Changes in cortisol release and heart rate variability in sport horses during long-distance road transport

A. Schmidt [a], S. Biau [b], E. Möstl [c], M. Becker-Birck [a], B. Morillon [b], J. Aurich [c], J.-M. Faure [b], C. Aurich [a,c,*]

[a] *Graf-Lehndorff-Institut für Pferdewissenschaften, 16845 Neustadt (Dosse), Germany*
[b] *Ecole Nationale d'Equitation, 49411 Saumur Cedex, France*
[c] *Veterinärmedizinische Universität, 1210 Vienna, Austria*

Abstract

It is widely accepted that transport is stressful for horses, but only a few studies are available involving horses that are transported regularly and are accustomed to transport. We determined salivary cortisol immunoreactivity (IR), fecal cortisol metabolites, beat-to-beat (RR) interval, and heart rate variability (HRV) in transport-experienced horses (N = 7) in response to a 2-d outbound road transport over 1370 km and 2-d return transport 8 d later. Salivary cortisol IR was low until 60 min before transport but had increased ($P < 0.05$) 30 min before loading. Transport caused a further marked increase ($P < 0.001$), but the response tended to decrease with each day of transport. Concentrations of fecal cortisol metabolites increased on the second day of both outbound and return transports and reached a maximum the following day ($P < 0.001$). During the first 90 min on Day 1 of outbound transport, mean RR interval decreased ($P < 0.001$). Standard deviations of RR interval (SDRR) decreased transiently ($P < 0.01$). The root mean square of successive RR differences (RMSSD) decreased at the beginning of the outbound and return transports ($P < 0.01$), reflecting reduced parasympathetic tone. On the first day of both outbound and return transports, a transient rise in geometric HRV variable standard deviation 2 (SD2) occurred ($P < 0.01$), indicating increased sympathetic activity. In conclusion, transport of experienced horses leads to increased cortisol release and changes in heart rate and HRV, which is indicative of stress. The degree of these changes tended to be most pronounced on the first day of both outbound and return transport.
© 2009 Elsevier Inc. All rights reserved.

Keywords: Horse; Transport; Cortisol; Heart rate variability

1. Introduction

The growing number of equestrian competitions has led to a rapid increase in transport of sport horses. Between 1997 and 2007, the number of international equestrian events has risen worldwide from approximately 550 to more than 2100 [1], and additional increases have taken place at national and regional levels. Sport horses are thus transported regularly and repeatedly over long distances.

Based on increases in cortisol release [2–9], it is widely accepted that transport is a stressful event for horses. So far, cortisol release in response to transport has been evaluated mainly in blood plasma. Noninvasive techniques such as cortisol analysis in saliva and analysis of cortisol metabolites in feces offer the

---

* Corresponding author. Center for Artificial Insemination and Embryo Transfer, University of Veterinary Sciences, Vienna, Austria. Tel.: +43 1 25077 6400; fax: +43 1 25077 5490.

E-mail address: christine.aurich@vetmeduni.ac.at (C. Aurich).

advantage of avoiding stress reactions of the animals to repeated venipuncture. In addition, plasma cortisol is bound mainly to carrier proteins, whereas salivary cortisol mirrors unbound, free cortisol [10,11]. Salivary cortisol concentrations increased in response to loading horses onto a trailer, but with adequate preparation of the animals, the stress response was reduced [12]. Increased salivary cortisol concentrations have also been found in horses transported over short and medium distances [9], but no data are available for long-term transports lasting several days. Although cortisol in saliva and in plasma reflects acute changes in cortisol release [13], cortisol metabolites in the feces of horses do not increase until 24 h after an increase in cortisol concentrations in blood and reflect mainly prolonged stressful situations [14–16]. Fecal cortisol metabolites have not been analyzed in studies on long-distance transport in horses so far.

Transport of horses also causes increased heart rate [4,17–19] and changes in heart rate variability [20]. Cardiac function is regulated by the autonomous nervous system (ANS). Heart rate variability—that is, short-term fluctuations in heart rate—is based essentially on the antagonistic oscillatory influences of the sympathetic and parasympathetic nervous system on the nodus sinu-atrialis of the heart. It thus reflects the prevailing balance of sympathetic and parasympathetic (vagal) tone. Heart rate variability is used as an indicator for assessment of ANS activity in response to acute and chronic stress. In general, reductions in the values of the HRV variable standard deviation of beat-to-beat interval (SDRR) and root mean square of successive RR differences (RMSSD) reflect a shift toward more sympathetic dominance, whereas increased values indicate a shift toward more parasympathetic dominance [21–23].

To assess stress in horses transported over several days, we determined salivary cortisol IR, fecal cortisol metabolites, beat-to-beat interval, and heart rate variability before, during, and after long-distance transport. The experimental setup closely resembled the situation horses are exposed to in conjunction with international equestrian events.

2. Materials and methods

2.1. Experimental design

Horses of the Cadre Noir de Saumur (classical dressage team of the French National School of Equitation) were studied before, during, and after road transport over 1370 km from Saumur, France, to a presentation in Neustadt (Dosse), Germany. The study period included 2 d before transport, 2 d of transport from Saumur to

Table 1
Time schedule of horse transports and presentations.

Day	Location and activity
-2	Saumur: establishment of baseline values, light exercise
-1	Saumur: establishment of baseline values, light exercise
1	Transport from Saumur, France to Aachen, Germany; 740 km (720 km on 4-lane motorways); 13 h; 2 breaks of 30 min duration after 5 (12:00 PM) and 10 h (5:00 PM) of transport; overnight stabling of horses in individual loose boxes at the Aachen International Horse Show Centre
2	Transport from Aachen to Neustadt (Dosse); 630 km (495 km on 4-lane motorway); 11 h; 2 breaks of 30 min duration after 5 (1:00 PM) and 9 h (5:00 PM) of transport; stabling of horses in individual loose boxes at the Brandenburg State Stud in Neustadt (Dosse)
3	Neustadt (Dosse): rest
4	Neustadt (Dosse): training and rehearsals
5	Neustadt (Dosse): training and rehearsals
6	Neustadt (Dosse): public equestrian presentation
7	Neustadt (Dosse): public equestrian presentation
8	Neustadt (Dosse): training and rehearsals
9	Neustadt (Dosse): public equestrian presentation
10	Transport from Neustadt (Dosse) to Aachen; 11 h; 2 breaks of 30 min duration after 5 (12:00 PM) and 7 h (2:00 PM) of transport; overnight stabling of horses in individual loose boxes at the Aachen International Horse Show Centre
11	Transport from Aachen to Saumur, France; 13 h; 2 breaks of 30 min duration after 5 (12:00 PM) and 10 h (5:00 PM) of transport
12	Saumur: light exercise
13	Saumur: light exercise
14	Saumur: light exercise

Neustadt (Dosse), 7 d at Neustadt (Dosse), 2 d of return transport, and 3 d thereafter (see Table 1). The study was approved by the Ethics and Animal Experimentation Committee of the Vienna University of Veterinary Sciences.

2.2. Experimental procedures

2.2.1. Animals and transport

Out of a total of 34 horses transported, 7 were selected for the study. Horses were transported in 4 custom-made vans designed for 10 horses each. Animals were placed in the same sequence as in their home stable, that is, between familiar neighbors. Horses included in the study were the first and the last animal of each van, which were easily accessible for sampling during transport. Horses were loaded at an angle of approximately 75° to the long axis of the van and were separated from each other by solid wooden partitions that were 160 cm high. Stalls were 75 cm wide and 205 cm long.

All animals were French sport horses trained in classical equitation to an advanced level. Age of the

horses was 13.7 ± 2.0 y (mean $\pm$ standard error of the mean [SEM], range 11-18 y). One of the horses was a mare, and 6 were geldings. All horses had been transported repeatedly before, including long-distance transports. The travel schedule is summarized in Table 1.

During transport, horses did not receive feed on the van but had access to water during the breaks. At Saumur, during the nights between the 2 transport days, and at Neustadt (Dosse) State Stud, the horses were stabled in individual boxes on wood chips. They were fed concentrates 3 times daily, hay twice daily, and had free access to water.

2.3. Salivary cortisol

Saliva was collected with specific cotton rolls (Salivette, Sarstedt, Nümbrecht-Rommelsdorf, Germany). The Salivette was grasped with a surgical arterial clamp, inserted at the angle of the lips into the mouth, and placed onto the tongue of the horses for 1 min until the cotton was well soaked with saliva. This procedure was well tolerated by all horses and was performed by 1 person without any restraint of the animals. The cotton roll was then returned to the Salivette polypropylene tube and stored at $4\,°C$ until centrifugation at the end of each transport day or centrifuged within 10 min at $1000 \times g$ for 10 min. At least 1 mL saliva per sample was obtained and frozen at $-20\,°C$ until analysis. Concentrations of cortisol in saliva were determined with a direct enzyme immunoassay (without extraction) [24] validated for equine saliva [13]. The antiserum shows cross-reactivity with cortisone and several corticosterone metabolites. Thus, values obtained have to be interpreted as cortisol immunoreactivity (IR). The intra-assay coefficient of variation was 5.0%, the interassay variation was 6.7%, and the minimal detectable concentration was 0.3 pg/well.

Saliva samples for basal cortisol determination were collected for 2 d before transport (Days -2 and -1). On each of these days, 4 samples were taken at 30-min intervals between 8:00 and 10:00 AM. On the transport days (Days 1, 2, 10, and 11), 2 samples were taken in the stable 60 and 30 min before loading; during the break, at approximately half the daily transport distance; at the end of transport, before unloading the horses; and at 5, 15, 30, 60, 90, 120, 180, and 240 min thereafter. Corresponding times of the day are given in Table 1. Because of technical problems (loading was performed 2 h earlier than scheduled), no samples at 60 and 30 min before loading could be taken on Day 2 of the outbound transport).

2.3.1. Fecal cortisol metabolites

Fecal samples for analysis of cortisol metabolites were collected daily from 2 d before the first transport until 3 d after the last transport, that is, over 16 d. On the transport days, samples were collected at 6:00 AM and 10:00 PM. On all other days, samples were collected at 6:00 AM, 2:00 PM, and 10:00 PM. Feces were collected from fresh fecal balls on the ground of the horses individual boxes, thus sampling did not interfere with the animals. Samples were frozen on dry ice immediately and were stored at $-20\,°C$ until analysis. Analysis by enzyme immunoassay was performed as described by Möstl et al [25]. The assay is directed against 11-etiocholanolone-CMO coupled to bovine serum albumin. The standard curve ranged from 2 to 500 pg/well, and the 50% intercept was at 20 pg. The interassay and intra-assay coefficents of variation were 11.2% and 8.7%, respectively.

2.3.2. Heart rate and heart rate variability

Heart rate was recorded with a mobile recording system (S810i, Polar, Kempele, Finland) attached to the thorax of the horse with an elastic girth. The positive electrode was located at the right shoulder and the negative electrode at the middle of the left thorax. The electrodes were affixed with a second girth, which also contained a pocket for the recording watch. The Polar device records changes in electrical potential to detect the R-peaks of the horses' electrocardiogram and stores the beat-to-beat (RR) intervals in digital form.

Basal heart rate was recorded on the day before each 2-d transport (Day -1 and Day 9); during transport on Days 1, 10, and 11; and for 2 h after unloading the horses. Because loading was performed 2 h earlier than scheduled on Day 2, no heart rate recordings could be obtained on that day. Mean RR interval and heart rate variability were calculated at 30-min intervals. Kubius HRV software (version 2.0, released 16.10.2008, Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) was used for heart rate variability (HRV) analysis. To remove trend components, data were detrended, and in addition, an artefact correction was made. Detrending followed the procedure described by Tarvainen et al [26]. Heart rate variability is usually nonstationary, and slow linear or more complex trends in the HRV signal can cause distortion of HRV analysis. The Kubios HRV program uses a detrending procedure based on the smoothness priors approach [26]. The smoothness parameter was set at 500 ms. For artefact correction, the custom filter of the program was set at 0.3, identifying RR intervals differing from the previous RR interval by more than 30% as arte-

facts. After abnormal interval removal, the program's algorithm substitutes detected errors with interpolated intervals calculated from differences between previous and next accepted RR intervals.

The time domain variables RR interval and its standard deviation (SDRR; square root of variance of all RR intervals for a given recording time), RMSSD (root mean square of successive RR differences), as well as the geometric mean standard deviation 1 (SD1) and 2 (SD2) were calculated. The means for all HRV variables were then recorded for subsequent periods of 30 min each. The RMSSD is determined by calculating the difference between consecutive RR intervals before squaring and summing them; the values are then averaged, and the square root is obtained. The RMSSD is the primary time domain variable used to estimate high-frequency, beat-to-beat variations that represent vagal regulatory activity [21]. For calculation of the geometric means, the duration of each RR interval is plotted against the duration of the preceding RR interval (Poincaré plot). To parameterize the shape of the plot, the Kubius HRV software fits an ellipse to the plot. The ellipse is oriented according to the line-of-identity (RRj = RRj+1) at 45° to the X axis. The standard deviation (SD) of the points perpendicular to the line-of-identity (SD1) describes short-term HRV mainly caused by parasympathetic activity. The standard deviation along the line-of-identity (SD2) describes long-term variability and is related to SDRR [21].

On Day 1 of outbound transport, recordings were obtained from all animals throughout the complete transport time (13 h). On Days 10 and 11, in individual horses, contact of the electrodes with the animals' skin was lost during the latter stages of transport. Because it was not possible to readjust the electrodes in the moving van, the number of animals was reduced to 4 on Day 10 after 7 h of transport and to 5 on Day 11 after 11 h of transport. For statistical analysis, only the period with recordings from all animals (N = 7) was included, but in the figures, data for the complete transport time are given.

2.3.3. Statistical analysis

Statistical analysis was performed with the SPSS statistics package (version 17.0, released 11.3.2009, SPSS, Chicago, IL, USA). All data were normally distributed (Kolmogorov-Smirnov test). Changes in salivary cortisol concentrations and heart rate variables over time were analyzed for each day of transport (including mean pretransport baseline values) by analysis of variance (ANOVA) using a general linear model for repeated measures. For fecal cortisol metabolite concentrations, data were available for the whole 16-d experimental period, and analysis of variance included all 16 d. In

the case of overall significant effects, values differing from the pretransport baseline were identified by testing for least significant differences. A P value < 0.05 was considered significant. All data given are means $\pm$ SEM.

3. Results

3.1. Salivary cortisol and fecal cortisol metabolite concentrations

On each 2-d transport (that is, outbound and return transport), cortisol IR concentrations increased significantly ($P < 0.001$ over time). Basal salivary cortisol IR concentrations were low on the day before transport (mean of Days -2 and -1: 0.38 ± 0.05 ng/mL). Values were still low on the first day of outbound transport (Day 1) at 60 min before loading (0.49 ± 0.09 ng/mL) but had significantly increased at 30 min before loading (1.07 ± 0.21 ng/mL, $P < 0.05$ vs baseline). During transport, salivary cortisol-IR increased further to 3.10 ± 0.42 ng/mL at mid-transport and was 2.83 ± 0.36 ng/mL at the end of transport ($P < 0.05$ vs baseline). After unloading, salivary cortisol IR decreased to near-baseline values within 30 min. Comparable changes occurred on the second day of outbound transport; no preloading values were available for that day (Fig. 1).

On the 2-d return transport (Days 10 and 11), salivary cortisol IR concentration had again increased at 30 min before loading (Day 10, 1.66 ± 0.38 ng/mL; Day 11, 1.37 ± 0.42 ng/mL; both $P < 0.05$ vs baseline). Cortisol IR concentration increased further during transport, with maximal concentrations measured at mid-transport (Day 10, 2.37 ± 0.43 ng/mL; Day 11, 2.43 ± 0.48 ng/mL; $P < 0.05$ vs baseline). After unloading of the horses, baseline values were again reached within less than 30 min (Fig. 1).

Mean basal concentrations of cortisol metabolites in feces on Days -2 and -1 were 85.9 ± 8.4 ng/g. Statistical analysis revealed significant overall differences between times ($P < 0.001$). Concentrations did not change significantly on the first day of outbound transport, but they increased on the second day of outbound transport ($P < 0.05$ vs baseline) and reached a maximum of 235.9 ± 47.5 ng/g at 2:00 PM on the day following outbound transport ($P < 0.05$ vs baseline). Cortisol metabolite concentrations in feces decreased thereafter, but they remained significantly elevated above baseline values ($P < 0.05$) at individual time points on Days 4, 7, and 9 (Fig. 2). During the return transport (Days 10 and 11), fecal cortisol metabolite concentrations were again low (mean daily concentrations on Days 10 and 11, 95.5 ± 12.1 and 101.0 ± 21.2 ng/g, respectively) and
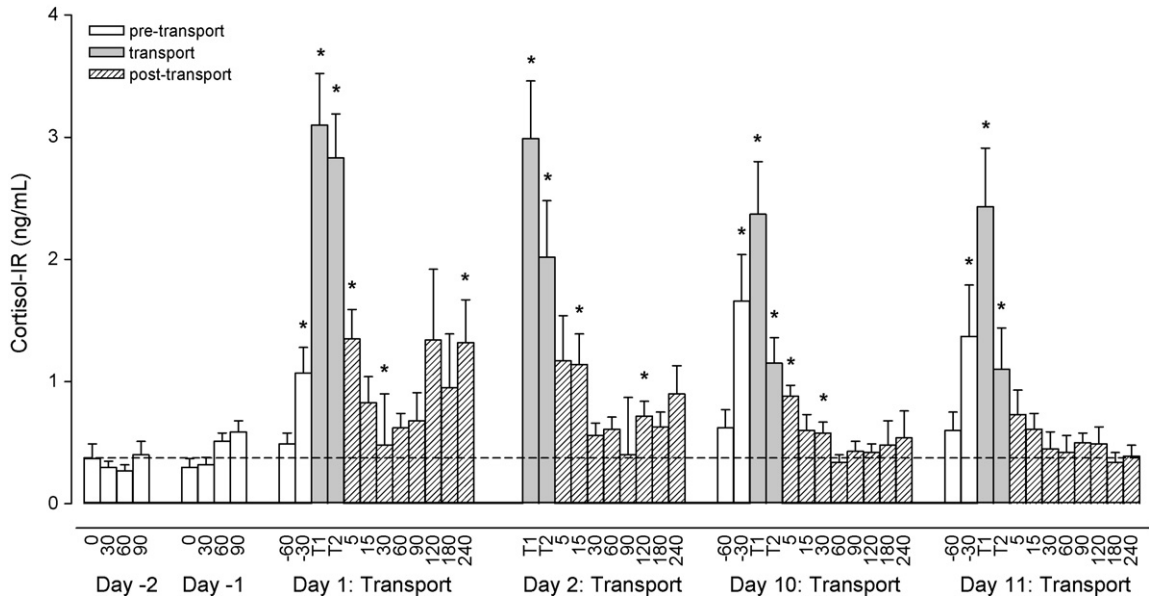
Fig. 1. Concentration of cortisol-IR in saliva of horses (N = 7) before, during, and after transport. Times on x axis indicate minutes, T1 = mid-transport, T2 = end of transport. Changes over time are significant for Days 1, 2, 10, and 11 (P < 0.001, analysis of variance). Values differing significantly from the pretransport baseline are indicated by asterisk (P < 0.05). Dashed line indicates mean pretransport baseline value.

increased significantly 1 d after transport (mean daily concentration on Day 12: 166.9 ± 31.5 ng/g; P < 0.05 over time, Fig. 2).

3.2. Heart rate and heart rate variability

On the first day of outbound transport (Day 1), mean RR interval decreased transiently during the first hour

of transport (from a baseline of 1635 ± 71 msec to a minimum of 1392 ± 99 msec, P < 0.05 vs baseline) and increased again within 1 h (P < 0.05 over time, Fig. 3). No significant changes in RR interval occurred on either day of the return transport (Fig. 3).

Standard deviations of RR interval decreased during the first 2 h on the first day of outbound transport
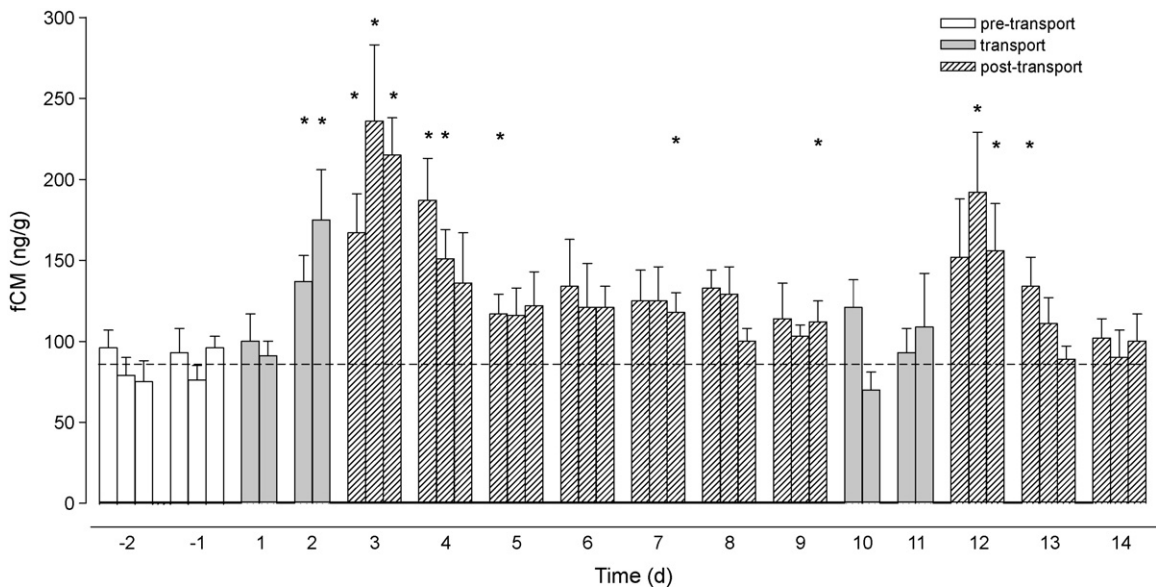

Fig. 2. Concentration of cortisol metabolite concentrations in feces of horses (N = 7) before, during, and after transport. Times on x axis indicate days (transports on Days 1,2, 10, and 11). Significant changes over time (P < 0.001, analysis of variance). Values differing significantly from the pretransport baseline are indicated by asterisk (P < 0.05). Dashed line indicates mean pretransport baseline value.
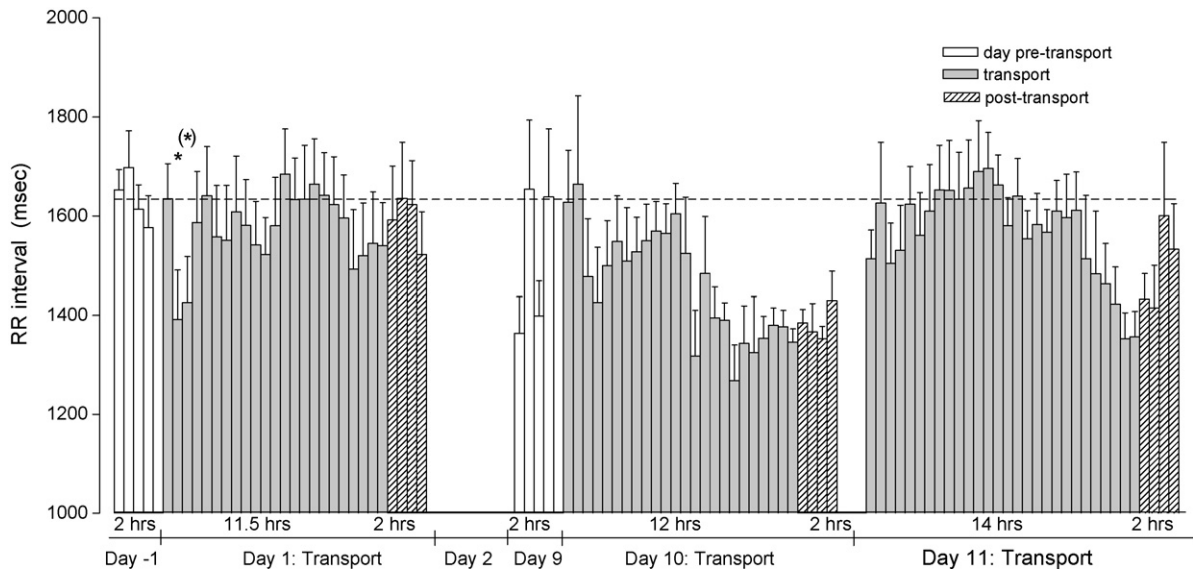
Fig. 3. Mean beat-to-beat (RR) interval in horses (N = 7) before, during, and after transport. Times on x axis indicate days and recording times divided into 30-min intervals. Changes over time are significant for Day 1 ($P < 0.05$, analysis of variance). Values differing significantly from the pretransport baseline are indicated by asterisk ($P < 0.05$; *, $P < 0.07$). Dashed line indicates mean pretransport baseline value.

(from a baseline of 66 ± 8 msec the day before transport to 47 ± 5 msec at 90-120 min of transport, $P < 0.05$) and increased slightly but near continuously thereafter ($P < 0.01$ over time). Standard deviations of RR interval followed a related pattern on the first day of return transport (Day 10; $P < 0.05$ over time), but post hoc tests revealed only a near significant difference ($P = 0.063$) from the pretransport baseline for the first 30 min of transport (Fig. 4a). Changes over time in RMSSD were similar to SDRR and reached statistical significance on the first day of both outbound (Day 1; $P < 0.01$) and return transports (Day 11; $P > 0.01$). On Day 1, mean basal RMSSD was 74 ± 12 msec, and values decreased to 48 ± 7 msec at 90 to 120 min of transport (Fig. 4b).

The geometric HRV variables SD1 and SD2, representing short-term and long-term changes in heart rate, respectively, changed significantly over time on both outbound and return transports ($P < 0.01$). On the first day of outbound transport, SD1 was less than the pretransport baseline, from 90 to 180 min of transport ($P < 0.05$ vs baseline). On the first day of return transport (Day 11), SD1 was reduced during the first 30 min of transport ($P < 0.05$ vs baseline; Fig. 5a). Geometric HRV variable standard deviation 2 markedly increased during the first hour of outbound (Day 1) and return transport (Day 10; $P < 0.001$ over time; Fig. 5b). Baseline values before transport were 272 ± 20 and 308 ± 70, respectively, and maximal values during the time period 30 to 60 min of transport were 475 ± 50 and 546 ± 48 for Days 1 and 10, respectively (Fig. 5b).

4. Discussion

In this study, salivary cortisol, fecal cortisol metabolites, heart rate, and heart rate variability were analyzed in horses exposed to a 2-d road transport interrupted by an overnight break. It was the aim of the experiment to analyze the stress experienced by the animals and to compare different methods of stress assessment. The conditions in our study closely mirror the factors to which experienced sport horses are exposed in conjunction with equestrian events.

Transport on all days caused a marked increase in salivary cortisol concentrations, which lasted at least several hours. This finding is in agreement with previous studies on transport-induced changes in blood plasma cortisol concentrations [7,27–29]. Cortisol release in horses follows a diurnal rhythm, with high values in the morning and a gradual decrease throughout the day [30–32]. Because baseline values in our study were obtained in the morning, that is, at the time of maximal endogenous cortisol release, it can be excluded that further increases thereafter were caused by diurnal changes. The cortisol response to transport tended to decrease with each day of transport, suggesting that a certain degree of adaptation might have occurred. The lesser salivary cortisol concentrations at the end of transport on each day compared to mid-transport might indicate depletion of readily available cortisol, causing cortisol concentrations to decrease even as the stress might continue. It cannot not be excluded that diurnal changes in cortisol
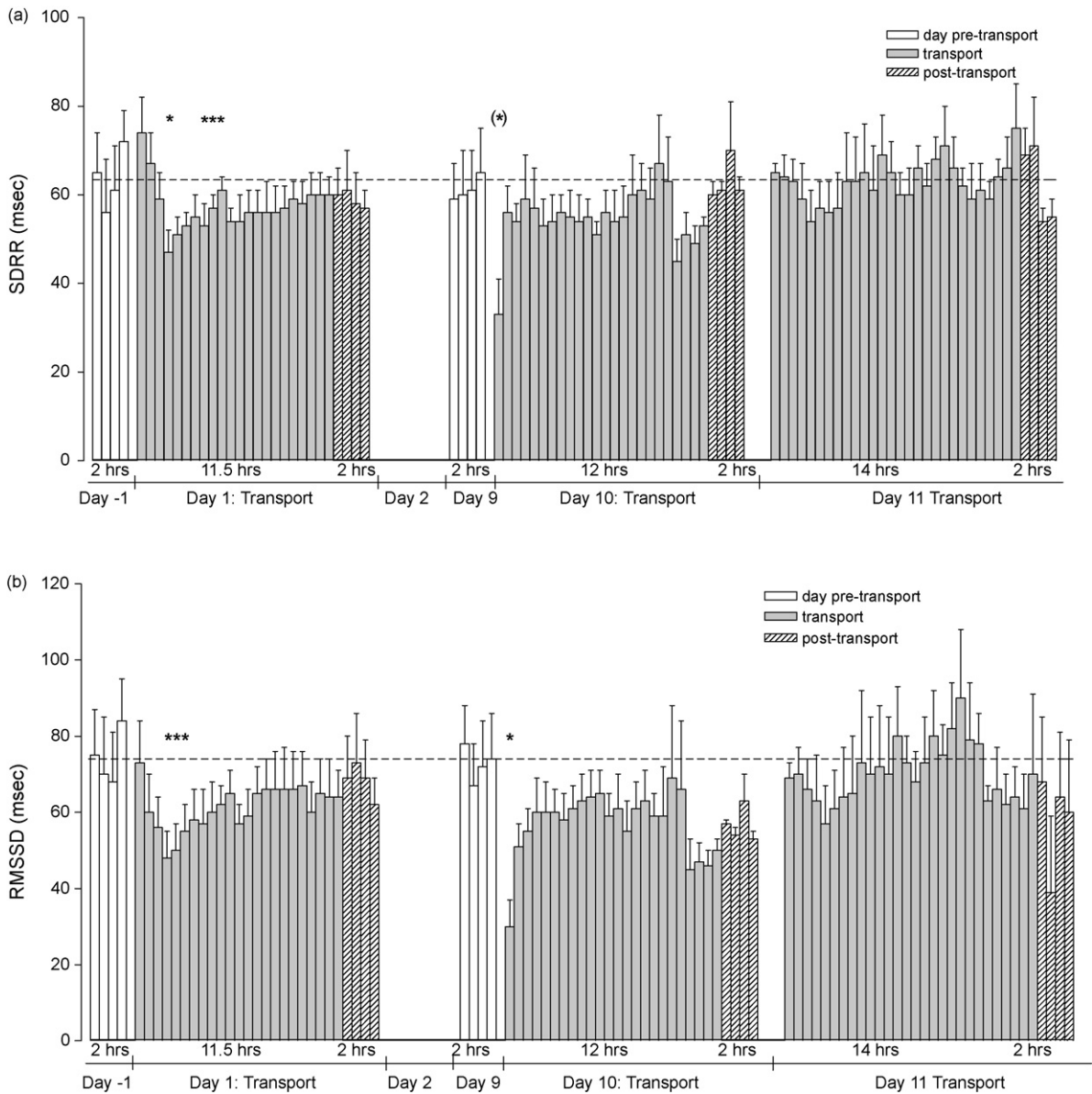
Fig. 4. (a) Mean standard deviation of beat-to-beat interval (SDRR) and (b) root mean square of successive beat-to-beat intervals (RMSSD) in horses (N = 7) before, during, and after transport. Times on x axis indicate days and recording times divided into 30-min intervals. (a) RR interval: changes over time are significant for Days 1 ($P < 0.01$; analysis of variance [ANOVA]) and 10 ($P < 0.05$; ANOVA). (b) Root mean square of successive beat-to-beat intervals: changes over time are significant for Days 1 and 10 (both $P < 0.01$; ANOVA). Values differing significantly from the pretransport baseline are indicated by asterisk ($P < 0.05$; *, $P < 0.07$). Dashed line indicates mean pretransport baseline value.

release [30–32] also contribute in part to the decrease in salivary cortisol concentrations with ongoing transport. Furthermore, a certain adaptation during the course of each transport day can also not be excluded. Highest plasma cortisol concentrations at mid-transport have also been found in mares transported for 12 h [2], whereas in another study on 24-h transport, the highest cortisol concentrations were found at the end of transport [7]. In a previous study from our group [9], during trans-

port of transport-inexperienced horses over distances up to 500 km, the highest cortisol concentrations in saliva were always found at the end of transport. In contrast, in the current study, the distance was considerably longer, but all horses were transport experienced.

Interestingly, salivary cortisol concentrations increased already within the last hour before transport, when horses were still in their stalls. Preparation of the transport vehicles, grooming of the horses, and
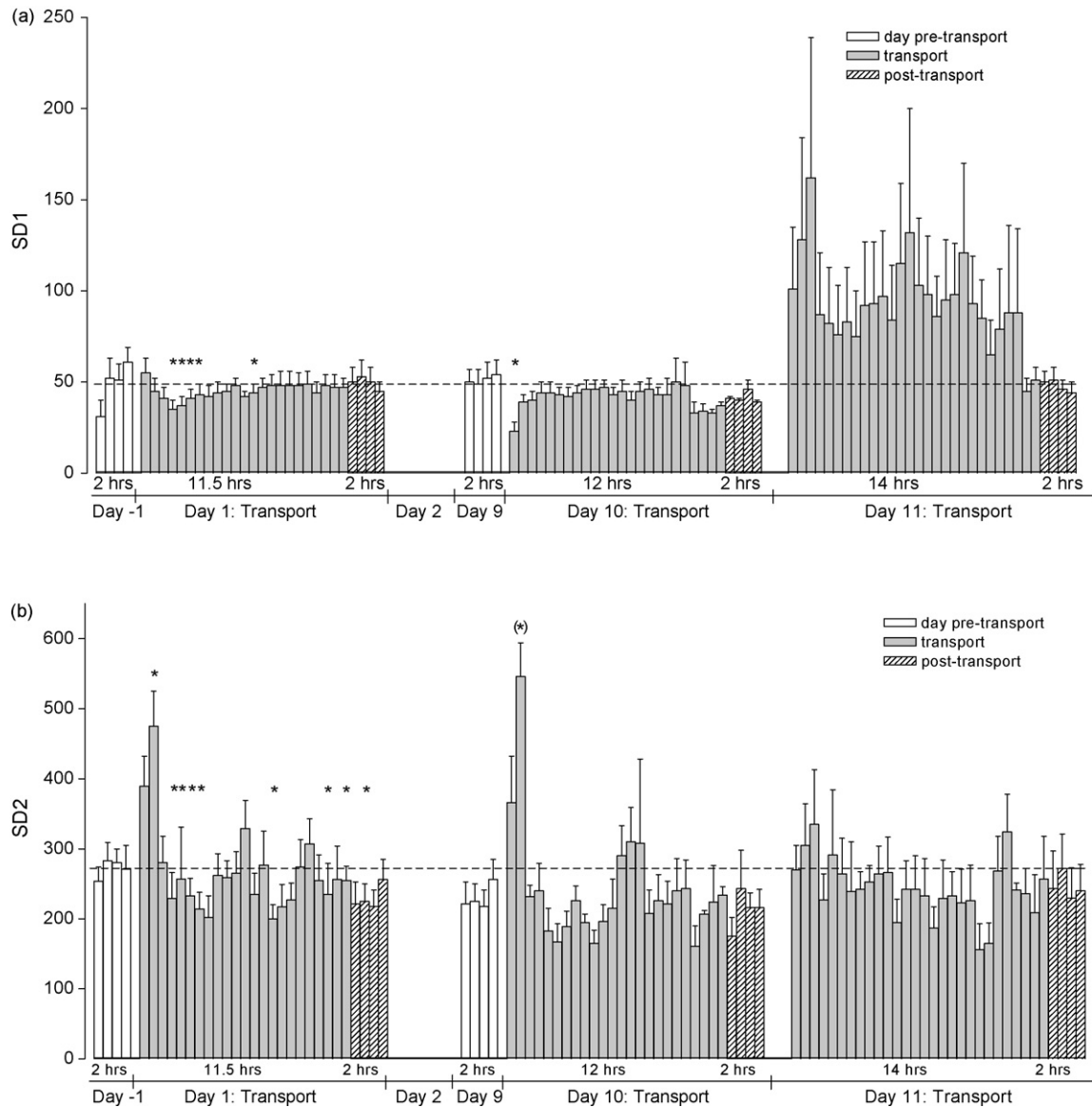
Fig. 5. (a) Geometric mean standard deviation (SD) 1 and (b) SD2 in horses (N = 7) before, during, and after transport. Times on x axis indicate days and recording times divided into 30-min intervals. (a) For SD1: changes over time are significant for Days 1 and 10 (both $P < 0.01$, analysis of variance). (b) For SD2: changes over time are significant for Days 1 and 10 (both $P < 0.001$, analysis of variance). Values differing significantly from the pretransport baseline are indicated by asterisk ($P < 0.05$; *, $P < 0.07$). Dashed line indicates mean pretransport baseline value.

preparation of the horses for transport (putting on protective transport boots and tail bandages) occurred at that time. Horses have a limited trainable prospective memory [33]. The increased cortisol release indicates that horses in our study were anticipating loading and subsequent transport. This anticipation does not necessarily mean that the horses were looking forward to a negative experience. The transport-experienced animals might also have been excited in a positive way, and changes in cortisol release may

help them to cope successfully with the demands of transport.

Transport also caused a clear increase in cortisol metabolite concentrations in feces. In agreement with previous studies in horses [17] and sheep [14], this increase occurred with a delay of 1 to 2 d, and maximal values were found only after transport. Fecal cortisol metabolites reflected the prolonged transport duration but did not allow distinguishing between different phases of transport. Fecal cortisol metabolite concentrations

decreased after transport but remained somewhat above pretransport baseline values between the outbound and the return transport. This elevation either might indicate long-term effects of the 2-d transport on cortisol release or could be caused by the exposure of the horses to a new environment, new stables, and to the equestrian presentations on Days 6, 7, and 9. However, if such additional external factors contributed to increased cortisol release, they were at all times less pronounced than the stress associated with transport.

In the present study, the heart rate and heart rate variability data are less clearly to interpret than the endocrine data on cortisol release. However, RR interval and heart rate variability were significantly affected by transport. During the first 90 min of outbound transport, RR interval decreased, and thus heart rate increased. An increase in heart rate indicates increased sympathetic activity and/or decreased parasympathetic (vagal) activity [21]. Unfortunately, no data were available for Day 2 of outbound transport. Because little physical activity was requested from the animals during transport, changes in heart rate were caused mainly by the need to adapt to the transport situation. However, road transport is not a continuous stressor, and coping with changes in speed, road surface, and turning will require some form of physical activity, which will affect heart rate and HRV. Although some changes in HRV variables were detected in our study, it thus cannot be excluded that others were masked by transport-induced activity of the horses.

A transient decrease in RR interval during transport is in agreement with results from previous studies [4,9,17–20,34], but in these studies, changes persisted for a longer time period. The rather rapid return to baseline values in our study is most likely a result of the transport experience of our horses, which are transported regularly in the same group of animals and on the same transport vehicles. This interpretation is also supported by the lack of major decreases in RR interval on the return transport. To our knowledge, data on transports lasting several days are not available from other studies.

Standard deviation of beat-to-beat interval (SDRR) decreased after approximately 1 h of the outbound transport. Because stress in horses [23] and calves [22] reduces variability of the RR interval, the decrease during transport is in agreement with the increase in cortisol release. In our study, HRV was analyzed for consecutive 30-min intervals. Within these intervals, RR intervals might change transiently in response to external factors requiring some physical activity of the animals. This change will affect HRV and lead to an increased over-

all SDRR for each 30-min period, which might not have occurred with shorter recording intervals.

The root mean square of successive RR differences decreased during the initial phase of transport on each day. Root mean square of successive RR differences is the primary time domain parameter used to estimate high-frequency, beat-to-beat variations that represent parasympathetic regulatory activity. A decrease in RMSSD reflects a reduced parasympathetic tone and can be caused by external stressors [21]. Also, SD1, representing short-term HRV and thus parasympathetic influence [21], decreased transiently on the first day of outbound and, less pronounced, return transport.

On the first day of each 2-d transport, a short but marked increase in the geometric mean SD2 also occurred. The variable SD2 represents changes in HRV caused predominantly by sympathetic regulation [21]. At rest, horses have a high parasympathetic tone, and sympathetic tone plays little role in determining heart rate [35,36]. The onset of transport is thus associated with transiently increased sympathetic and reduced parasympathetic activity, but these changes apparently became less evident on subsequent days of transport. A reduced parasympathetic tone in transported horses, as indicated by a decrease in RMSSD, is in agreement with reduced high-frequency power during transport, when HRV was determined by power spectrum analysis [20]. However, in that study, 2-yr old horses were transported. Most likely they were less experienced than our horses and thus may have perceived transport as more stressful.

In conclusion, a 2-d road transport of transport-experienced horses leads to increased cortisol release and to changes in heart rate and HRV, which are indicative of stress in the animals. The degree of these changes tended to be most pronounced on the first day of transport. During an overnight rest, cortisol concentrations rapidly returned to normal. Thus, long-distance transports of sport horses to equestrian competitions should include recovery times to minimize potential cortisol-induced effects in the animals. Salivary cortisol concentrations are a sensitive parameter to detect acute increases in cortisol secretion, whereas cortisol metabolite concentrations in feces increase with a delay of approximately 24 h and detect only prolonged increases in cortisol release. Changes in heart rate variability during transport indicate increased sympathetic activity and decreased vagal tone only at the onset of transport.

Acknowledgments

References

[1] International Equestrian Federation. 2007 Annual Report of the FEI (Fédération Equestre Internationale); 2007. Available at: http://www.fei.org. Accessed July 21, 2008.

[2] Baucus KL, Squires EL, Ralston SL, McKinnon AO, Nett MT. Effects of transportation on the estrous cycle and concentrations of hormones in mares. J Anim Sci 1990;68:419–26.

[3] Baucus KL, Ralston SL, Nockels CF, McKinnon AO, Squires EL. Effects of transportation on early embryonic death in mares. J Anim Sci 1990;68:345–51.

[4] Clark DK, Friend TH, Dellmeier G. The effect of orientation during trailer transport on heart rate, cortisol and balance in horses. Appl Anim Behav Sci 1993;38:179–89.

[5] Smith BL, Jones JH, Hornof WJ, Miles JA, Longworth KE, Willits NH. Effects of road transport on indices of stress in horses. Equine Vet J 1996;28:446–54.

[6] Grandin T. Assessment of stress during handling and transport. J Anim Sci 1997;75:249–57.

[7] Stull CL, Rodiek AV. Physiological responses of horses to 24 hours of transportation using a commercial van during summer conditions. J Anim Sci 2000;78:1458–66.

[8] Fazio E, Medica P, Aronica V, Grasso L, Ferlazzo A. Circulating β-endorphin, ACTH and cortisol levels of stallions before and after short road transport: stress effect of different distances. Acta Vet Scand 2008;50:6.

[9] Wehnert C, Schmidt A, Möstl E, Aurich J, Müller J, Aurich C. Heart rate and cortisol release in horses during road transport of 1, 3.5 and 8 hours duration. Proceedings of the International Equine Science Meeting, Regensburg, Germany; October 2008; 44.

[10] Riad-Fahmy D, Read GF, Walker RF. Salivary steroid assays for assessing variation in endocrine activities. J Steroid Biochem 1983;19:265–72.

[11] Möstl E, Palme R. Hormones as indicators of stress. Domest Anim Endocrinol 2002;23:67–74.

[12] Shanahan S. Trailer loading stress in horses: behavioural and physiological effects of nonaversive training (TTEAM). J Appl Anim Welf Sci 2003;6:263–74.

[13] Schmidt A, Aurich C, Neuhauser S, Aurich J, Möstl E. Comparison of cortisol levels in blood plasma, saliva and faeces of horses submitted to different stressors or treated with ACTH. Proceedings of the Fifth International Symposium on Equitation Science, Sydney, Australia; July 2009; 53.

[14] Palme R, Möstl E. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Int J Mammal Biol 1997;62(Suppl II):192–7.

[15] Merl S, Scherzer S, Palme R, Möstl E. Pain causes increased concentrations of glucocorticoid metabolites in horse faeces. J Equine Vet Sci 2000;20:586–90.

[16] Berghold P, Möstl E, Aurich C. Effects of reproductive status and management on cortisol secretion and fertility of oestrous horse mares. Anim Reprod Sci 2007;102:276–85.

[17] Waran NK. The behaviour of horses during and after transport by road. Equine Vet Educ 1993;5:129–32.

[18] Smith BL, Jones JH, Carlson G. Pascoe JR Effect of body direction on heart rate in trailered horses. Am J Vet Res 1994;55:1007–11.

[19] Waran NK, Cuddeford D. Effects of loading and transport on the heart rate and behaviour of horses. Appl Anim Behav Sci 1995;43:71–81.

[20] Ohmura H, Hiraga A, Aida H. Changes in heart rate and heart rate variability in Thoroughbreds during prolonged road transport. Am J Vet Res 2006;67:455–62.

[21] Von Borell E, Langbein J, Despres G, Hansen S, Leterrier C, Marchand-Forde J, Marchand-Forde R, Minero M, Mohr E, Prunier A, Valance D, Veissier I. Heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals—a review. Physiol Behav 2007;92:293–316.

[22] Mohr E, Langbein J, Nürnberg G. Heart rate variability—a non-invasive approach to measure stress in calves and cows. Physiol Behav 2002;75:251–9.

[23] Visser EK, van Reenen CG, van der Werf JTN, Schilder MBH, Knaap JH, Barneveld A, Blokhuis HJ. Heart rate and heart rate variability during a novel object test and a handling test in young horses. Physiol Behav 2002;76:289–96.

[24] Palme R, Möstl E. Measurement of cortisol metabolites in faeces of sheep as a parameter of cortisol concentration in blood. Proceedings of the First International Symposium on Physiology and Ethology of Wild and Zoo Animals, Berlin, Germany; September 1996; 84.

[25] Möstl E, Maggs JL, Schrötter G, Besenfelder U, Palme R. Measurement of cortisol metabolites in faeces of ruminants. Vet Res Commun 2002;26:127–39.

[26] Tarvainen MP, Ranta-aho PO, Karjalainen PA. An advanced detrending method with application to HRV analysis. IEEE Trans Biomed Eng 2002;49:172–5.

[27] Friend TH. Dehydration, stress and water consumption of horses during long-distance commercial transport. J Anim Sci 2000;78:2568–80.

[28] Stull CL, Rodiek AV. Effects of cross-tying horses during 24 h of road transport. Equine Vet J 2002;34:550–5.

[29] Stull CL, Morrow J, Aldridge BA, Stott JL, McGlone JJ. Immunophysiological responses of horses to a 12-hour rest during 24 hours of road transport. Vet Rec 2008;162:609–14.

[30] Zolovick A, Upson DW, Eleftheriou BE. Diurnal variation in plasma glucocorticosteroid levels in the horse. J Endocrinol 1996;35:249–53.

[31] Bottoms GD, Roesel OF, Rausch FD, Akins EL. Circadian variation in plasma cortisol and corticosterone in pigs and mares. Am J Vet Res 1972;33:785–90.

[32] Hoffsis GF, Murdick PW, Tharp VL, Ault K. Plasma concentrations of cortisol and corticosterone in the normal horse. Am J Vet Res 1978;31:1379–87.

[33] Murphy J. Assessing equine prospective memory in a Y-maze apparatus. Vet J 2009;181:24–8.

[34] Waran NK, Robertson V, Cuddeford D, Kokoszko A, Marlin DJ. Effects of transporting horses facing either forwards or backwards on their behaviour and heart rate. Vet Rec 1996;139:7–11.

[35] Hamlin RL, Klepinger WL, Gilpin KW, Smith CR. Autonomic control of heart rate in the horse. Am J Physiol 1972;222:976–8.

[36] Kuwahara M, Hashimoto S, Ishii K, Yagi Y, Hada T, Hiraga A, Kai M, Kubo K, Oki H, Tsubone H, Sugano S. Assessment of autonomic nervous function by power spectral analysis of heart rate variability in the horse. J Auton Nerv Syst 1996;60:43–8.