.27 ng/mL, $P = 0.96$, and YHC: 4-yr-old 0.81 ± 0.12, 5-yr-old 0.61 ± 0.18, 6-yr-old 0.80 ± 0.13 ng/mL, $P = 0.59$). The effect of age on EXDIF could not be investigated as the level of exercise differed between the age groups.

3.2. The relationship between competition scores and salivary cortisol concentrations

There was no correlation between baseline cortisol concentrations and competition scores at the events SH13 (Fig. 1A) and SH14 (Fig. 1B). However, at YHC, there was a positive correlation between baseline cortisol concentrations and competition scores (Fig. 1C). The correlation between EXDIF and competition scores was low and nonsignificant for all the events ($r < 0.14$; $P > 0.58$).

3.3. Diurnal rhythm in salivary cortisol concentrations in the home environment and at events

Saliva cortisol concentrations followed a diurnal rhythm, with the highest concentrations measured in the morning and the lowest concentrations in the evening both at home (SH13) and at the events (SH13 and YHC; Fig. 2). There was no effect of age, sex, or discipline (data not shown).

3.4. Baseline cortisol concentrations at home and at the event

The effect of day (home and Day 1, 2, 3, 4 at the events) on the baseline saliva cortisol concentrations is shown in Table 2. Baseline saliva cortisol concentrations were lower

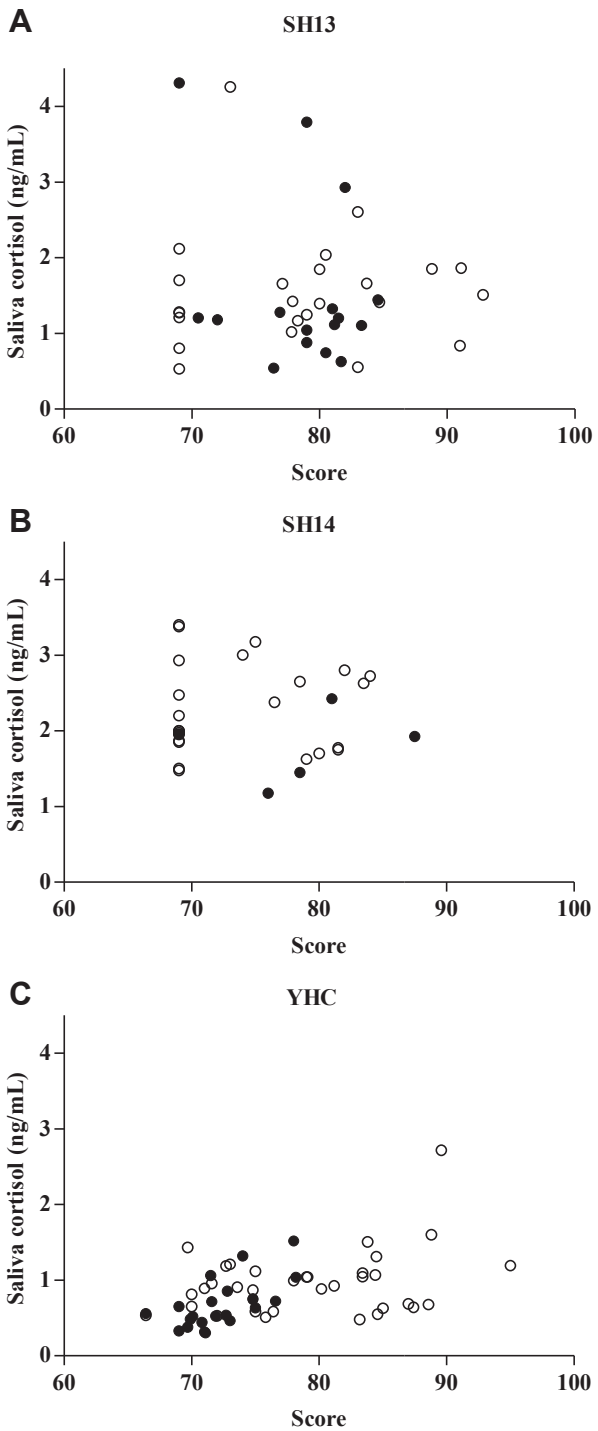

Fig. 1. The correlation between baseline cortisol concentrations (ng/mL) and competition scores; (A) SH13 (overall r = 0.08, $P = 0{,}63$, in dressage r = 0.08, $P = 0.7$ and showjumping r = 0.02, $P = 0.95$), (B) SH14 (overall r = −0.05, $P = 0{,}81$, in dressage r = 0.04, $P = 0.84$ and showjumping r = 0.60, $P = 0.35$), and (C) YHC (overall r = 0.53, $P = 0.0003$, in dressage r = 0.33, $P = 0.05$ and showjumping r = 0.63, $P = 0.002$). The symbols ○ and ● indicate dressage horses and showjumpers, respectively. YHC, Young Horse Championship.

at home than at event in SH13 and SH14 for both dressage horses and showjumpers (Table 2), whereas at event YHC, only dressage horses had a greater baseline cortisol concentrations at the event than at home (dressage horses; home: 0.61 ± 0.07, event: 0.93 ± 0.07, $P < 0.0001$, and showjumpers; home: 0.55 ± 0.09, event: 0.59 ± 0.09, $P = 0.61$). The average saliva cortisol concentrations increased during the Days at SH13 and were significantly greater on Day 3 than on Day 1. For the event SH14, there was no difference in saliva cortisol concentrations between Day 1, 2, 3, and 4 at the event (Table 3). Neither age nor sex (SH13 and YHC) had an effect on baseline cortisol concentrations.

3.5. The effect of discipline on baseline cortisol concentrations and EXDIF

The effect of discipline on the baseline cortisol concentrations and EXDIF is shown in Table 3. In SH13, we found that exercise at home increased salivary cortisol concentrations by 89% compared with pre-exercise concentrations, and during the 3 d of the event, exercise increased salivary cortisol by 135%–139% compared with baseline cortisol concentrations. In SH13, there was no difference between the disciplines for the 3-yr-old stallions, but the 5-yr-old dressage horses had greater baseline and EXDIF cortisol concentrations than showjumpers (Table 3). In SH14, dressage horses had greater baseline and EXDIF cortisol concentrations than showjumpers (Table 3). There was also an effect of discipline on baseline cortisol concentrations at YHC with dressage horses having greater baseline cortisol concentration than showjumpers, and dressage horses tended to have a greater EXDIF than showjumpers (Table 3).

4. Discussion

The results from this study indicate that young sports horses in particular dressage horses have an increased cortisol response to an unfamiliar environment compared with home. However, the diurnal rhythm was maintained in the novel environment. In general, there was no effect of age and sex on salivary cortisol concentrations but the results suggest that especially 4–6-yr-old showjumpers had lower saliva cortisol concentrations than dressage horses.

4.1. The relationship between salivary cortisol concentrations and competition scores

In the present study, we found no significant correlation between EXDIF and competition scores in any of the 3 events, nor between competition scores and baseline cortisol concentrations for the horses participating at SH13 and SH14. However, at event YHC, there was a significant but rather weak biological relationship between competition score and baseline saliva cortisol concentrations. A study by Peeters et al [24], found that the number

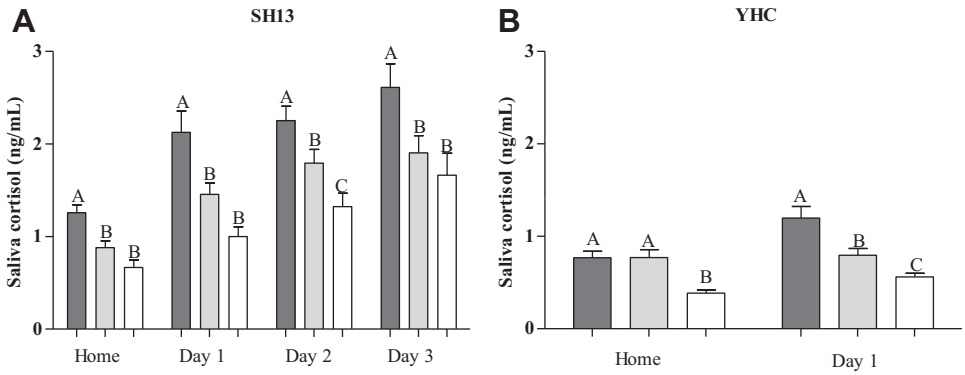

Fig. 2. The effect of time of the day (morning, midday, or evening) on saliva cortisol concentrations (ng/mL) at home and at the events (A) SH13 and (B) YHC (within days, columns with different letters differ significantly [$P < 0.05$]). YHC, Young Horse Championship.

of penalties in a showjumping competition was negatively correlated (r = −0.44) to salivary cortisol concentrations measured 20 min postexercise, however, they did not correlate scores to baseline cortisol or EXDIF in their study. The negative correlation was suggested to reflect that the stress induced during the competition (in a familiar environment, without any transport or change in stabling) was lower than the limit where stress should become distress and potentially have a negative influence on performance.

It has been suggested in human studies that cortisol is related to behavioral patterns important for competitions including aggression, arousal, and mobilization of physiological resources [20–22]. This could also be the situation for the horses in YHC where the competition score was based on physical exercise, sensitivity to rider cues and movements. In regard to the 3-yr-old stallions in SH13 and SH14, part of the competition score was based on conformation to which cortisol is likely unrelated.

It could be assumed that a high-cortisol response to exercise would be associated with a high-competition score in competitions depending on physical parameters but in this study, we did not find a positive relationship between EXDIF and cortisol. Since EXDIF was measured only on one (SH13) or two days (SH13) of the event whereas competition scores related to the entire event, it is not surprising to find that these parameters were unrelated. Further studies that control for the level of physical exercise and include behavioral parameters are required to investigate the potential relationship between competition scores and cortisol responses in horses.

4.2. Diurnal rhythm in saliva cortisol concentrations in the home environment and at events

In the present study, we found a diurnal rhythm in baseline cortisol concentrations in the home environment. There was a difference between morning and evening samples, with a decrease in salivary cortisol concentrations throughout the day. This is in accordance with other studies [25,27,31]. It has been reported that stressful events can disrupt the diurnal rhythm of cortisol concentrations in animals [29,32]. However, this disruption was not apparent in the present study, where horses were housed in an unfamiliar environment at an event for one day (YHC) to three days (SH13). At the events, horses were kept in temporary stables where they could see and hear other horses and with physical and vocal contact with unfamiliar neighbor horses. Despite these conditions, the diurnal rhythm was preserved at the events. The diurnal rhythm of plasma cortisol was also demonstrated in trained racehorses both before and after the exposure to jetlag (change of daylight cycle) [25] and in horses housed in individual stalls or in groups [29]. Other studies have failed to detect a diurnal rhythm in an indoor-stabled group and an outdoor control group of horses [33]. The lack of diurnal rhythm in these studies was interpreted by the authors as being the result of an underlying stressful situation, however, cortisol concentrations can be influenced by feeding time and metabolic requirements that have not been controlled in the studies. In our study, the diurnal rhythm was not disrupted, despite a significant increase in cortisol concentrations at the events, compared with at home (SH13 and YHC).

Table 2
Baseline saliva cortisol concentrations (ng/mL) at home and during 3 different events: the Danish National Stallion Show and National Championship for 5-yr-old Danish Warmblood horses in 2013 (SH13), the Danish National Stallion Show in 2014 (SH14).

Event	Baseline cortisol					
	Home	Day 1	Day 2	Day 3	Day 4	*P*-value
SH13	0.94 ± 0.06^{C}	1.53 ± 0.13^{B}	$1.77 \pm 0.12^{A,B}$	2.03 ± 0.18^{A}	-	<0.001
SH14	1.34 ± 0.32^{B}	2.29 ± 0.22^{A}	2.21 ± 0.14^{A}	2.35 ± 0.22^{A}	2.23 ± 0.18^{A}	<0.001

Values are presented as mean ± SEM.
Home, home environment; Day 1, 2, 3, 4, days of the event. Baseline saliva cortisol concentrations represent midday cortisol concentration in event SH14 and mean cortisol concentration (morning, midday, and evening) for event SH13.
^{A,B,C}Values with no common letters differed significantly within the event ($P < 0.001$).

Table 3
The effect of discipline on baseline saliva cortisol concentrations (ng/mL) and on exercise-induced increase (EXDIF) in saliva cortisol concentrations (ng/mL) in the different age groups at the 3 events: the Danish National Stallion Show and National Championship for 5-yr-old Danish Warmblood horses in 2013 (SH13), the Danish National Stallion Show in 2014 (SH14), and the Young Horse Championship in 2014 (YHC).

Event	Age	Baseline cortisol		*P*-value	EXDIF cortisol		*P*-value
		Dressage	Showjumping		Dressage	Showjumping	
SH13	3	1.76 ± 0.14	1.73 ± 0.21	0.904	1.91 ± 0.23	3.02 ± 0.99	0.281
	5	2.28 ± 0.23	1.40 ± 0.19	0.012	2.56 ± 0.41	1.30 ± 0.33	0.007
SH14	3	2.30 ± 0.12	1.64 ± 0.14	0.015	4.04 ± 0.77	0.73 ± 0.43	0.045
YHC	4	0.93 ± 0.10	0.71 ± 0.18	0.009	1.80 ± 0.37	2.09 ± 0.68	0.074
	5	0.87 ± 0.13	0.61 ± 0.16		2.43 ± 0.51	0.83 ± 0.62	
	6	1.15 ± 0.14	0.69 ± 0.12		2.45 ± 0.54	1.39 ± 0.46	

Values are presented as mean ± SEM.

4.3. Baseline salivary cortisol concentrations at home and during consecutive competition days

We further aimed to investigate the extent to which competition horses adapt to an unfamiliar environment over consecutive days at an event. At SH13, the baseline cortisol concentrations were greater at the event than at home, and it increased over the 3 consecutive days. At SH14, the baseline cortisol concentrations were also greater at the event than at home and remained at the same elevated level across the 4 consecutive competition days. It should be noted, however, that we only obtained midday samples at SH14, whereas the results from SH13 are based on three daily samples and may therefore be more precise. Thus, there were no indications that the horses habituated to the competition environment during the events. At YHC only dressage horses had an increased baseline cortisol concentration, and this could relate to the age distribution of the horses at this event. The 5- and 6-yr-old showjumpers would potentially have more competition experience than the dressage horses from the same event and than the younger horses from SH13 and SH14. Other studies have shown that repeated exposure to a stressor (such as training and transportation) in some cases will lead to a reduced cortisol response, possibly due to habituation [3,4,34,35].

4.4. Cortisol response to exercise

In SH13, the increase in cortisol related to exercise between home (89%) and the event (135%–139%) was most likely to be caused by the additional psychological stress perceived by the horses. It was not possible to determine the extent of the physical response, since we could not measure parameters related to physical activity such as blood lactate or heart rate. However, an exercise-induced cortisol increase of 135%–139% compared with pre-exercise concentrations is not unusually high when compared with the results of other studies, where an increase of 340% was measured in horses after finishing a cross-country course [9], an increase of 150%–360% when showjumping in an unfamiliar environment [7,24,31], or an increase of 200% during a dressage competition [31]. Transportation is regarded as mainly a psychological stressor, and transport has been found to increase cortisol concentrations by 600% compared with pre-transport concentrations [3,4].

The event with the highest exercise-induced increases in salivary cortisol was YHC, where the increase was 220% compared with pre-exercise concentrations, respectively (all age groups and both disciplines included). We did not measure any physical parameters, however, the physical demands at YHC were expected to be greater than for the horses at SH13 and SH14 since competition level increased with age, and there were no 3-yr-old horses at YHC.

4.5. Cortisol response in different disciplines

Showjumpers had lower baseline cortisol concentrations than dressage horses during events, except that there was no difference between dressage and showjumpers for the 3-yr-old stallions at event SH13. This corresponds well with the results from Cayado et al [8], who found that dressage horses had a greater plasma cortisol concentration than showjumpers after the transportation to a new environment, as well as an increased plasma cortisol concentration before exercise at the competition. This may suggest that dressage horses are more sensitive to these types of stressors.

Von Borstel et al [36] investigated the difference in reactivity and time to resume feeding after exposure to a moving object in showjumpers and dressage horses and found that showjumpers reacted less than dressage horses. This difference in fear reactions was independent of training level and therefore genetic heredity was suggested as a reason. Similarly in this study, the difference in baseline cortisol between dressage horses and showjumpers found at SH13, SH14, and YHC may also reflect genetic heredity, but this requires further studies. Management could also explain the difference in cortisol concentrations between dressage horses and showjumpers, because even though the majority of horses at SH13 and SH14 were 3-yr-old horses, they could already have been trained differently.

Furthermore, we found differences in cortisol responses to exercise between disciplines as dressage horses had a greater EXDIF than showjumpers in two of three events (SH13, 5-yr-old only, and SH14) and a tendency in one event (YHC). However, since the values are based on a very low number of horses (only 6 showjumpers participated in SH14), and there is a lack of standardization of exercise between the events the results should be interpreted with caution.

Studies [37,38] have reported that dressage horses tested with and without spectators (only 6 horses), or

showjumpers tested in a competitive and a non-competitive setting did not have significantly different postexercise cortisol responses, suggesting that spectators may not be perceived as a stressor in horses. However, it is difficult to distinguish between the physiological cortisol release related to exercise and the psychological-induced cortisol release caused by environmental factors and since it was not possible to standardize exercise and warm up in our study and we did not have any measures on physical performance, the influence of discipline on EXDIF in this study should be interpreted with caution and require further studies.

5. Conclusion

A diurnal rhythm in salivary cortisol concentrations was confirmed in the home environment and also when stabled in an unfamiliar environment during consecutive days at competition. As expected, baseline salivary cortisol concentrations were greater at the events than at home. Dressage horses generally had greater baseline cortisol concentrations at competition than showjumpers and an acute bout of exercise lasting approximately 30 min, significantly increased cortisol concentrations, with a generally greater response in dressage horses. However, due to the nature of this exploratory study, a large number of factors could not be controlled for, and further studies are required to investigate the potential relationship between competition results, discipline, and cortisol responses.

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