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Plasma cortisol concentration during standardized exercise in Standardbred racehorses within a racing season

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Summary: Performance diagnosis of Standardbred racehorses needs to be improved. It was examined whether parameters derived of the blood plasma cortisol concentration (CORT) during a standardized exercise test (SET) used to determine the endurance parameter v_4 (velocity run under defined conditions inducing 4 mmol/L blood lactate concentration) provided additional information for performance diagnosis of two-, three-, and four-year-old Standardbred horses during a racing season. The CORT parameters were $CORT_{start}$ (CORT after warm-up but before starting), $CORT_{max}$ (maximal CORT) and $CORT_{max-min}$ (difference between the $CORT_{max}$ and $CORT_{min}$). The v_4 of the horses was related to all CORT derived parameters and the effect of testing in April, June and August on the CORT derived parameters was examined for all age-groups. The date of testing did not influence CORT derived parameters in any age group ($P > 0.05$ all). $CORT_{max-min}$ of the four-year-old horses was the only CORT derived parameter showing a significant relationship with v_4 ($P = 0.048$; $r^2 = -0.27$). None of the CORT derived parameters was different among age-groups. In conclusion, further studies need to be done to clarify whether the CORT parameters defined are useful for performance or poor performance diagnosis.

Keywords: horse, exercise, fitness, lactate, performance, test

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Introduction

The objective of training is to improve the performance of athletes. The blood plasma cortisol concentration (CORT) seems worthy for consideration as a parameter for performance diagnosis due to its wide spectrum of actions within the energy delivering systems and many other functions (*Ferlazzo* and *Fazio* 1997, *Ambrojo* et al. 2018, *Ferlazzo* et al. 2020). In addition, CORT is widely accepted as a marker of stress (*Cayado* et al. 2006, *Schmidt* et al. 2010, *Lorello* et al. 2017, *Bohák* et al. 2018) and used as a marker of overtraining (*Golland* et al. 1999, *de Graaf-Roelfsema* et al. 2007) which is frequent in Standardbred racehorses (*Tyler* et al. 1996). In practice, standardized exercise tests (SET) are used for diagnosing endurance and assessing effects of training (*Couroucé* et al. 1997, *Lindner* 2010, *Lorello* et al. 2017). The variable derived from the SET that has most frequently shown a strong relationship with parameters of sports performance is v_4 (velocity run under defined conditions inducing 4 mmol/L blood LA) (*Erickson* et al. 1991, *Casini* and *Greppi* 1996, *Couroucé* et al. 1997, *Lindner* 2010, *Fraipont* et al. 2012).

Few studies relating performance of sport horses to plasma CORT derived parameters were found in the literature. *Persson* et al. (1980) noted that poor performing Standardbred racehorses had a lower mean CORT than normally performing horses after ACTH injection. *Grosskopf* et al. (1983) found smaller CORT increases, while *Dybdal* et al. (1980) did not observe differences in horses with a better performance after endurance competition compared to horses with lower performance. *Munk* et al. (2017) did not observe a consistent relationship between baseline salivary CORT and competition scores of show-jumping and dressage horses and *Ferlazzo* et al. (2012) found no CORT difference between show-jumping horses with or without faults during competition. Finally, *Witkowska-Piłaszewicz* et al. (2021) describe that the best performing Purebred race Arabians experience a lower increase of serum cortisol than worse runners after racing.

Lindner et al. (2002) examined the relationship between the v_4 of Standardbred racehorses of two racing yards with parameters derived from CORT during SET. They describe a significant positive relationship between v_4 and CORT after the last interval of SET in the horses older than two years, but not in two-year-old horses. The objective of the actual study was to verify the results of the study of *Lindner* et al. (2002) and to examine the effect of the testing time during a racing season on parameters of CORT derived during SET of horses of differing age. It was hypothesized, that the CORT derived parameters change during the racing season and that there would be significant relationships between them and the v_4 of two-, three- and four-year old Standardbred racehorses in training for competition or racing already. In addition, the data of all age-groups were compared to the values of the highest money earning Standardbred racehorse in Germany during the season documented.

Materials and methods

This research was a spin-off of veterinary service for the racing yard described. The objective of it was to monitor the athletic and health development of the horses continuously throughout the year. The amount of blood collected for performance diagnosis was sufficient to analyse other variables than blood lactate only. Thus, an approved statement of ethics was not necessary for the present work.

Trainer

The trainer was one of the most successful in Germany in the decade 1990–2000. He allowed full insight into his training. The training was made into a protocol in the year 1999 (*Wegener* 2000, *Wegener* et al. 2012). Several of his horses won the German Derby and competed at the highest international levels. From 1997 to 2000, between 25% and 30% of his starters won the race in which they were started (*Hauptverband für Traber-Zucht* 1997–2000). By comparison, this proportion was between 17% and 20% for the five trainers with the most winnings/year.

Horses

The training yard had 20 two-year-old, 16 three-year-old, 15 four-year-old and eight horses older than four years. The health and performance of all horses was monitored continuously by veterinary surgeons. The monitoring involved determing, as regularly as possible, v_4 without interfering in the competition schedule. The data of horses tested within three days before and after April 2, June 20 and August 29, 1999, were used for this study only. In total data of four Standardbred racehorses of two-, three- and four-years of age each and of a three-year-old stallion, that became the most money winning horse in Germany during the year, were available. The latter horse was called BEST for the purpose of this study. All horses had been reared and were being managed under similar conditions, kept in 3 × 4 m boxes on straw bedding and with pasture access for a couple of hours on most days. All horses were trained for racing, but distance, speed and frequency of exercise varied according to the race dates selected for them. The training of the horses has been described in detail (*Wegener* 2000, *Lindner* et al. 2019). Horses involved in the study are those that were submitted to SET at the time periods selected (early April, mid-June and late August). These were healthy horses only, not receiving medication.

Standardized exercise test

The SET prescription differed between the two-year-old and the older horses (Table 1). Horses were tested always in groups of up to three horses of the same age at the same time of day at each SET date. SETs were performed between 7:30 and 10:30 hours only. The older horses always exercised first, the younger last.

During the SET, the drivers constantly used a stop-watch to control the prescribed pace. Before each SET, the 800 m long track was prepared to ensure regular lane conditions. The horses were warmed up for between 10 and 15 minutes at a walk and slow trot before starting the SET. The increase in speed from interval to interval was such that a continuous increase in the blood LA from the concentration before exercise but after warm-up to ≥ 4 mmol/L was obtained in no less than four intervals of approximately 5 minutes each (Table 1). This was done to obtain at least four values to describe the blood LA-running speed relationship (BLRS) and to run the horses as slow as possible to obtain the 4 mmol/L of blood LA. The SET was discontinued when the blood LA of the horses was at or above 4 mmol/L (determined on site with Accusport™; Roche Diagnostics, Mannheim, Germany) (*Lindner* 1996). The horses always started and finished an interval at the same place. This place was near to the entrance track, so that the horses could be taken out of the track within seconds of finishing an interval and blood samples could be collected generally within 20 s after an interval. The intervals were separated by a period of 3 minutes. An independent observer, with a stop-watch accurate to 0.1 seconds, timed every lap of each interval. In Table 1 are shown the mean speeds in each SET interval derived from the time clocked by the independent observer and the distance run by the horses. With the measured blood LA and running speed for each interval of the SET, v_4 was calculated from the BLRS relationship by an exponential regression analysis (*Galloux* 1991).

Table 1 Standardized exercise test prescribed for 2-year-old and older Standardbred racehorses. The track was 800 m long (means ± standard deviation). | *Standardisierter Belastungstest für 2-jährige und ältere Trabrennpferde. Die Rennbahn war 800 m lang (Mittelwert ± Standardabweichung).*

Interval	2-year-olds			> 2-year-olds		
	Distance (m)	v (m/s)		Distance (m)	v (m/s)	
		Prescribed	Run (n)		Prescribed	Run (n)
1.	2,400	6.00	6.11 ± 0.39 (12)	2,400	6.67	6.84 ± 0.19 (27)
2.	2,400	6.67	6.64 ± 0.11 (12)	2,400	7.50	7.53 ± 0.11 (27)
3.	2,400	7.33	7.31 ± 0.05 (12)	2,400	8.33	8.32 ± 0.18 (27)
4.	2,400	8.00	7.92 ± 0.15 (12)	3,200	9.17	9.13 ± 0.12 (27)
5.	2,400	8.67	8.72 ± 0.12 (12)	3,200	10.00	9.92 ± 0.19 (27)
6.	3,200	9.33	9.34 ± 0.28 (12)	3,200	10.83	10.8 ± 0.29 (13)

v = velocity / n = number of SETs / v Run = v derived from the time clocked by the independent observer and the distance run by the horses

Cortisol

Blood (5 ml) was sampled by venepuncture from the jugular vein after warm-up but before commencing the SET ($CORT_{start}$) and as soon as possible after each interval of the SET, normally within 20 seconds, into Na-heparinized evacuated tubes (Becton Dickinson, Heidelberg, Germany). Due to the working routine in the yard, some $CORT_{start}$ samples were missed. The collected blood samples were centrifuged for 10 min at 5,000 g (environmental temperature ranging between 15 and 25 °C). Plasma was then transferred to vials and stored at −80 °C until analysis within 4 wk. Plasma samples were analysed in duplicate for CORT using a competitive enzyme assay (RADIM, Pomezia, Italy). The assay sensitivity was 13.8 nmol/L. The intra- and inter-assay coefficients of variance were 4 % and 6.9 %, respectively. The lowest and the highest CORT during the SET were defined as $CORT_{min}$ and $CORT_{max}$. $CORT_{start}$, $CORT_{max}$, and $CORT_{max-min}$ were used to compare among age-groups, SET-dates, and with BEST.

Statistical analyses

All statistics were run on Statview 5.0 (SAS, Cary, NC, USA). The normality of the data was confirmed using the Kolmogorov-Smirnov test. All data are expressed as means ± standard deviation (SD). A repeated-measures, two-way analysis of variance (ANOVA) with the independent parameters date of testing (April, June, August) and age-group (two-, three-, four-year-old) was used to test differences in all CORT derived parameters. A one-way repeated measures analysis of variance was applied to investigate the effects of the age groups on CORT during SET. The relationships between v_4 and the CORT derived parameters were investigated by means of linear regression analysis and Pearson's correlation. When a significant F ratio was achieved with the level of significance fixed at $P < 0.05$, post hoc comparisons were accomplished via Fisher's least significant test to locate specific significant differences.

Results

v_4 of horses

Age significantly affected the mean v_4 of the horses ($P = 0.0002$): v_4 was lower in two-year-old than in three-year-old and four-year-old horses ($P = 0.0006$ and 0.0001 respectively). The v_4 was not different between three- and four-year-old horses. The v_4 of the 2-year-old horses increased during the season, that of the 3- and 4-year-olds did not (*Lindner* et al. 2019). The v_4 of BEST remained similar during the observation period and was at the higher range of the three-year-old horses.

Relationship between v_4 and blood plasma cortisol derived parameters

There was no relationship between v_4 and the CORT derived parameters (all $P > 0.05$) apart from a negative relationship between v_4 and $CORT_{max-min}$ in for the four-year-old horses ($P = 0.048$, $r^2 = -0.27$).

Development of the blood plasma cortisol concentration during standardized exercise tests

$CORT_{max}$ was in 87.7 % of the SETs after the last interval and $CORT_{min}$ in 75.5 % of the SETs after the first interval. The mean CORT increased linearly during the SET in the horses of all age-groups (Fig. 1; $P \leq .0001$ between interval and blood LA of two-, three-, and four-year-old horses). There were no differences among the testing dates. The relationship between plasma CORT and the running speed during SET was examined by linear and exponential regression for each horse and SET separately. In 20 out of 39 SETs, a significant linear and positive relationship was found. Exponential regression analysis did not increase the number of significant relationships. The development of CORT during SET of BEST appeared to be closer to that of horses aged two-years than of the older horse groups.

Effect of the standardized exercise test date and age of Standardbred racehorses on blood plasma cortisol derived parameters

The SET date and the age of the horses did not have an effect on the CORT derived parameters (all $P > 0.05$; Table 2). $CORT_{start}$ and $CORT_{max}$ of BEST appeared to be lower than the values of the horses in the different age-groups, $CORT_{max}$ being closest to those of the two-year-old horses, while $CORT_{max-min}$ was higher than the mean values of the horses in the age-groups.

Discussion

This study examined on three dates during a racing season the concentration of CORT parameters derived from values before and during a SET to determine v_4 of two-, three- and

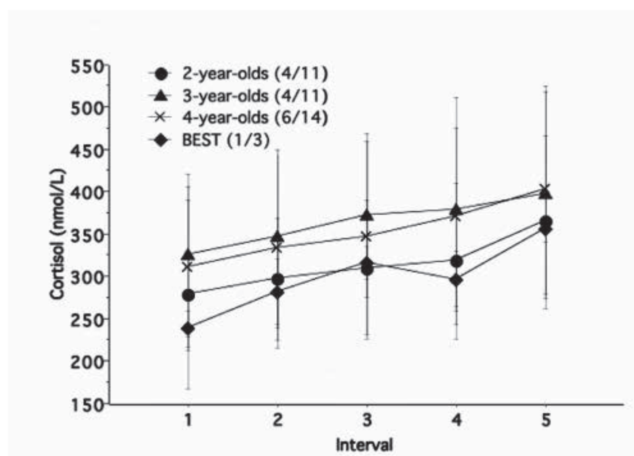

Fig. 1 Development of the blood plasma cortisol concentration during standardized exercise tests in Standardbred racehorses of different ages and the horse BEST (means ± SD; in brackets: number of horses/number of SETs; BEST = Horse winning the most money of all racehorses during the season; $P > 0.05$ among all horse groups; at least $P = 0.004$ among intervals for each group). | *Entwicklung der Kortisolkonzentration im Blutplasma von Trabrennpferden unterschiedlichen Alters und dem Pferd BEST (3-Jähriger mit der höchsten Gewinnsumme des Jahres) während eines Belastungstests (Mittelwert ± Standardabweichung; In Klammern Anzahl Pferde/Belastungstest; P > 0,05 zwischen Altersgruppen; mindestens P = 0,004 zwischen Testintervallen bei jeder Altersgruppe).*

four-year-old Standardbred racehorses. In addition, the relationship between the CORT derived parameters and the v_4 of the horses was investigated.

The date of SET did not affect the CORT parameters during the season nor were differences found between the age groups. *Witkowska-Piłaszewicz* et al. (2021) observed in 3-year-old Purebred Arabian racehorses that the basal level of serum cortisol remained almost unchanged during the entire training season.

Certainly, the low number of horses for data analysis in the actual study may have contributed to these results. The reason for the low number of horses available for the statistical analysis, despite the many more horses tested regularly in the racing yard, was that there were no more horses tested within three days before and after the three defined dates during the season because of their racing dates and health status. The selection of other dates would not have yielded more horses for data analysis. Nevertheless, the information provided could be of value for performance and poor performance diagnosis because it was obtained from horses trained in a remarkably successful racing yard including the best horse of the season.

The development of markers of the autonomic nervous system during a competitive season has been examined in Standardbred racehorses (*Kinnunen* et al. 2006), Purebred Arabian racehorses (*Janczarek* et al. 2003) and eventing horses (*Lorello* et al 2017). In all these studies the heart rate variability (HRV) was determined and showed a reduction of the parasympathetic and an increase of the sympathetic tone towards or at the end of the respective competitive seasons. The HRV findings of those studies cannot be discussed with own results because HRV was not determined. *Lorello* et al. (2017) only measured plasma CORT in addition to HRV. CORT after SET did not change during the season in their eventing horses. $CORT_{max}$ behaved in this study similarly to CORT after SET in the study of *Lorello* et al. (2017), but the three-year-old Standardbred racehorses are comparable with the eventing horses only. This is because the training programs of these horse groups in both studies had the objective to maximize the performance level towards late summer and early autumn while the training of the two-year-old Standardbred racehorses was designed to compete later in the year and even prepare them for their three-year-old racing career, and the four-year-old horses were competing when the study started already (*Wegener* et al. 2012, *Lindner* et al. 2019). There are several reasons, besides the low number of horses, that could explain the lack of significant effects of the testing dates and age groups of the horses on the CORT derived parameters in this study. The different objectives of the training programs among age groups was addressed above already. The quality of the management of the horses including their training program could be another factor to consider. It may have allowed the horses to regenerate sufficiently after training and racing regardless of age keeping their autonomic nervous system balanced. *Janczarek* et al. (2003) as well as *Kinnunen* et al. (2006) consider the changes of the HRV parameters of their horses to be a reaction to increasing stress due to training and racing which may not have been the case for the horses of this study. Finally, it is known that the basal and post-exercise CORT values of horses taken at the same time on different days vary significantly (*Linden* et al. 1990, *Lindner* et al. 2000). This high variability could account for the lack of significant effects too. The well documented circadian and ultradian rhythm of CORT in horses may have played a role too, but to a lesser extent because all testing was done between 7:30 hours and 10:30 hours, starting always with the older horses and finishing with the younger ones (*Irvine* and *Alexander* 1994).

The only significant relationship found in this study was between the v_4 of four-year-old horses and their $CORT_{max-min}$: the higher the v_4 the smaller the $CORT_{max-min}$ value. This result may indicate that the four-year-old horses were less stressed by the training and racing management than the younger horse groups (*Sauer* et al. 2019), or were overtrained (*Golland* et al. 1999). However, there was no indication for this based on the health and performance of the horses. Certainly, this result is in contrast to those described by *Lindner* et al. (2002). They found a positive linear relationship between v_4 of Standardbred racehorses older than two years and the difference between CORT after the last interval of SET and CORT after warm-up in one racehorse yard while no relationship in another yard. The CORT parameters defined in both studies are not exactly the same, but very similar: CORT after the last interval of SET was in more than 87% of the cases $CORT_{max}$, and CORT after warm-up was in 75% of the cases $CORT_{min}$. The SET prescriptions used in both studies had the same criteria. Thus, there is no conclusive explanation for the differences in results. Differences in the management of the horses may have played a role, but also different blood sampling times due to the circadian rhythm: In this study between 7:30 hours and 10:30 hours in the morning, the older horses

Table 2 Comparison of blood plasma cortisol concentration (CORT) derived parameters of two-, three-, and four-year-old Standardbred racehorses and BEST (3-year-old horse winning the most money of all racehorses in the racing season; $CORT_{start}$ = CORT after warm-up but before starting standardized exercise test (SET); $CORT_{max}$ = maximal CORT during SET; $CORT_{max-min}$ = difference between $CORT_{max}$ and the lowest CORT during SET; means ± SD; in brackets number of horses/tests; P > 0.05 among age-groups). | Vergleich der Kortisolgehalte im Blutplasma (CORT) von 2-, 3- und 4-jährigen Trabrennpferden und dem Pferd BEST (3-Jähriger mit der höchsten Gewinnsumme des Jahres) zu Beginn der Belastungstests ($CORT_{start}$), dem höchsten Wert ($CORT_{max}$) und der Differenz zwischen dem höchsten und dem niedrigsten Wert während der Belastungstests ($CORT_{max-min}$; Mittelwert ± Standardabweichung; In Klammern die Anzahl Pferde/Belastungstest; P > 0,05 zwischen den Altersgruppen).

Blood plasma cortisol derived parameter (nmol/l)	Horse groups			BEST
	Two-year-old	Three-year-old	Four-year-old	
$CORT_{start}$	281 ± 97.0 (4/12)	299 ± 78.0 (4/12)	301 ± 141 (4/12)	237 ± 21.0 (1/3)
$CORT_{max}$	370 ± 93.5 (4/12)	401 ± 124 (4/12)	412 ± 122 (4/12)	366 ± 3.61 (1/3)
$CORT_{max-min}$	108 ± 27.6 (4/12)	89.7 ± 51.5 (4/12)	107 ± 39.2 (4/12)	127 ± 18.5 (1/3)

first, the two-year-old horses last, while horses examined by *Lindner* et al. (2002), older than two years, were tested in the morning, at noon, and early afternoon, and the two-year-old horses were all examined between noon and early afternoon.

Concerning the effect of the horses' age on CORT during SET, it has to be taken into consideration that the prescription differed between the two-year-old and the older horses. For practical reasons only, the SET speeds of the horses older than two years were higher than those of the two-year-old horses to keep the total time for running a SET to reach a blood LA of 4 mmol/L similar to that of the two-year-old horses. Therefore, testing all horses with the same SET prescription might have yielded different results. This is certainly the case for v_4 (*Lindner* et al. 2019).

During SET there was a moderate and linear increase of CORT in all age-groups without statistical differences among them. Similar results are reported by *Lindner* et al. (2000, 2002) for Thoroughbreds and Standardbreds, and *Church* et al. (1987) for Thoroughbreds, while *Cravana* et al. (2006) report that two-year-old Thoroughbreds have higher CORT increases than three-year-old horses during simulated races, and *Horohov* et al. (1999) as well as *Malinowski* et al. (2006) describe that, in Standardbred mares older than 20 years, CORT increases less than in younger mares during SET on treadmills. The differing results may be attributed to the different types of exercise, and to the large age differences between the horse groups in the latter referenced studies.

The horse BEST won by far, the largest purse in Germany in the year of the study and went on to be a superior performer as a four-year-old and older horse too. His v_4 was above the average of his age peers during the three-years-old season and he was lighter than them. BEST was not trained differently from the other horses, which allows the assumption that he might have reacted better than the others to the applied training (*Wegener* et al. 2012, *Lindner* et al. 2019). The CORT derived parameters of BEST appeared to be quite different to those of his age group. He had by far the lowest $CORT_{start}$ of all age groups and his mean $CORT_{max}$ and CORT during the SET were lower than that of the horses of his age-group. *Witkowska-Piłaszewicz* et al. (2021) described a similar behavior of the CORT in Purebred Arabian racehorses performing better than their peers. These results could mean that the autonomic nervous system of BEST was better balanced than that of other horses in the racing yard. His $CORT_{max-min}$ was higher than that of the horses in all age-groups which could indicate that he was able to mobilize more CORT to benefit of its effects on the energy metabolism during competition as discussed by *McBride* and *Mills* (2012) and *Janczarek* et al. (2016) under the term flightness. Certainly, there is the possibility that BEST was genetically superior to other horses, but his race yard was part of a breeding stud and the majority of the horses being trained there had a similar genealogy. Future studies with elite horses are needed to provide insight in the practical importance of his CORT profile.

Conclusion

The CORT parameters derived of a SET to determine v_4 may have the potential of being useful for diagnosing performance and follow training effects including overtraining in Standardbred horses, but many more horses need to be studied. The CORT profile of BEST may be of value to find horses with superior competitive quality.

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Erweiterte Zusammenfassung