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ORIGINAL ARTICLE

# Cortisol release, heart rate and heart rate variability, and superficial body temperature, in horses lunged either with hyperflexion of the neck or with an extended head and neck position

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## Summary

Bringing the head and neck of ridden horses into a position of hyperflexion is widely used in equestrian sports. In our study, the hypothesis was tested that hyperflexion is an acute stressor for horses. Salivary cortisol concentrations, heart rate, heart rate variability (HRV) and superficial body temperature were determined in horses ($n = 16$) lunged on two subsequent days. The head and neck of the horse was fixed with side reins in a position allowing forward extension on day A and fixed in hyperflexion on day B. The order of treatments alternated between horses. In response to lunging, cortisol concentration increased (day A from 0.73 ± 0.06 to 1.41 ± 0.13 ng/ml, $p < 0.001$; day B from 0.68 ± 0.07 to 1.38 ± 0.13 ng/ml, $p < 0.001$) but did not differ between days A and B. Beat-to-beat (RR) interval decreased in response to lunging on both days. HRV variables standard deviation of RR interval (SDRR) and RMSSD (root mean square of successive RR differences) decreased ($p < 0.001$) but did not differ between days. In the cranial region of the neck, the difference between maximum and minimum temperature was increased in hyperflexion ($p < 0.01$). In conclusion, physiological parameters do not indicate an acute stress response to hyperflexion of the head alone in horses lunged at moderate speed and not touched with the whip. However, if hyperflexion is combined with active intervention of a rider, a stressful experience for the horse cannot be excluded.

## Introduction

Although domestic horses have been selected over centuries for characteristics that fit their equestrian use and are able to perform high-level physical exercise, a variety of challenges to which horses are exposed are potential stressors for the animals. This includes training and competitions (Dybdal et al., 1980; Snow and Rose, 1981; Lange et al., 1997; Schmidt et al., 2010a), transport (Baucus et al., 1990; Clark et al., 1992; Schmidt et al., 2010b,c,d), veterinary examinations (Berghold et al., 2007) and exposure to new groups (Alexander and Irvine, 1998). However, the stress experienced by horses in response to different training methods has rarely been studied.

The head and neck position of the ridden horse from initial training to dressage at advanced level has been a topic of debate for centuries (De la Guerinière, 1733; Baucher, 1852; Podhajsky, 1965). Classical equitation postulates that by extending the neck of the young horse forward and downwards, a suspension bridge function of its back is achieved. In the ridden horse, the atlanto-occipital joint should be the highest point, and a line from the forehead down to the nose should be never behind the vertical (Podhajsky, 1965). However, bringing the head and neck of the ridden horse into a position of hyperflexion enables the rider to exert extreme control over the animal and – although in contrast to classical equitation theory – is widely used in the training of dressage horses. Hyperflexion is discussed controversially, and effects on health and well-being of horses have been addressed (Sloet van Oldruitenborgh-Oosterbaan et al., 2006; von Borstel et al., 2009; McGreevy et al., 2010; Wijnberg et al., 2010). In one of the few studies measuring physiological stress parameters in association with hyperflexion, mean blood lactate concentration immediately after a trot and canter phase in hyperflexion was higher than in the same horse ridden with light rein contact (Sloet van Oldruitenborgh-Oosterbaan et al., 2006). Based on heart rate and heart rate variability, it has also been suggested that training procedures involving hyperflexion are no stressor for dressage horses (van Breda, 2006).

In this experiment, salivary cortisol concentration, heart rate and heart rate variability were determined as physiological stress parameters in horses lunged either with hyperflexion or with an extended position of the head and neck. The position of the head and neck may affect blood circulation in the musculature. Therefore, the superficial thermographic pattern of the neck and head region was assessed by infrared thermography. Our hypothesis was that lunging in hyperflexion induces a transient stress response compared to lunging with an extended position of the head and neck in horses.

## Materials and methods

### Animals

A total of 16 adult German sport horses of the Brandenburg State Stud were available for the study. The horses belonged to the riding school of the stud and were used to daily equestrian exercise. None of the horses was used to being lunged or ridden in forced hyperflexion. For 29 days before the experiment, horses were kept as one group on pasture. During this time, they were neither ridden nor lunged and did not receive any feed in addition to pasture. Two days before the experiment, horses were transferred to identical, individual loose boxes on straw and were fed concentrates three times daily and hay twice daily. Water was available at all times. During the study period, the horses were not exposed to any exercise except lunging in the experiment. The age of the horses was 7.7 ± 0.7 years (±SEM), and 14 animals were geldings and two mares.

### Experimental design

For the experiment, horses were lunged on two subsequent days. After a warm-up phase, on 1 day (A), the head of the horses was fixed with side reins in a position allowing a forward extension of the neck, and on the other day (B), the head and neck of the horse was fixed in hyperflexion. Half of the horses received experimental treatment A on the first day and treatment B on the second day, and the other half was treated in opposite order. Salivary cortisol concentrations, heart rate and heart rate variability (HRV) were determined repeatedly before, during and after the experiments. Superficial body temperature was determined by infrared thermography before and during the experiments. The study was approved by the competent authority for animal experimentation in Brandenburg State, Gernany (*Landesamt für Verbraucherschutz, Landwirtschaft und Flurneuordnung*, licence number 23-2348-8a27-2008).

## Experimental procedures

#### *Lunging of horses*

Horses were lunged on 2 days in an indoor riding arena (15 × 35 m) separated from other sections of the stud. The building was insulated (19th century structure with thick stone walls) and had a near-constant temperature and humidity throughout the experiment. Horses were wearing a bridle with a double-jointed bit and saddle. On each day, lunging was begun with an 11-min warm-up phase with loose side reins attached between a lunging-girth fixed above the saddle and the ring of the bridle allowing a totally free position of the head and neck (Table 1). The horses were then stopped, and side reins were tightened. On 1 day (day A), side reins were shortened to reach a head position closely at the vertical (Fig. 1a). On the other day (day B), the

**Table 1** Experimental schedule

| | |
|---|---|
| Warm-up phase: no side reins | 5-min walk |
| | 2-min trot |
| | 2-min canter |
| | 2-min walk (cool down) |
| Break | Fixing of side reins |
| Experimental phase: side reins attached | 2-min walk |
| Day A: extended head and neck position | 5-min trot |
| Day B: hyperflexion | 1-min walk (cool down) |
| | 5-min canter |
| | 24 min total time |

side reins were shortened to bring the head of the horse into a position clearly behind the vertical (hyperflexion of the neck; Fig. 1b). After fixation of the side reins, the horses were again lunged for 13 min (Table 1). Horses were always lunged on the left hand (anticlockwise direction). The order of experimental days A and B was arranged in a Latin square design with eight horses being lunged with an extended neck on the first day and a hyperflexion position the other day, and 8 horses being studied in reverse order. The animals thus served as their own controls. Experiments A and B in each horse were always carried out at exactly the same time on both days.

*Salivary cortisol*

Saliva for determination of cortisol concentrations was collected on 2 days before the first experiment at 6:00, 6:30; 14:00, 14:30, 22:00 and 22:30 to obtain baseline values. Saliva was then collected at 60 and 30 min before each experiment, during the break for fixing the side reins and at 0, 5, 15, 30, 60, 90, 120, 150 and 180 min after the end of lunging.

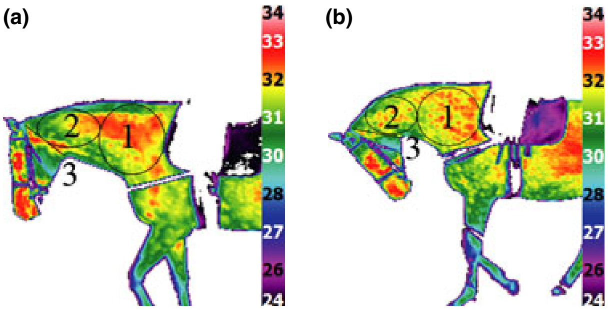

**Fig. 1** Thermographic image of horse prepared for the experiment with long side reins (left) allowing extension of the neck (a) and with short side reins, fixing the neck in hyperflexion position (b). The areas chosen for statistical analysis of thermographical data are indicated: (1) caudal part of neck, (2) cranial part of neck and (3) line from guttural angle to nape.

Collection of saliva was performed as described (Schmidt et al., 2010d). In brief, cotton rolls (Salivette; Sarstedt, Nümbrecht-Rommelsdorf, Germany) were placed loosely onto the tongue of the horse for 1 min with the help of a surgical arterial clamp until the swab was well soaked. Concentrations of cortisol were determined with a direct enzyme immunoassay without extraction (Palme and Möstl, 1997) validated for equine saliva (Schmidt et al., 2009). The antiserum cross-reacts with cortisone and some cortisone metabolites. Thus, the values have to be interpreted as cortisol immunoreactivity (IR). The intra-assay coefficient of variation was 5.0%, the inter-assay variation was 6.7%, and the minimal detectable concentration was 0.3 pg/well.

*Heart rate and heart rate variability*

Heart rate and heart rate variability were determined with a mobile recording system (S810i; Polar, Kempele, Finland) as described (Schmidt et al., 2010d). Beat-to-beat (RR) intervals were recorded on the day before the first experiment for 2 h to obtain baseline values. On both experimental days, RR intervals were recorded continuously from 60 min before to 60 min after lunging. Heart rate variability was analysed with the Kubios HRV software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland). To remove trend components, data were detrended and, in addition, an artefact correction was made following established procedures (Tarvainen et al., 2002). In our study, RR interval, standard deviation of RR interval (SDRR) and RMSSD (root mean square of successive RR differences) were calculated. The means for HRV variables were calculated for periods of 5 min each in the following phases: 60–55 and 30–25 min before lunging, warm-up phase (walk), trot phase of lunging with side reins, canter phase of lunging with side reins and 0–5 and 25–30 min after lunging.

*Thermography*

For thermography analysis, an uncooled mircrobolometer thermal imaging camera (type IR-TCM 640 high resolution, Jenoptik, Jena, Germany) was used. Standard thermal images were taken, while the horse was standing including thermograms of the head, neck, shoulder, abdomen and hip from the left and right side in addition to usual digital pictures of the same districts. Subsequently, thermal images of the moving horse were taken from the centre of the lunging circle in a constant distance of 6.20–6.40 metres from the horse. The camera was focused on the forehand including the left side of the head, neck and shoulder and both front limbs. Thermal images were automatically triggered every 10 s producing at least 144 thermal images (thermograms) of the moving horse in one lunging period. The thermograms were assigned to and saved with a special thermographic software (EXAM 5.6; Inframedic, Moersfelden, Germany). Measurements in the head and neck region were obtained at the same temperature spread and level. For the three gaits such as walk, trot and canter, one picture at the end of each phase (except the walk phases for cool down) was selected for both the warm-up and experimental phase on day A and day B. The following areas on the left side of the horse were analysed for minimum, maximum and average temperature and minimum–maximum difference with the EXAM 5.6 software: (i) a circle reaching from the cranial brim of the saddle flap onto the neck and from the height of the shoulder joint to the upper aspect of the mane (base of the neck); (ii) an ellipse on the cranial part of the neck positioned between the caudal aspect of the mandible, and the ventral and dorsal lines of the neck; and (iii) a line drawn from the highest point of the horses' nape of the neck to the ventral guttural angle (Fig. 1).

### Statistical analysis

Statistical analysis was carried out with the PASW 17.9 statistics package SPSS (Chicago, IL, USA). All data were normally distributed (Kolmogorov–Smirnov test). Differences between days A and B and changes over time within experimental days A and B were analysed by ANOVA using a general linear model for repeated measures with day (A vs. B) as between subject factor. In case of overall significant effects, values differing from each other were identified by *post hoc* analysis of main effects with Bonferroni correction of p-values. All data given are means ± SEM.

## Results

### Degree of hyperflexion and behaviour of horses

On day A, the inward angle (determined from the thermograms) between the vertical and a line from the forehead to the nose was 4 ± 1°, while the corresponding value for day B was 31 ± 2° ($p < 0.001$). All horses tolerated the experiment without any obvious resistance and moved forward in the gaits and paces requested. Movement was easily controlled with the lunge, and a lunging whip moved carefully towards the animal. At no times horses were touched with the whip.

### Salivary cortisol

Baseline cortisol IR concentrations on the 2 days before the experiment were highest in the morning and decreased throughout the day. In response to lunging, cortisol concentrations increased during the warm-up phase and further when the horses were lunged with the head and neck brought into the respective experimental positions by use of side reins (day A: 60 min before lunging 0.73 ± 0.06, immediately after warm-up 0.95 ± 0.10, immediately after end of lunging 1.41 ± 0.13 ng/ml; day B: 60 min before lunging 0.68 ± 0.07, immediately after warm-up 1.04 ± 0.13, immediately after end of lunging 1.38 ± 0.13 ng/ml, $p < 0.001$ over time for both days). At no time, before, during and after lunging cortisol concentrations differed between day A (lunging with extended neck) and day B (lunging in hyperflexion; Fig. 2), and no interactions time of day × treatment existed.

### RR interval and heart rate variability

The RR interval decreased in response to lunging during the warm-up phase and decreased further when horse were lunged with their head and neck fixed by side reins in a position either before the vertical (day A) or beyond the vertical (day B;

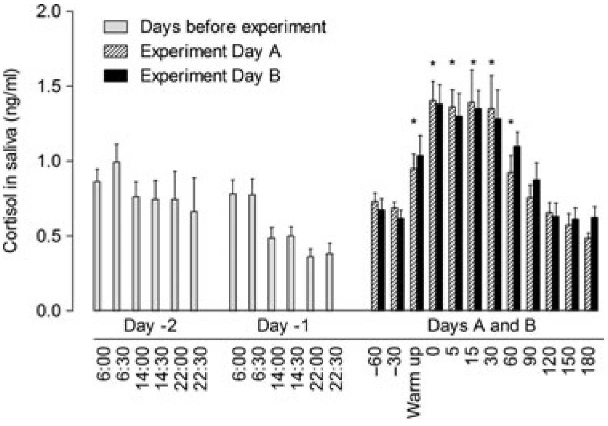

**Fig. 2** Cortisol IR in saliva of horses on 2 days before being lunged and from 60 min before to 180 min after lunging with an extended neck (day A) and in hyperflexion position (day B; $n = 16$). Significant differences over time on both experimental days ($p < 0.001$, *individual values differing from baseline time −60 on days A and B). No significant differences between experimental days A and B and no interactions time × treatment.

Fig. 3a, $p < 0.001$ over time for both groups). During the canter segment at the end of the experimental phase, the RR interval tended to be lower when horses were lunged with the head and neck in hyperflexion (nose line beyond the vertical; 686 ± 49 ms) than when the same horses were lunged with the head and neck in an extended position (nose line before the vertical; 821 ± 66 ms; overall difference between days $p = 0.15$; no significant interactions time × treatment; Fig. 3a). For the HRV variables SDRR and RMSSD, the same changes over time were found as for RR interval, that is, a decrease during the warm-up phase and a further decrease during the experimental phase with the side reins fixing the head and neck either in a position before (day A) or beyond the vertical (day B; Fig. 3b,c, $p < 0.001$). For both HRV variables, neither significant differences between days A and B nor interactions time × treatment were found.

### Thermography

Mean temperature (Fig. 4a–c), minimal temperature and maximal temperature (data not shown) in all three areas of the neck analysed decreased from walk to trot and canter both in the warm-up phase and in the phase with the head and neck fixed by side reins in the respective experimental positions ($p < 0.001$). The minimum–maximum difference (Fig. 4d–f) changed over time only in the cranial region of the neck ($p < 0.01$; Fig. 4e). In the cranial region of the neck, the difference between the maximum and minimum temperature was also higher when horses were lunged with the head and neck in hyperflexion (day A) compared to the day when horses were lunged with the head and neck in an extended position (day B; $p < 0.05$, Fig. 4e). For none of the other parameters and none of the localizations analysed, thermographic results differed between days A and B.

## Discussion

In this study, lunging of horses caused an increase in salivary cortisol concentrations, a decrease in RR interval corresponding to an increase in heart rate and a decrease in heart rate variability. In agreement with previous studies on exercised horses, these changes are caused by a combination of physiological stress responses and physical activity (Marc et al., 2000; Becker-Birck et al., 2010; Schmidt et al., 2010a) that are not easy to differentiate. Glucocorticoids improve fitness of an animal during short-term stress (Raynaert et al., 1976). Heart rate variability, that is, short-term fluctuations in beat-to beat (RR) interval, reflects the stress response of the autonomic nervous system. Decreases in the values of the HRV variables SDRR and RMSSD reflect a shift towards sympathetic dominance (von Borell et al., 2007). Determination of cortisol in saliva is a well-accepted parameter for analysis of adrenocortical response to potential stressors, and cortisol concentrations in saliva reliably mirror its concentrations in plasma (Schmidt et al., 2009; Peeters et al., 2010). Cortisol analysis in saliva avoids the need of repeated venipuncture or prolonged catheterisation. In addition, plasma cortisol is mainly bound to carrier proteins, while salivary cortisol mirrors unbound, that is, free, and thus biologically active cortisol (Riad-Fahmy et al., 1983). Overall, salivary cortisol

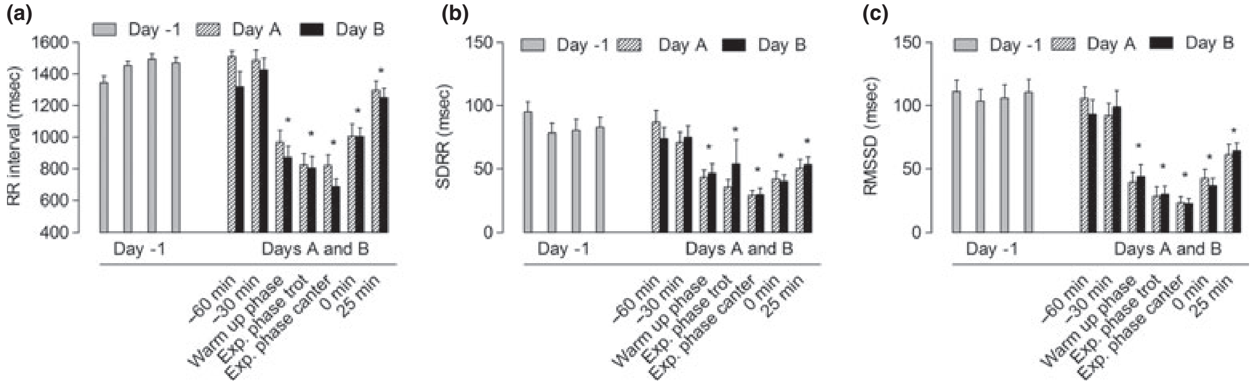

**Fig. 3** (a) RR interval and heart rate variability variables (b) SDRR and (c) RMSSD in horses lunged with the neck extended (day A) and in hyperflexion position (day B; $n = 16$). Significant differences over time on both experimental days for RR interval, standard deviation of RR interval (SDRR), root mean square of successive RR differences (RMSSD) and SD1 ($p < 0.001$, *individual values differing from baseline time −60 on days A and B). No significant differences between experimental days A and B and no interactions time × treatment.

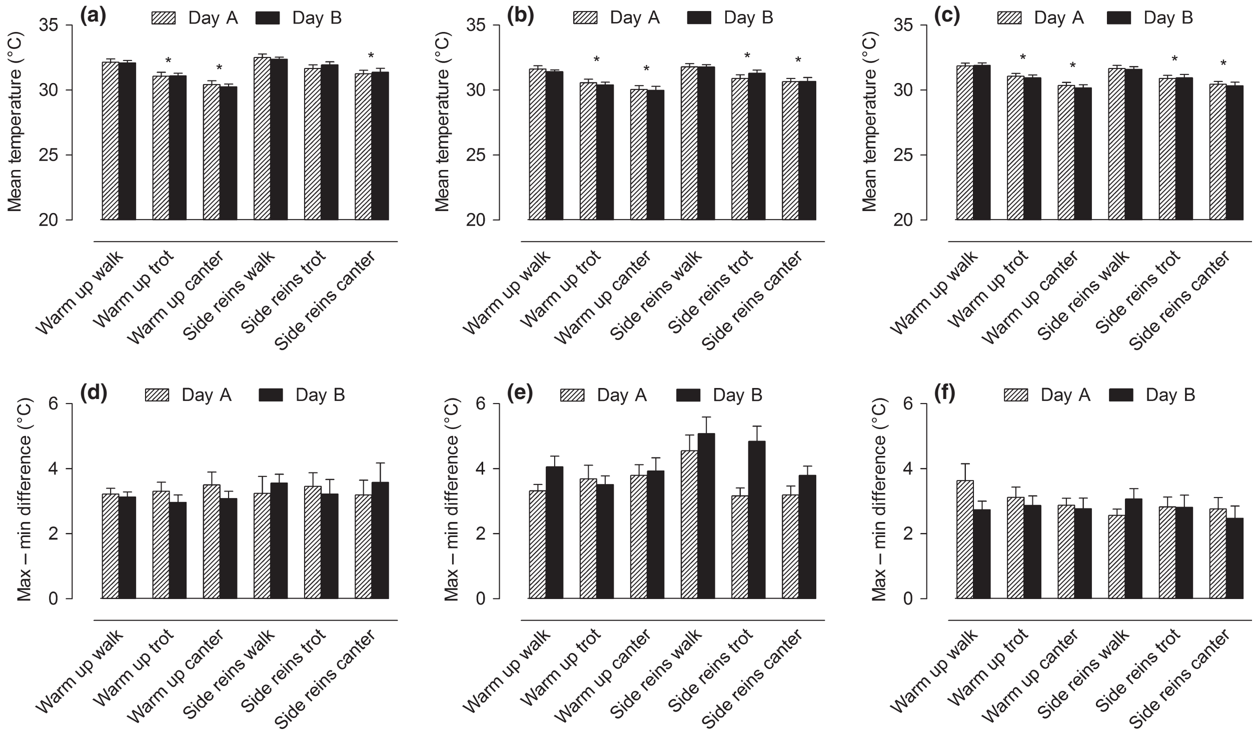

**Fig. 4** Superficial body temperature of horses lunged with the neck extended (day A) and in hyperflexion position (day B; $n = 16$). (a, d) Caudal part of the neck, (b, e) cranial part of the neck, (c, f) guttural angle to nape, (a–c) mean temperature, (d–f) maximal–minimal difference. Significant differences over time on both experimental days for mean temperature at all location measured (a, b, c; $p < 0.001$) and for minimum–maximum difference in the cranial part of the neck (e; $p < 0.01$, *individual values differing from baseline time −60 on days A and B), significant differences between days A and B for minimum–maximum difference in the cranial part of the neck (e; $p < 0.05$), no interactions time × treatment.

concentrations in response to lunging irrespective of the head and neck position were lower than concentrations determined with the same analytical techniques in horses during road transport (Schmidt et al., 2010c,d), initial equestrian training (Schmidt et al., 2010a) or weaning of foals (Erber et al., 2011b). Road transport over 1 h (Schmidt et al., 2010d), first mounting of young horses by a rider which took <15 min (Schmidt et al., 2010a) and even handling for foal identification for <5 min (Erber et al., 2011a) were sufficient to induce a pronounced increase in salivary cortisol concentrations. The duration of these stressors is thus well comparable to the current study. Thus, lunging of horses in our study cannot be considered a major stressor compared to other challenges to which horses are exposed regularly.

Lunging of horses with the neck in either an extended position with the nose line before or at the vertical or in hyperflexion with the nose line clearly beyond the vertical was without differential effect on cortisol release, HRV and most of the thermography variables analysed. However, lunging in hyperflexion was associated with a less homogenic thermographic pattern in the cranial part of the neck compared to lunging with an extended neck.

Based on the physiological stress parameters cortisol and HRV, our data do not indicate a specific stress reaction of horses in response to lunging in a hyperflexion position. With regard to HRV, this is in agreement with findings by Mohr et al. (2000), who found changes in HRV in horses caused by physical exercise but not by potential psychological stressors such as changes in environment. At first view, our data also appear to confirm previous studies on hyperflexion in the equestrian discipline of dressage (Sloet van Oldruitenborgh-Oosterbaan et al., 2006; van Breda, 2006). Van Breda (2006) analysed heart rate and HRV between 30 and 45 min after the end of riding. Based on these data, it was claimed that the stress response in elite dressage horses ridden in hyperflexion is lower than in recreational horses ridden in a position with an extended neck. It was concluded that hyperflexion in elite horses is less stressful than non-hyperflexion in recreational horses. Recently, we could show that riding elicits only short-term changes in heart rate, HRV and cortisol release (Becker-Birck et al., 2010; Schmidt et al., 2010a). These changes indicate a certain stress response of the animals during the riding phase but values returned to normal within 30 min or less after the end of riding. Determination of heart rate and HRV 30 min or later after the end of a training unit will thus not detect differences between experimental groups or procedures unless horses have been exposed to extreme workloads or challenges. This level is usually not reached in dressage competitions or presentations (Becker-Birck et al., 2010). Horses adapt rapidly to many anthropogenic stressors (Schmidt et al., 2010a,c). We therefore suggest that the difference found by others in an earlier study (van Breda, 2006) is potentially caused more by the difference in experience between elite dressage and recreational horses than by the different training methods studied.

Plasma cortisol concentrations, metabolic and haematology parameters and heart rate but not HRV have been compared in horses ridden in hyperflexion either with the aid of draw reins or with an extended neck and head (Sloet van Oldruitenborgh-Oosterbaan et al., 2006). Only plasma lactate concentrations and mean heat rate were slightly higher when the horses were ridden in hyperflexion. The authors concluded convincingly that hyperflexion obtained by draw reins was without major effects on physiological parameters. Our study, with additional parameters and different experimental set-up, extends previous work. In ridden horses, hyperflexion is associated with active intervention of the rider who forces the horse to move forward and to collect. The combination of hyperflexion and propulsive aids of the rider is termed ‘rollkur’ or ‘low, deep and round’ (see Wijnberg et al., 2010). While it was attempted previously (Sloet van Oldruitenborgh-Oosterbaan et al., 2006) to standardize the influence of the rider, in our study, this influence was completely eliminated and thus allowed studying hyperflexion independent from a rider. Any experimental set-up with horses ridden by different riders will result in a variability that must be considered larger than that in a lunging situation with the same person (Wijnberg et al., 2010). Hyperflexion, at least for a time period of 13 min, was without major effects on the response of horses to lunging. Heart rate at all times remained within the physiological range for horses submitted to moderate exercise.

Infrared thermography revealed no differences in mean superficial temperature of the neck and shoulder between lunging in hyperflexion or with an extended head and neck. However, the temperature range (difference between minimum and maximum temperature in the individual horse) in the cranial part of the neck was more pronounced when horses were lunged in hyperflexion. This indicates a less homogenous skin temperature. Equestrian training should result in an even blood perfusion which may have not been fully achieved in hyperflexion. Thus, thermoregulation might have been less evenly distributed in this area when horses were lunged in hyperflexion. Effects of lunging in hyperflexion on neuromuscular functionality of the horses neck have recently been demonstrated. Results indicate an increased muscular workload, a less synchronous arrival of individual motor unit action potentials and partially delayed neuromuscular transmission. However, in part, these parameters were also affected when horse were lunged with the head and neck in different positions (Wijnberg et al., 2010), and whether certain head and neck positions were perceived as stressful was not analysed.

In conclusion, a transient and moderate hyperflexion in horses lunged at moderate speed and not touched with the whip did not elicit a pronounced stress response in horses. We did also not observe overt aversive or stress behaviour of the horses. In equestrian sports, hyperflexion is often combined with forceful and aggressive weight and leg aids over prolonged periods of time. This may represent a different situation than lunging in hyperflexion, and our study thus does not exclude stressful or potentially negative effects of hyperflexion employed in such training regimes.

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