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Effect of age, time record and v_4 on plasma cortisol concentration in standardbred racehorses during exercise

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Summary

The objective of the study was to examine whether age, racing time record or v_4 of standardbred horses had an effect on the changes of cortisol concentration in plasma during exercise. For this purpose standardbred horses of two race barns were submitted to a standardized exercise test (SET). Speed and duration of each run of a SET were such that the blood lactate concentration of each horse exceeded 4 mmol/l after the fourth run or later only. The blood lactate concentration measured after each run was plotted against running speed to derive v_4 from the blood lactate-running speed relation.

The linear relation between speed and plasma cortisol concentration in all horses during SET was low but significant (in race barn A: $r^2 = 0.16$, $p < 0.001$; in race barn B: $r^2 = 0.18$, $p < 0.001$). When the relation between speed and plasma cortisol concentration during SET was examined for each horse individually it was found that in all two-year-olds the linear coefficient of determination was above 0.50 ($r^2 = 0.75 \pm 0.18$), whilst for the older horses it was often below 0.50 ($r^2 = 0.50 \pm 0.34$).

Age did not have a significant effect on mean plasma cortisol concentration in horses after warm-up. But after all runs of SET mean plasma cortisol concentration of two-year-old horses was lower than in horses older than 2 years.

The highest linear coefficient of determination between the racing time record and plasma cortisol concentration was calculated after the last run of SET for horses in one of the race barns ($r^2 = 0.57$). All other coefficients of determination were below 0.40. The highest linear coefficient of determination between v_4 and plasma cortisol concentration was determined after the last run of horses older than 2 years ($r^2 = 0.63$). In conclusion, age affects the development of cortisol concentration during exercise. Cortisol measurements may provide useful information for describing racing time record and v_4 of standardbred racehorses.

Keywords: cortisol, horse, blood, exercise, training, age

Wirkung des Alters, des Rennrekords und von v_4 auf den Kortisolgehalt im Plasma von Trabrennpferden bei Belastung

Ziel der Untersuchung war es zu prüfen, ob das Alter, der Rennrekord oder die v_4 einen Einfluss auf das Ausmaß der Veränderungen des Kortisolgehalts im Plasma von Trabrennpferden bei Belastung haben. Dafür wurden Trabrennpferde aus zwei Rennställen einem Belastungstest unterzogen. Die Geschwindigkeit und Dauer der einzelnen Intervalle jedes Tests waren so gewählt, dass die Laktatkonzentration im Blut frühestens nach dem vierten Intervall den Wert von 4 mmol/l überstieg.

Das Bestimmtheitsmaß für die lineare Beziehung zwischen Laufgeschwindigkeit und Kortisolgehalt im Plasma aller Pferde während des Tests war niedrig aber signifikant (in Rennstall A: $r^2 = 0{,}16$, $p < 0{,}001$; in Rennstall B: $r^2 = 0{,}18$, $p < 0{,}001$). Das Bestimmtheitsmaß für die lineare Beziehung zwischen Laufgeschwindigkeit und Kortisolkonzentration im Plasma während eines Tests betrug für das einzelne 2-jährige Pferd immer mehr als 0,50 ($r^2 = 0{,}75 \pm 0{,}18$). Dagegen war das Bestimmtheitsmaß für dieselben Beziehungen beim einzelnen Pferd, das älter als 2 Jahre war, häufig unter 0,50 ($r^2 = 0{,}50 \pm 0{,}34$).

Die mittlere Kortisolkonzentration im Plasma nach dem Aufwärmen unterschied sich nicht zwischen 2-jährigen und älteren Pferden. Dagegen war der Kortisolgehalt nach allen Intervallen des Belastungstests bei den 2-jährigen Pferden niedriger als bei den älteren Pferden.

Der höchste Wert für das Bestimmtheitsmaß der Beziehung zwischen Rennrekord und Kortisolgehalt im Plasma wurde für das letzte Intervall des Tests berechnet ($r^2 = 0{,}57$). Alle anderen Beziehungen hatten Bestimmtheitsmaße unter 0,40. Den höchsten Wert der Bestimmtheitsmaße für die Beziehungen zwischen v_4 und der Kortisolkonzentration im Plasma errechnete sich auch für das letzte Intervall des Belastungstests bei den Pferden älter als 2 Jahre ($r^2 = 0{,}63$). Schlussfolgernd kann festgestellt werden, dass Alter einen Effekt auf das Verhalten der Kortisolkonzentration im Plasma von Trabrennpferden bei Belastung hat. Die Messung der Kortisolkonzentration kann nützliche Information zur Einschätzung des Rennrekords und der v_4 geben.

Schlüsselwörter: Kortisol, Pferd, Blut, Belastung, Training, Alter

Introduction

Physical stress influences the cortisol response of the horse. This has been well documented (*Thornton 1985, Ferlazzo and Fazio 1997*). What is missing are studies on factors like age, duration and speed of exercise, athletic capacity, time of day, which may influence the changes of blood cortisol concentration in exercising horses. Effects of these factors may contribute to the difficult interpretation of results in performance diagnosis studies (*Persson et al. 1980, Baker et al. 1982, Grosskopf et al. 1983, Dybdal et al. 1990*).

The objective of this study was to examine whether age, racing time record or v_4 of standardbred horses had an effect on the changes of cortisol concentration in plasma during exercise.

Material and Methods

Horses and study design

The same study design was applied in two race barns. In race barn A thirteen healthy standardbred horses were involved (mean SD body weight: 455± 34 kg; table 1). The seven horses of this group older than 2 years already participated in races and had a racing time record. In race barn B 18 horses were tested, of which all 12 horses older than 2 years also had racing time records (body weight 482± 23 kg; table 1).

All horses were conditioned for competition for at least half a year before the study. All horses were submitted to standardized exercise tests (SET), but the guidelines differed according to the length of the oval dirt track available: in race barn A it was 1,000 m long, while in race barn B it was 800 m long. In race barn B the guideline of the SET for the two-year-old horses and for the older ones was different (table 2). During the tests, the drivers used a watch to set the pace. Before each test, the track was groomed to ensure regular lane conditions. In both cases all horses of a barn involved in the study were tested on the same day under similar weather conditions (mean SD of environmental temperature at race barn A 21.6 ± 2.5°C, relative humidity 70.4 ± 6.0%, at race barn B 22.3 ± 2.0° C and 65.2 ± 5.7 % respectively).

Standardized exercise test (SET)

The speed and duration of each run of the SET was conceived so that the blood lactate concentration did not reach a lactate concentration of 4 or more mmol/l before the fourth run (table 2). The test was discontinued when the blood lactate concentration of the horses was at or above 4 mmol/l only. Horses always started and finished a run at the same place. Runs were separated by a period of three minutes. During this time horses were stopped, blood samples taken and horses walked back to the start point. Velocity was maintained by the drivers who timed their progress, using a watch. Additionally, each run was timed by an independent observer with a stopwatch accurate to 0.1 seconds. Before the test but after warm-up (4 to 6 min walking to the track and 5 to 10 min of slow trotting on it), and within 30 seconds after each run, blood samples were collected for determination of lactate concentration. With the measured blood lactate concentration and running speed for each run of the SET v_4 was calculated from the blood lactate-running speed relation by exponential regression analysis.

Blood sample collection

The jugular vein was punctured for blood collection into Na-heparinized evacuated tubes.

Lactate analysis

Twenty microliters of blood was immediately transferred to vials with 200 µl of 0.6 n perchloric acid. Samples were centrifuged at 12,000 rpm for 10 minutes, and the supernatant was transferred to empty vials. Samples were stored at 4° C for up to 4 days until analysis, using an enzymatic test kit (Boehringer Mannheim No.1178750). The intra-assay coefficient of variation for this enzymatic method was 3.2% at a lactate concentration of 2.15 mmol/l and was 4.0% at a concentration of 4.4 mmol/l. Beginning with the third run blood lactate concentration of horses after each run was measured on site with Accusport® (*Lindner 1996*). When the blood lactate concentration was above 4 mmol/l the exercise test was finished.

Cortisol analysis

Samples were placed in a refrigerator immediately after collection and centrifuged within 4 hours after collection at 10,000 rpm for 10 minutes. The plasma was transferred to empty vials and stored at –20° C until analysis, using a commercial test kit (Boehringer Mannheim No. 649945). The intra-assay and inter-assay coefficient of variation for the method was 8%.

Tab. 1: Age, gender, racing time record, v_4 and daytime when warm-up for test was started for Standardbred racehorses

Alter, Geschlecht, Rennrekord und v_4 von Trabrennpferden sowie Tageszeit zu Beginn der Aufwärmphase für Tests

Race Barn	Horse	Parameter				
		Age	Gender	Race record min:s:ms	v_4 m/min	Daytime of start of test
A	1	7	stallion	1:12:9	615	8:30
	2	5	stallion	1:14:9	630	12:00
	3	4	mare	1:20:3	596	12:00
	4	4	mare	1:23:1	560	8:30
	5	4	stallion	1:20:6	580	9:30
	6	3	mare	1:23:1	572	13:30
	7	3	mare	1:18:1	560	8.30
	8	2	stallion	-	576	15:30
	9	2	mare	-	545	17.00
	10	2	mare	-	500	15:30
	11	2	mare	-	527	14:30
	12	2	stallion	-	572	14:30
	13	2	mare	-	505	17:00
B	1	3	stallion	1:16:3	617	11:00
	2	4	stallion	1:15:8	604	12:00
	3	4	stallion	1:14:7	606	9:30
	4	4	stallion	1:15:0	603	8:30
	5	3	stallion	1:17:7	595	11:00
	6	3	stallion	1:18:8	607	8:30
	7	5	stallion	1:12:0	667	9:30
	8	5	stallion	1:15:1	640	9:30
	9	3	stallion	1:17:8	575	12:00
	10	3	stallion	1:17:3	570	12:00
	11	4	mare	1:17:0	590	11:00
	12	4	stallion	1:16:4	579	8:30
	13	2	stallion	-	574	15:00
	14	2	stallion	-	570	15:00
	15	2	stallion	-	580	14:00
	16	2	stallion	-	583	14:00
	17	2	stallion	-	551	14:00
	18	2	mare	-	539	15:00

Tab. 2: Requested speed and number of laps per run for performance testing standardbred racehorses

Vorgeschriebene Geschwindigkeit und Anzahl Runden jedes Intervalls der Belastungstests von Trabrennpferden

Run	Race barn								
	A (1,000 m track)			B (800 m track)					
	All horses (n = 13)			> two-year-olds (n = 12)			two-year-olds (n = 6)		
	v m/s	Number of laps	Total time run min:s	v m/s	Number of laps	Total time run min:s	v m/s	Number of laps	Total time run min:s
1	6.00	2	5:34	7.67	3	5:12	7.00	3	5:42
2	7.00	2	4:46	8.33	4	6:24	7.67	3	5:12
3	8.00	3	6:15	9.00	4	5:56	8.33	3	4:48
4	9.00	3	5:33	9.67	4	5:28	9.00	4	5:56
5	10.00	3	5:00	10.33	4	5:12	9.67	4	5:28
6	11.00	3	4:33	11.00	4	4:52	10.33	4	5:12

v = velocity min:s = minutes:seconds

Driving accuracy of drivers

Two drivers exercised all horses in race barn A, and three in race barn B. All were experienced, active race drivers. The mean difference between asked and driven speed was 0.12 ± 0.12 m/s for drivers in race barn A (0.23, 0.05, 0.16, 0.04, 0.05 and 0.10 m/s for the six requested speeds respectively), and for drivers in race barn B 0.10 ± 0.04 m/s (0.13, 0.15, 0.07, 0.07, 0.12, 0.13 and 0.05 m/s for the six requested speeds respectively).

Data analysis

Analysis of variance for repeated measures was used to analyze the effect of trotting consecutive runs at incrementing speeds on cortisol concentration of horses of two years of age and older than two years of age (race barn A horses only). To determine where data between the two age groups was significantly different the t-test for non-dependent samples was applied. The relation between cortisol concentration at defined phases of SET or of cortisol concentration changes between defined phases of SET and racing time record as well as v_4 was evaluated by means of linear and exponential regression. The following differences were calculated to examine changes of the cortisol concentration during SET:

- absolute difference = value after 4th run – value after warm-up
- relative difference = value after run at which blood lactate concentration exceeded 4 mmol/l – value after warm-up.

$p < 0.05$ was accepted as significant for all statistical methods.

Results

The cortisol concentration in plasma of 11 out of 12 two-year-old horses increased after the first run of SET, and only in one did it decrease (Figure 1 and 2). In contrast, an increase of plasma cortisol concentration after the first run of SET was only observed in twelve of the 19 horses older than 2 years tested (table 3). The plasma cortisol concentration in the other horses decreased (n = 1), or was U-shaped (n = 1), bell-shaped (n = 4), or horizontal (n = 1).

The coefficient of determination for the linear regression between speed and plasma cortisol concentration in all horses during SET was low but significant (in race barn A $r^2 = 0.16$, $p < 0.001$; in race barn B $r^2 = 0.18$, $p < 0.001$; almost identical values for exponential regression). When the relation between speed and plasma cortisol concentration during SET was examined for each horse individually it was found that in all

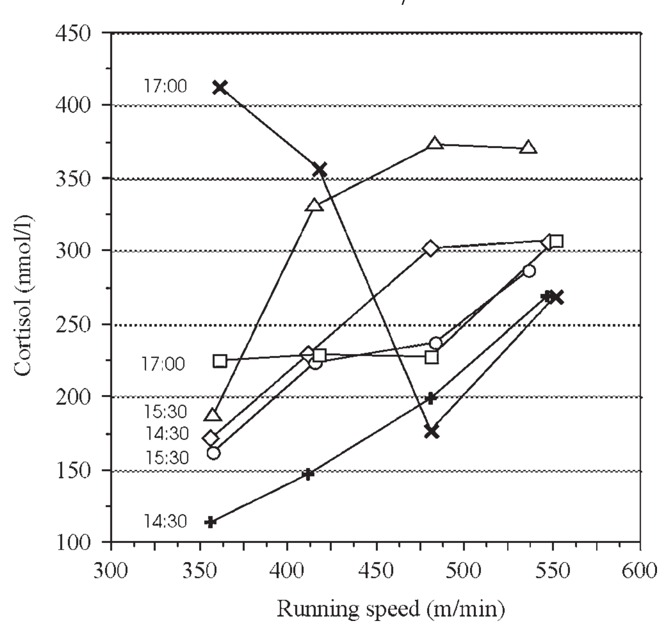

Fig. 1: Cortisol concentration in plasma of two-year old standardbred horses of race barn A during a standardized exercise test (6 horses; numbers before first value of each horse show the time when test was started)

Kortisolgehalt im Blutplasma der 2-jährigen Trabrennpferde von Rennstall A während eines Belastungstests (6 Pferde; Zahl vor erstem Wert zeigt an wann Test begann) two-year-olds the linear and exponential coefficient of determination was above 0.50 ($r^2 = 0.75 \pm 0.18$ and $r^2 = 0.88 \pm 0.17$ for linear and exponential regression respectively), whilst for the older horses it was often below 0.50 ($r^2 = 0.50 \pm 0.34$ and $r^2 = 0.71 \pm 0.29$ for linear and exponential regression respectively).

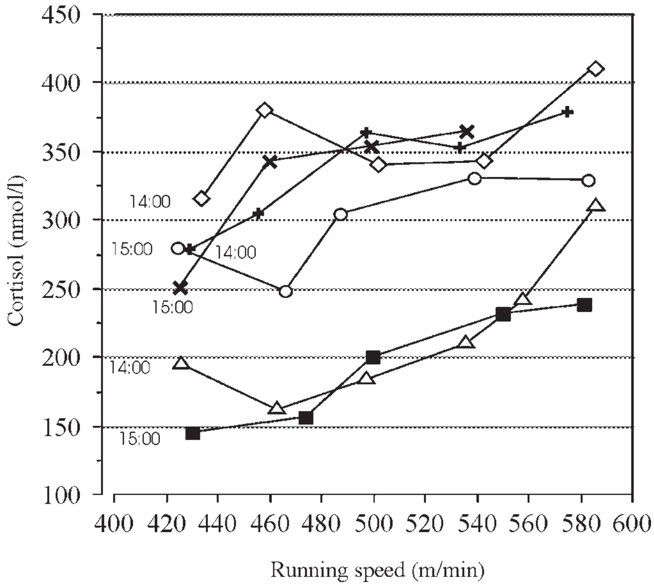

Fig. 2: Cortisol concentration in plasma of two-years old standardbred horses of race barn B during a standardized exercise test (6 horses; numbers next to first value of each horse show the time when test was started)

Kortisolgehalt im Blutplasma der 2-jährigen Trabrennpferde von Rennstall B während eines Belastungstests (6 Pferde; Zahl vor erstem Wert zeigt an wann Test begann)

Age did not have a significant effect on plasma cortisol concentration after warm-up in horses of either race barns. After all runs of SET plasma cortisol concentration of two-year-old horses was lower than in horses older than 2 years (Figure 3; horses of race barn A were only comparable because the same test guideline was used for all horses).

Exponential regression analysis of the relation between racing time record or v_4 and plasma cortisol concentrations did not

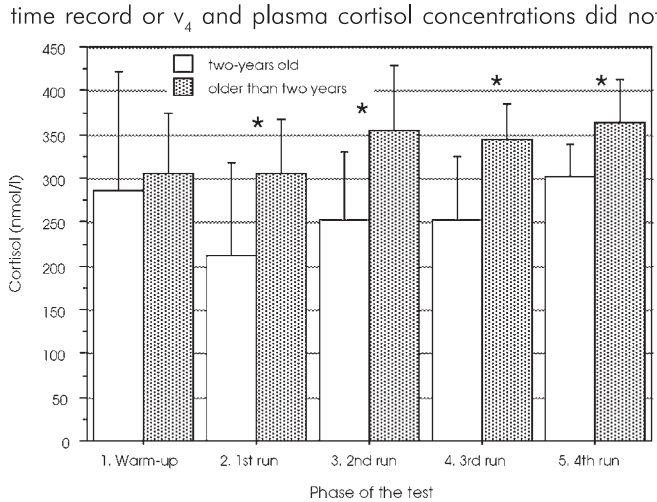

Fig. 3: Cortisol concentration in plasma of two-year-olds (n = 6) and older standardbred horses (n = 7) of race barn A during a standardized exercise test (mean± standard deviation: * = $p < 0.05$)

*Kortisolgehalt im Blutplasma von 2-jährigen (n = 6) und älteren Trabrennpferden (n = 7) während eines Belastungstests (Rennstall A; Mittelwert ± Standardabweichung; * = p < 0.05)*

provide a better interpretation of results than linear regression analysis and therefore only the latter will be referred to.

The highest coefficient of determination between the racing time record and plasma cortisol concentration was calculated after the last run of SET for horses in race barn A ($r^2 = 0.57$; table 4). All other coefficients of determination were below 0.40.

The highest coefficient of determination between v_4 and plasma cortisol concentration was also determined after the last run of horses older than 2 years in race barn A (table 4). The same holds for horses older than 2 years in race barn B but with a lower value. In horses of this age group in race barn A v_4 also explained a large proportion of the change of cortisol concentration between the last interval and warm-up ($r^2 = 0.49$; table 4).

In two-year-olds all coefficients of determination between v_4 and plasma cortisol concentrations were below 0.32 (table 5).

Discussion

The mean cortisol concentration in plasma of horses submitted to incremental exercise tests increased during exercise. This behaviour of cortisol is generally described in literature (*Church et al. 1987, Nagata et al. 1999, Lindner et al. 2000, Tedeschi et al. 2000*). But the individual pattern of the cortisol concentration in plasma showed an increase with each consecutive interval in only 23 out of 31 horses involved in this study and in 11 out of 12 two-year-old horses. The reason for the different patterns of the plasma cortisol concentration during SET is unknown. A factor which may play a role is the psychological stress, whose importance has not been cleared in the horse. Some of the horses studied started with rather high plasma cortisol concentrations after warm-up. It could be that the cortisol secretion after psychologic stimulus inducing a large increase of the cortisol concentration in blood is re-duced or at least delayed. This has been demonstrated for adrenaline and nor-adrenaline in plasma of horses by *Kurosawa et al. (1997)*.

Different patterns of the cortisol response during and after exercise are induced by the type of exercise (*Thornton 1985, Ferlazzo and Fazio 1997, Nagata et al. 1999*). But so far standardized differential studies on the reaction of plasma cortisol concentration in horses to duration and speed of exercise have not been published. Another explanation for a reduced or lacking increase in plasma cortisol concentration during exercise could be insufficient exercise stress (*Thornton 1985*). However, in this study metabolic stress induced by SET was controlled for each horse by its blood lactate concentration. Thus at least metabolic stress was similar for the horses of a barn, or of an age group in the case of horses of barn B.

The time of the day when the test was performed by the horses could affect the cortisol response to exercise due to its circadian rhythm (*Evans et al. 1977*). Studies on this aspect are not available. Most horses older than two years were tested in the morning, some at noon and early afternoon, while the two-year-olds were all examined in the afternoon. Among the older horses the influence of daytime on the cortisol response to exercise was not evident. However, it could well provide for the more homogeneous reaction in two-year-olds. Superimposed upon the circadian rhythm of cortisol is an ultradian rhythm, which is variable within the same horse at least among mares that were not in sportive condition (*Evans et al. 1977*). This observation may be more extreme in sport horses. Single plas-

Tab. 3: Plasma cortisol concentration of standardbred horses after warm-up and after each run of a standardized exercise test

Kortisolgehalt im Plasma von Trabrennpferden nach dem Aufwärmen und nach jedem Intervall der Belastungstests

Race Barn	Horse	Daytime of start of test	Cortisol (nmol/l)						
			After warm-up	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6
A	1	8:30	217	208	260	294	266	308	-
	2	12:00	242	279	289	315	355	298	-
	3	12:00	327	340	355	391	391	349	-
	4	8:30	258	298	298	372	419	324	412
	5	9:30	370	391	398	387	371	-	-
	6	13:30	404	264	448	307	355	432	428
	7	8.30	317	355	432	345	391	444	-
B	1	11:00	266	225	299	342	319	405	-
	2	12:00	188	198	211	248	253	287	-
	3	9:30	271	292	312	293	363	329	-
	4	8:30	255	292	297	310	346	394	-
	5	11:00	280	214	207	213	292	334	-
	6	8:30	344	355	360	384	399	389	-
	7	9:30	284	344	344	341	401	380	383
	8	9:30	391	391	352	444	353	392	-
	9	12:00	242	271	259	245	285	-	-
	10	12:00	371	347	385	388	367	-	-
	11	11:00	248	277	329	379	318	334	-
	12	8:30	407	293	286	318	334	-	-

Tab. 4: Dependence of plasma cortisol concentration at different times of blood sampling during SET from racing time record and v_4 of standardbred racehorses older than two years

Abhängigkeit des Kortisolgehalts im Plasma zu verschiedenen Zeitpunkten des Belastungstests vom Rennrekord und v_4 von Trabrennpferden älter als 2 Jahre

Time of blood sampling	Race barn			
	A (1.000 m track: n = 7)		B (800 m track: n = 12)	
	Race record	v_4	Race record	v_4
After warm-up	r^2 =0.10	r^2 =0.00	r^2 =0.01	r^2 =0.00
After 4th run	r^2 =0.04	r^2 =0.28	r^2 =0.10	r^2 =0.19
After last run	r^2 =0.57 *	r^2 =0.63 *	r^2 =0.10	r^2 =0.25 **
Difference 4th run - warm-up	r^2 =0.03	r^2 =0.14	r^2 =0.15	r^2 =0.14
Difference last run - warm-up	r^2 =0.08	r^2 =0.49%	r^2 =0.08	r^2 =0.14

* = $p < 0.05$ ** = $p = 0.10$

ma cortisol values during and after exercise will most likely mislead diagnosis. With repeated measures of cortisol concentration during exercise, as done in this study, and subsequently also, this drawback may be overcome. To take several blood samples during SET was preferred in this study. The reason is

Tab. 5: Dependence of plasma cortisol concentration at different times of blood sampling during SET from v_4 of two-year-old standardbred racehorses

Abhängigkeit des Kortisolgehalts im Plasma zu verschiedenen Zeitpunkten des Belastungstests vom Rennrekord und v_4 von 2-jährigen Trabrennpferden

Time of blood sampling for cortisol measurements	Race barn	
	A (1,000 m track; n = 6)	B (800 m track: n = 6)
After warm-up	r^2 = 0.06	r^2 = 0.23
After 4th run	r^2 = 0.28	r^2 = 0.31
After last run	r^2 = 0.28	r^2 = 0.17
Difference 4th run - warm-up	r^2 = 0.15	r^2 = 0.00
Difference last run - warm-up	r^2 = 0.15	r^2 = 0.22

that exact timing of repeated blood sampling in sport horses after exercise is almost impossible because access to them is difficult during cooling down and rehabilitative measures to which they are and need to be submitted.

The more uniform pattern of the plasma cortisol concentration changes during exercise in two-year-olds compared to the older horses indicates that there was an age effect. This effect of age was reflected also in the lower cortisol concentrations in two-year-olds compared to the older horses during all runs of the test. *Horohov et al. (1999)* observed that in Standardbred mares older than 20 years the cortisol concentration increased less than in mares with an average age of 5 years after continuous exercise on treadmill. Therefore, the age of a horse has to be considered when cortisol measurements during exercise are done for performance diagnosis.

Between the racing time record of horses in race barn A and their plasma cortisol concentration after the last run there was a relative high relation. This result was encouraging but could not be verified in horses of race barn B, or for other times of blood sampling. So far the validity of measuring cortisol concentration in blood of horses during and after exercise for performance diagnosis has not been studied intensively. Only two studies on this subject were found in literature, but in both, horses were examined with non-standardized exercise. *Grosskopf et al. (1983)* found smaller increases, while *Dybdal et al. (1990)* did not observe differences in horses with a better performance after endurance competition compared to horses with lower performance. In some studies blood cortisol concentration was measured for poor performance diagnosis (*Persson et al. 1980, Baker et al. 1982*) or to monitor overtraining (*Bruin et al. 1994, Golland et al. 1996)*. The results in these studies are contradictory and demand further research.

The value of determining v_4 of horses for competitive performance diagnosis has been demonstrated several times (*Lindner 1997*). A good agreement between racing time record and v_4 of horses in race barn A and race barn B has been demonstrated (*Lindner 1998, Lindner 2000*). Thus the finding that v_4 and plasma cortisol concentration were highly related after the last run of SET in horses of race barn A indicated its potential use for performance diagnosis. But, again this result could not be verified in horses of race barn B. Further studies have to be done to clarify whether cortisol is a useful variable for performance diagnosis.

In conclusion, age affects the development of cortisol concentration during exercise, and has to be considered for the interpretation of results and design of studies. In such case cortisol measurements may provide useful information for describing racing time record and v_4 of standardbred racehorses.

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