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Evaluation of the effect of tongue ties on stress parameters, behaviour and heart-rate variability in racehorses

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Abstract

Fixation of the tongue to the mandible using so-called tongue ties (TTs) is common practice in Standardbred (SB) and Thoroughbred (TB) racing, but little is known about their impact on animal welfare. In this study, the influence of TTs on heart-rate variability (HRV), stress parameters in plasma (cortisol, glucose, lactate) and behaviour was evaluated in 30 SBs and 29 TBs (n = 59) presenting with exercise insufficiency. Overall, 36/59 horses (24 SBs, 12 TBs) were familiar with TTs. Blood was taken at rest, after TT application and after racing in all horses, additionally samples were taken without TT in SBs another day. HRV was calculated over 3 min before, during and after racing. Additionally, SBs' behaviour during TT application and racing was documented in real time. TT application did not increase cortisol levels significantly, while highly significant increases in cortisol levels were found after racing. Lactate levels were not influenced by TT application, but also significantly increased after racing. No significant differences were found for glucose. Seventeen out of 30 SBs showed mild (n = 8), moderate (n = 8) and severe (n = 1) reactions during TT application, none during or after race training. At rest, 23/30 SBs had a low/high frequency (LF/HF) ratio < 1.5 (1.05 [± 0.61], n = 30, dominating parasympathetic activity). After TT application, the LF/HF ratio increased to 1.4 (± 0.45) (increased sympathetic activity). In TBs, sympathetic activity dominated at rest. No differences in LF, HF and LF/HF were found after TT application or comparing HRV after racing with/without TT. The stress response (blood parameters and HRV) was not influenced by horses' naivety to TTs, however an increased stress response was observed in SB mares. Overall, obvious adverse behaviour, but only slight evidence of an increased systemic stress response, was found in this study. These results might provide objective evidence for future decisions from equine sports organisations concerning further regulations on TTs.

Keywords: *animal welfare, cortisol, heart-rate variability, racing, stress, tongue tie*

Introduction

Tongue ties (TTs) are devices used to fixate the tongue to the mandible and hold it in this position during exercise with the goal of facilitating control of the horse and as a conservative treatment for dorsal displacement of the soft palate (DDSP; Barakzai & Dixon 2005). They have been used in racehorses for over 100 years. Conflicting evidence regarding their efficacy in preventing DDSP, combined with public concerns of potential welfare implications, led to the banning of TT use in show horses by the Fédération Equestre Internationale (FEI) in 2004. However, TTs are still approved for use in racing in many countries. The prevalence of TT use is approximately 5% in racehorses in Great Britain, rising to 89% in horses diagnosed with DDSP (Barakzai *et al* 2009a,b), so their use is commonplace and often in combination with other equipment (Chalmers *et al* 2013). In Germany, in June 2018, they were banned in Thoroughbreds (TB) racing nationally due to ongoing public debate, but are still approved for Standardbreds (SB) and remain part of the official regulations on racing equipment.

Stress in animals is of increasing research interest and been the focus of multiple studies. Cortisol concentrations have been measured in plasma, saliva and faeces to evaluate the stress response during transport (Stull & Rodiek 2002; Fazio *et al* 2008; Schmidt *et al* 2010b,c,d; Medica *et al* 2019) and physical exercise under training and performing conditions (Golland *et al* 1999; Cayado *et al* 2006; Malinowski *et al* 2006; Ferlazzo *et al* 2009; Cravana *et al* 2010; Schmidt *et al* 2010a). Endogenous cortisol increases glucose levels in plasma to supply energy for the central nervous system, which has no energy stores itself (Harbuz & Lightman 1992). In horses, this hyperglycaemia has been shown to correlate with hospitalisation, anaesthesia and slaughter (Luna *et al* 1996; May 2007; Walther 2017), but glucose levels can be affected by various factors including fasting or feeding, training, circadian rhythm of cortisol levels, stress, disease, gestation and bodyweight (Evans 1971; Ralston 2002; Bertin *et al* 2016).

Lactate levels also increase during training and physical stress (Hodgson *et al* 1987; Harris & Snow 1988; Rose *et al*

1988), but immediate sampling is of utmost importance (Marlin *et al* 1991; Lindner *et al* 1992; Rainger *et al* 1994; Roberts *et al* 1999). Lactate levels are commonly used to evaluate training condition after standardised exercise tests.

Heart-rate variability (HRV) reflects the interaction between the sympathetic and parasympathetic autonomous nervous system and its regulatory effect on the cardiovascular system (Sammito & Bockelmann 2015). HRV parameters have been shown to be a good option for non-invasive evaluation of the sympathetic-parasympathetic balance and the baroreceptor activity in different stress and pain situations (Malik 1996; Berntson *et al* 1997; Kucera 2006) with HRV decreasing with pain and stress, reflecting autonomous dysregulation. Therefore, the RR-interval between R-waves of the QRS-complex are analysed, if they are proceeded by a P-wave reflecting the depolarisation of the sinus node. These RR-intervals are also called NN- (normal to normal) intervals, as they appear physiologically due to the activity of the sinus node (Rompelman *et al* 1977; Tarvainen *et al* 2014). The spectral analysis allows analysis between sympathetic and parasympathetic activity (Pomeranz *et al* 1985; Malliani *et al* 1991), with HF (high frequency) reflecting parasympathetic activity calming the heart rate and LF (low frequencies) representing sympathetic activity (Pagani *et al* 1986). Other authors also discuss parasympathetic involvement in these LF (Houle & Billman *et al* 1999), which are representative for the activity of baroreceptors influencing the vascular pressure (Brüggemann *et al* 1995; Kuwahara *et al* 1996; Hottenrott 2002). The LF/HF ratio shows the combined effect of the autonomous nervous system (sympathetic/parasympathetic activity) on the heart (Eckberg 1997). which increases reflecting overwhelming sympathetic activity. As in other species, parasympathetic activity dominates in horses at rest (Kuwahara *et al* 1999). Several training methods have been evaluated using HRV, including hyperflexion of the neck (Becker-Birck *et al* 2013; Zebisch *et al* 2014) and influence of equipment (Fenner *et al* 2016). In these studies, neither lunging nor riding in hyperflexion led to changes in HRV, while tight nosebands reduced HRV severely. As previous studies showed common discolouration of the tongue during TT application (Barton *et al* 2019), TTs can be expected to lead to poor perfusion and to reduce HRV as a consequence of stress or pain.

Horses' behaviour during TT application and 30 min afterwards was documented at rest in animals both familiar with TTs and naïve to them (Latimer-Marsh *et al* 2017). Increased frequencies of head-shaking and gaping were found in horses with previous experience of TT use in comparison to naïve horses. Previous studies on the tolerance of tight nose-bands had found behavioural changes in chewing, licking, yawning and swallowing (Fenner *et al* 2016). During the application of discomforting equipment, tail twitching and flattening the ears often indicate discomfort in horses (Wagner 2010).

In this study, the effect of TTs on stress parameters (cortisol, lactate and glucose) as well as HRV, was evaluated in 59 SB and TB racehorses under high-intensity exercise. Horses' behaviour during TT application and during strenuous exercise was scored. We hypothesised that TT application and racing under TT use would increase stress parameters and decrease HRV as a consequence of overwhelming sympathetic activity compared to horses without TT, in particular in those familiar with TT application.

Materials and methods

Ethical statement

According to their training records, all horses included in this study were presented for exercise insufficiency, with respiratory noises recorded during exercise for 3/59 horses. Orthopaedic problems had been excluded by the referring veterinarians. A clinical examination including auscultation of heart and lungs, blood work (red and white blood cell counts, arterial blood gas analysis), electrocardiogram (ECG) at rest and during exercise and dynamic endoscopy were performed for diagnostic work-up. Data and blood samples were used for this study, but no additional samples were taken. Therefore, no ethical approval was essential according to §8a of German Animal Welfare Law.

Study animals

The population included in the study consisted of 59 horses (30 SB, 29 TB) with a mean (± SD) age and weight of 4 (± 2) years and 432 (± 31) kg, respectively. We examined 25 mares, 26 geldings and eight stallions trained by nine different trainers. Overall, the majority of horses (24 of 30 SBs and 12 of 29 TBs) were accustomed to the use of TTs (36/59 overall) with TT use overwhelming in SBs. All horses had already been trained for racing or had raced previously. Horses familiar with TT use were included, as well as horses where trainers wished to evaluate the use of a TT to improve the clinical problem of the horse. All horses had a history of exercise insufficiency and were not randomly chosen. Three horses had a recorded respiratory noise and some had had other examinations before, ie examination of the musculoskeletal system, but these had not yielded a diagnosis to explain their problems.

Equipment and racing conditions

All horses were equipped with their usual training tack, including bits and other equipment, such as check reins in SBs or martingales in TBs. SBs were driven and TBs ridden by their usual trainers and jockeys. Distance, type of racetrack and speed varied between horses due to differences in TB and SB racing, age and training condition, but all horses raced under the same conditions with and without TT. Comparable exercise intensity with and without TT was ensured by measuring speed using a GPS system (TBs and SBs), heart rate using the mobile ECG system (TBs and SBs) and venous lactate immediately after exercise (SBs). SBs were evaluated on two subsequent days at their normal training times and were sampled on one day randomly before and after racing without TT (t1a-control and t2-control) and on the other day before application and after application of the TT and after racing (t1a, t1b and t2). TBs were not trained on two subsequent days at racing intensity therefore they were only sampled on one day before and after application of the TT and after racing (t1a, t1b and t2).

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TT application and behaviour

An elastic bandage (Meproflex, Bela Pharm, Vechta, Germany) was used as a TT and applied by the trainer/driver, so the material was standardised, although pressure on the tongue was not. It was applied approximately 15 min prior to maximum exercise intensity, when occurrence of DDSP was deemed most likely. In all 30 SBs, defence behaviour during TT application was scored as absent (presentation of head and/or tongue for TT application), neutral (no obvious defence behaviour), mild (turning away the head, mild tail twitching, no flattening of the ears), moderate (rapid head movements, ongoing tail twitching, mild flattening of the ears) or severe (aggressive behaviour, rising, biting, severe flattening of the ears). It was also documented whether this behaviour remained ongoing on the way to the race-track and/or during/after exercise. Observations were carried out in real time by one of the authors (IL).

Plasma samples

Blood samples were taken by jugular venipuncture at rest (in the stable, t1a_control and t1a), directly after application of the TT (t1b) and after racing a maximum speed (t2_control and t2). Blood was filled into serum (cortisol) and fluoride tubes (lactate, glucose) (Sarstedt AG & Co KG, Sarstedt, Germany) and stored on ice until assayed. Analysis of lactate and glucose was performed in the clinic's laboratory using a blood gas analyser (Cobas b123, Roche, Mannheim, Germany), while serum tubes were centrifuged (3,800 U per min; 15min), stored in 500 µl aliquots at –80°C until assayed by the Institute of Veterinary Physiological Chemistry of the University of Leipzig, Germany.

ECG

Telemetric holter ECGs were recorded with a two-channel recorder (Televet 100, Engel Engineering Services GmbH, Offenbach, Germany) during race training as part of the diagnostic work-up of exercise insufficiency. The electrodes were placed under the saddle pad and girth to ensure they were kept in place during race training (red: left thoracic wall 30 cm below the withers, black: 10 cm below the red electrode, yellow: right thoracic wall 30 cm below the withers and green: 2–3 cm lateral to the sternum). The ECG data were also used to evaluate the effect of TTs on HRV. The ECG was fitted and remained *in situ* for the entirety of the race. Recordings were analysed starting 5 min before and after the application of the TT at rest (both at the stable), during exercise until 5 min after maximum exercise (at the race-track). Three minutes of each phase were included in the HRV analysis.

Analysis of HRV

RR intervals were exported from the Televet software and imported to the 'Heart rate variability Analysis Software 1.1' (Biomedical Signal Analysis Group, University of Kuopio, Finland) and a time and frequency domain analysis was calculated. LF and HF frequencies are defined in the horse as 0.01–0.07 and 0.07–0.6 Hz, respectively, as previously described (Kuwahara *et al* 1996).

Table 1 Mean (± SD) and statistical significance (Wilcoxon test for paired samples) of stress parameters in SBs on two subsequent training days after racing with and without application of TTs.

	Cortisol (ng ml^{-1})	Lactate (mmol l^{-1})	Glucose (mmol l^{-1})
After training with TT	116.7 (± 33.60)	8.8 (± 7.20)	5.23 (± 2.06)
After training without TT	122.2 (± 30.80)	6.7 (± 6.80)	4.99 (± 1.59)
Significance	$P = 0.185$	$P = 0.683$	$P = 0.558$
Before training with TT (before application)	73.6 (± 28.10)	0.9 (± 0.0)	4.64 (± 0.83)
Before training without TT	78.7 (± 27.60)	0.9 (± 0.0)	4.52 (± 0.72)
Significance	$P = 0.141$	$P = 1.000$	$P = 0.108$

Statistical analysis

This was carried out using IBM SPSS® software (version 24). SB and TB data were tested for normal distribution using the Kolmogorow-Smirnov test and visual inspection. All variables with the exception of lactate concentrations were normally distributed.

A series of *t*-tests for paired samples compared variables (serum cortisol/lactate/glucose concentration, LF, HF, LF/HF ratio) from baseline (t1a_control and t1a) to the time-point after application of the TT (t1a tot1b), from baseline to the time-point after racing (t1a_control and t1a to t2_control and t2) and from the time-point of TT application to the time-point after racing (t1b to t2). Wilcoxon test for paired samples was used to investigate whether differences between before and after racing were higher with TT compared to without. These investigations were carried out with the data from 30 SBs.

Analysis of variance (ANOVA) models with repeated measurements were adapted to investigate the influence of previous training with TT, sex, breed, age, and bodyweight on changes to the respective dependent variable before and after application of TT, as well as after racing. For cortisol, lactate, glucose, HF, LF, and HF/LF ratio separate models were analysed. Interactions were included in the model, non-significant interactions were excluded step-by-step. Model diagnostics included normality and homoscedasticity of residuals. The level of significance was set at $P \leq 0.05$. Since six models included the same set of influence variables, the level of significance was adjusted using Bonferroni method and the level of significance was adjusted to 0.008 using Bonferroni correction (0.05/number of comparisons).

Results

Stress parameters in standardbreds

In SBs (n = 30), the serum cortisol concentration increased by 55% during exercise ($P < 0.001$) with mean (± SD) values of 78.7 (± 27.6) ng ml^{-1} without the application of the TT (t1a_control), and 122.3 (± 30.8) ng ml^{-1} after racing (t2_control), when no TT was applied ($P < 0.001$; *t*-test for paired samples; Table 1. On the other day, there was no significant increase in cortisol levels before (73.6 [± 28.1] ng ml^{-1}; t1a) and after the application of the TT (81.8 [± 32.2] ng ml^{-1}; t1b). The ANOVA model with repeated measurements revealed significantly different cortisol levels between the time-points 'after application of TT' (t1b) and 'after racing' (t2; $P = 0.001$) that additionally interacted between animals that were already used to TT or naive ($P = 0.008$) and between SB and TB ($P = 0.001$). Independent of the time-point, cortisol levels were mainly influenced by the question of whether animals were already used to TT or not (Figure 1), and the interaction between this factor and age. Sex and breed did not influence cortisol levels.

Significant differences could be detected in the cortisol concentrations after racing with TT (116.7 [± 33.6] ng ml^{-1}; t2) or without TT (122.3 [± 30.8] ng ml^{-1}; t2_control; $P = 0.032$, Wilcoxon test for paired samples; Table 1. No statistically significant differences between races with and without TT could be detected for lactate ($P = 0.175$) or glucose concentrations ($P = 0.863$; both Wilcoxon tests for paired samples) (Figure 2).

Lactate concentrations were 0.9 (± 0.0) mmol l^{-1} before (t1a), 1.0 (± 0.4) mmol l^{-1} after TT application (t1b) and 8.8 (± 7.2) mmol l^{-1} after racing (t2). The horses that were investigated without TT also showed significant differences before and after racing ($P < 0.001$; *t*-test for paired samples, mean difference: 5.78 mmol l^{-1}). In ANOVA analysis, there were significant differences between the time-points ($P < 0.001$) that were not able to be explained in more detail by the factors investigated (Figure 3).

Glucose concentrations were 4.6 (± 0.8) mmol l^{-1} before (t1a), 4.66 (± 0.9) mmol l^{-1} after TT application (t1b) and 5.5 (± 2.4) mmol l^{-1} after racing (t2). As for cortisol and lactate, values also differed significantly in horses without TT before and after racing ($P = 0.003$; *t*-test for paired samples). The ANOVA model showed again that significant differences occurred between 'after application of TT' and 'after racing' ($P < 0.001$) with an average increase of 1.3 mmol l^{-1} after racing. Significant interactions were found between time-point and breed (TB had higher values than SB; $P < 0.001$, and TB had higher increase than SB; $P < 0.001$) as well as time-point and TT naivety (steeper increase in non-naivety; $P = 0.0036$). Glucose increases after racing did not differ significantly with or without TT in SBs ($P = 0.991$; Wilcoxon test for paired samples) (Figure 4).

Stress parameters in thoroughbreds

In TBs (n = 29), the serum cortisol concentration increased after exercise ($P = 0.001$) with mean values of 66.74 (± 17.18) ng ml^{-1} before the application of the TT (t1a), 71.96 (± 17.78) ng ml^{-1} after the application (t1b; 7.8%) of the TT and 98.29 (± 19.71) ng ml^{-1} after racing (t2; difference 47.3% compared to values before the application of TT). As in SBs, the rise in cortisol concentration before and after application of the TT remained insignificant. Lactate concentrations were 0.67 (± 0.12) mmol l^{-1} before (t1a), 1.51 (± 0.77) mmol l^{-1} after TT application (t1b) and 10.28 (± 7.2) mmol l^{-1} after racing (t2). Again, there was no significant increase with application of the TT ($P = 0.063$), but after racing ($P < 0.001$). As in SBs, no differences were found for glucose with 5.54 (± 0.71) mmol l^{-1} before (t1a), 5.39 (± 0.95) mmol l^{-1} after TT application (t1b), but a significant increase compared to baseline was found after racing (t2; 7.68 [± 1.21] mmol l^{-1}; $P < 0.001$).

Analysis of HRV

Without TTs, in SBs (n = 30) there was an insignificant decrease of HF after racing ($P = 0.346$) as well as an insignificant increase of LF ($P = 0.110$; *t*-tests for paired samples each). The LF/HF ratio differed significantly between before and after racing ($P = 0.041$; Wilcoxon test for paired samples) with higher values after racing. ANOVA models revealed no significant differences between the time-points nor age, but LF values in SB mares were higher than in geldings (not significant; $P = 0.03$).

No significant differences before and after racing between with and without TT were detected regarding HF and LF/HF ratio ($P = 0.417$; $P = 0.65$, respectively), but for LF the increase was significantly higher when the animals wore TT ($P = 0.003$; Wilcoxon test for paired samples).

In SBs (n = 30), horses showed slightly higher HF values at rest before the application of TTs (LF 53.15 [± 19.36] normalised units [nu], HF 55.63 [± 19.36] nu) representing dominant parasympathetic activity. Overall, 23/30 horses had an LF/HF ratio < 1.5 with mean value of 1.05 (± 0.61) over all 30 horses. After application of the TTs, LF values dominated (LF 69.39 [± 14.29] nu, HF 48.28 [± 19.05] nu) representing increased sympathetic activity. Ten out of 30 horses showed LF/HF ratios > 1.5 with the TTs, and the mean value of LF/HF was 1.4 (± 0.45) over all 30 horses when having the TT in place. After racing, LF values dominated also (LF 73.51 [± 23.10] nu, HF 55.03 [± 22.53] nu), representing ongoing sympathetic stimulation. Ten out of 30 horses had LF/HF ratios > 1.5 after exercise with a mean value of 1.42 (± 0.78) over all 30 horses (summarised in Table 2[a]). Data of two subsequent training days (training without and with TT) showed significantly higher HF values ($P = 0.05$) and lower LF/HF ratios after race training without TTs (Table 3).

In contrast, LF values dominated in TBs already before and after application of TTs as well as after racing and tended to be higher than in SBs. We found no significant differences in LF, HF and LF/HF (Table 2[b]).

Behaviour in SBs

Two horses showed no defence behaviour and presented head or tongue for TT application and eleven showed neutral behaviour. The remaining 17 SBs showed mild (n = 8), moderate (n = 8) and severe (n = 1) reactions during the application of the TT. None of the horses showed obvious defence behaviour during or after race training.

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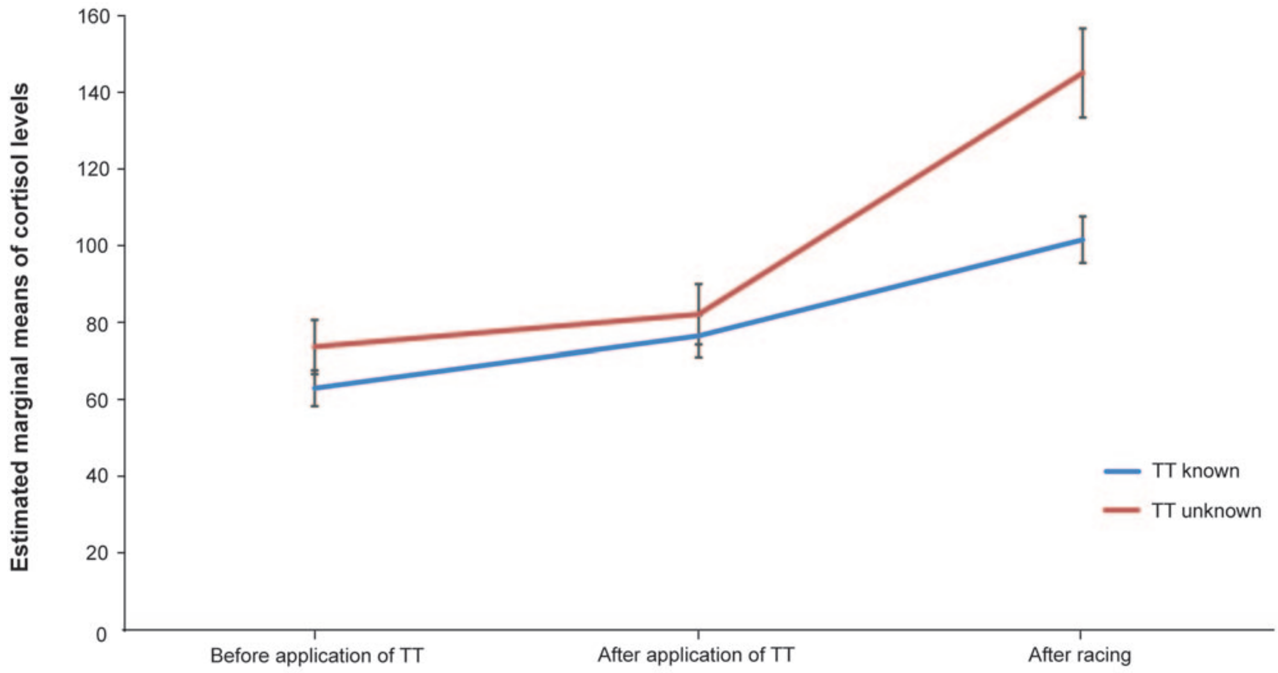
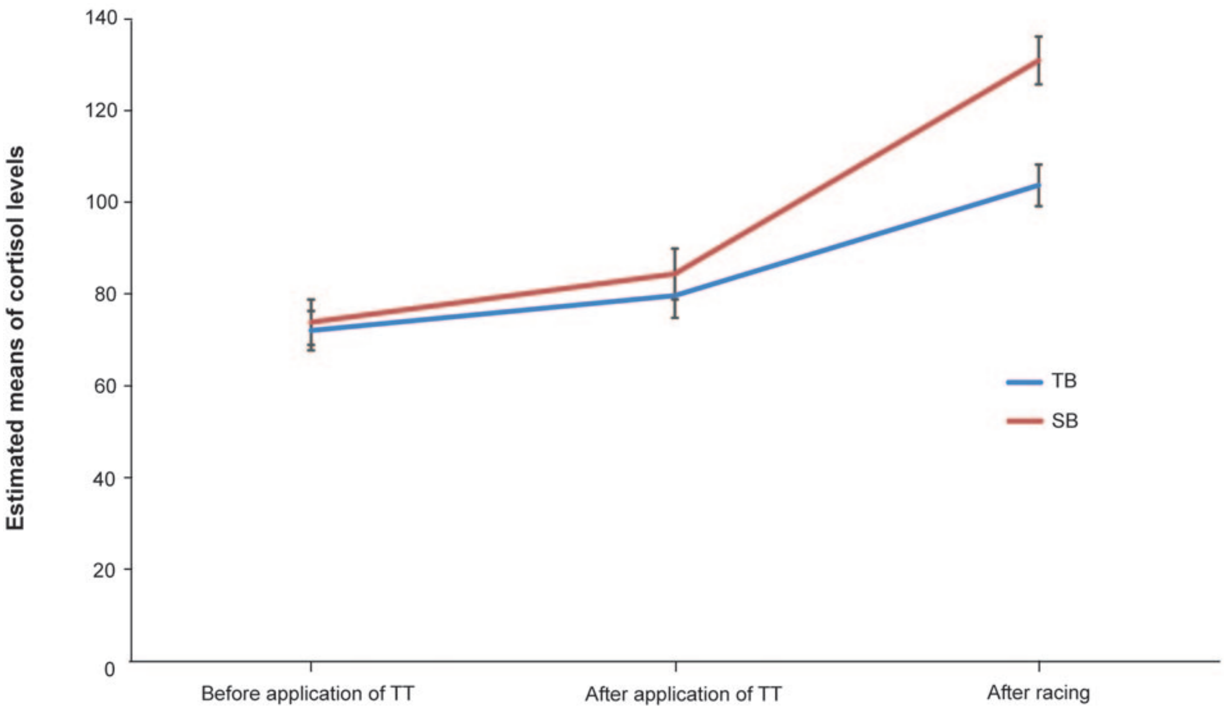
Figure 1



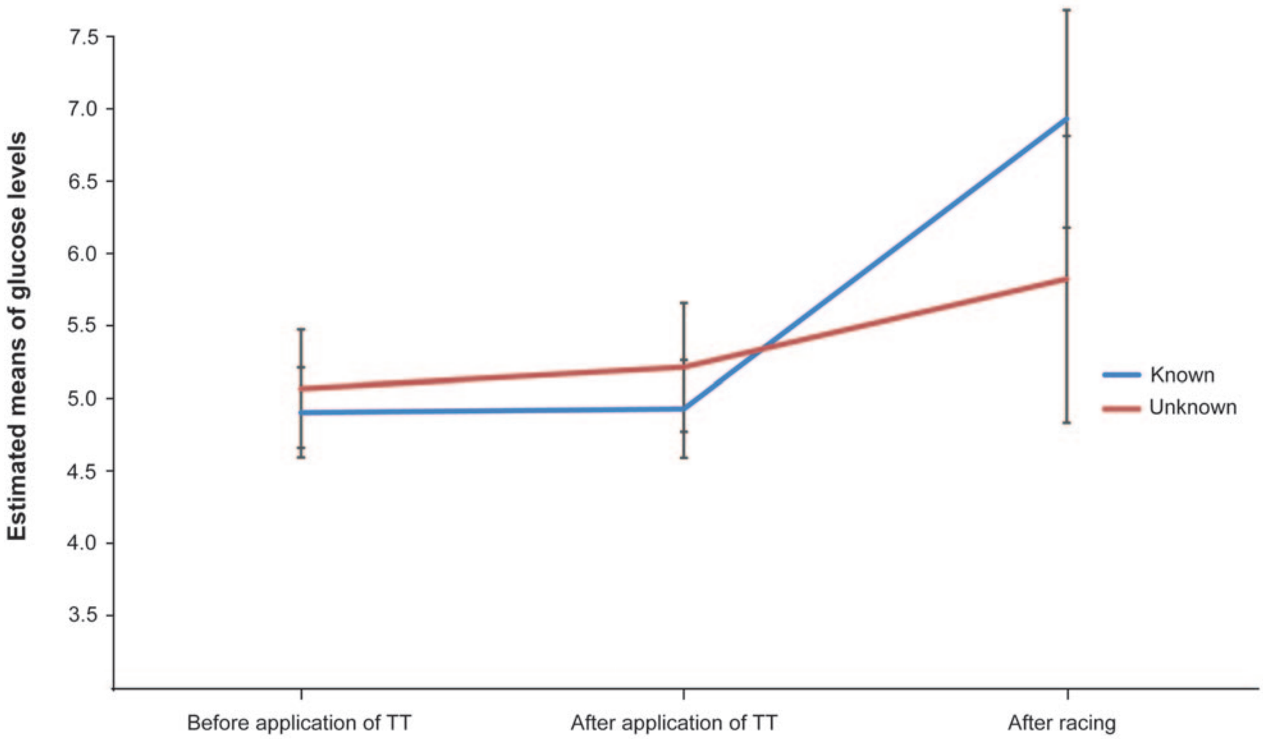
Estimated marginal means of cortisol levels at different time-points in all horses (SBs and TBs) familiar with TT (blue) compared to those that are not (red); $P = 0.016$ for interaction between TT naivety and time-point; $P = 0.001$ for TT naivety (ANOVA with repeated measurements).

Figure 2



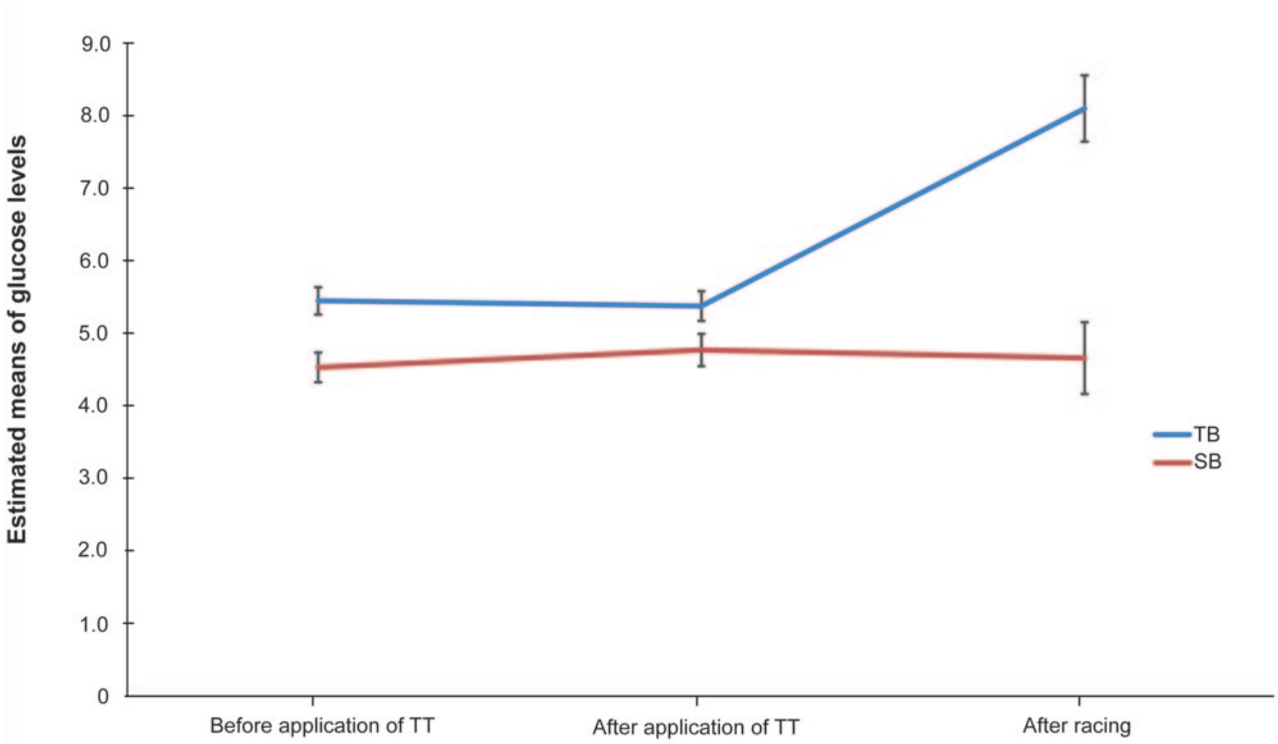
Estimated marginal means of cortisol levels at different time-points in SBs (red) versus TBs (blue). $P < 0.001$ for interaction between breed and time-point, $P = 0.059$ for breed (ANOVA with repeated measurements).

Figure 3



Estimated marginal means of glucose levels at different time-points in all horses (SBs and TBs) familiar with the TT (blue) compared to unfamiliar (red). $P = 0.033$ for interaction between TT naivety and time-point, $P = 0.179$ for TT naivety (ANOVA with repeated measurements).

Figure 4



Estimated marginal means of glucose levels at different time-points in SBs (red) versus TBs (blue). $P = 0.009$ for interaction between breed and time-point, $P = 0.001$ for breed (ANOVA with repeated measurements).

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Discussion

Overall, the majority of racehorses showed adverse behaviour in response to TT application, but only slight quantitative evidence of an increased stress response was found in this study by measuring heart-rate variability and cortisol levels. The higher LF values reported in SB mares suggest that TT application might affect them more than SB geldings.

Horses

The fact that all the horses participating in this study had health problems (exercise insufficiency and/or respiratory noise) might be a serious concern for the results. Unfortunately, the trainers' interest in participating in this study was based on these issues. We tried to address this problem by evaluating at least the SBs with and without TT, so that the health problem would fall out as a factor, since it would be unlikely for this problem to be alleviated by application of a TT. For example, an orthopaedic problem might lead to subtle lameness with and without TT application, therefore factors attributing to exercise insufficiency, but not impairing upper airway function and not influenced by TT application, will cancel themselves out. In addition, there is no indication for TT application in a horse without any problems; therefore it seems reasonable to study horses which are exercise insufficient, but do not have a diagnosis to explain it. Trainers often use TTs in response to poor training or racing performance since upper airway pathologies are common in racehorses with exercise insufficiency. Therefore, the results of this study are surely valuable, although the gold standard would be evaluation of randomly chosen racehorses in a real racing situation, were they able to be secured for research purposes.

Parameters

In this study, we focused on cortisol and HRV analyses as they are considered useful for the evaluation of stress in multiple studies (Alexander *et al* 1996; Hydbring *et al* 1996; Alexander & Irvine 1998; Golland *et al* 1999; Horohov *et al* 1999; Möstl & Palme 2002; Ohmura *et al* 2002; Bachmann *et al* 2003; Cottin *et al* 2005; Cayado *et al* 2006; Cottin *et al* 2006; Fazio *et al* 2008; Ferlazzo *et al* 2009; Quick & Warren-Smith 2009; Cravana *et al* 2010; Ohmura *et al* 2012; Becker-Birck *et al* 2013). The evaluation of cortisol in saliva might have been even less invasive and stressful for the horses (Schmidt 2010[a],[b],[c],[d]; Becker-Birck *et al* 2013). Cortisol can also be measured in faeces (Möstl & Palme 2002; Schmidt *et al* 2010[b],[c],[d]), but this is of higher value in chronic stress and not representative of the stress stimulus of short race training and the application of TTs for approximately 20 min.

Equipment and racing conditions

An influence of further equipment (bit, sulky, saddle, whip and rider/driver) is possible, but was not part of this study. The use of this equipment reflects the racehorses' daily reality and they were familiar with it. Other TT studies (eg Chalmers *et al* 2013) were accompanied by trainers with horses equipped with their usual training gear since a racehorse would never be trained using a TT alone. Although not feasible in TBs, we tried to exclude the influence of equipment in SBs by examining them on two subsequent days, one with application of the TT and the other day under the influence of their normal equipment. This enabled us to see in this breed that HF was higher without TT. Therefore, we believe the effect of other equipment to be minimal. Although the use of TTs is very common (Barakzai *et al* 2009[a],[b]; Findley *et al* 2016), little data have been published supporting this practice. It was shown sonographically at rest that TT influence the position of basihyoid and thyroid cartilage and an increased stress-response was documented in horses at rest (Latimer-Marsh *et al* 2017), confirming our finding of decreasing HF after the application of TT and considerable sympathetic activity thereafter.

Differences in distance, type of race-track and speed were clearly methodology limitations here, however, each horse raced with and without TT on the same race-track and over the same distance to minimise intra-individual variance.

Table 2(a) Mean (± SD) LF, HF and LF/HF ratios in SBs before and after application of TTs and after race training.

	LF (nu)	HF (nu)	LF/HF (nu)
Before application of TT	53.15 (± 19.36)	55.63 (± 19.57)*	1.05 (± 0.61)
After application of TT	69.39 (± 14.29)*	48.28 (± 19.05)	1.40 (± 0.45)
After race training with TT	73.51 (± 23.10)*	55.03 (± 22.53)	1.42 (± 0.78)

Table 2(b) Mean (± SD) LF, HF and LF/HF ratios in TBs before and after application of TTs and after race training.

	LF (nu)	HF (nu)	LF/HF (nu)
Before application of TT	60.08 (± 14.88)*	52.17 (± 14.84)	1.27 (± 0.55)
After application of TT	66.98 (± 14.22)*	52.53 (± 15.70)	1.42 (± 0.62)
After race training with TT	74.89 (± 13.12)*	59.87 (± 17.09)	1.41 (± 0.64)

* Overwhelming activity.

Table 3 Mean (± SD) and statistical significance (Wilcoxon test for paired samples) of LF, HF and LF/HF ratios in SBs on two subsequent training days after racing with and without application of TTs.

	LF (nu)	HF (nu)	LF/HF (nu)
With TT	68.01 (± 19.84)	43.72 (± 14.84)	3.78 (± 11.33)
Without TT	73.51 (± 23.10)	55.03 (± 22.53)	1.42 (± 0.79)
Significance	$P = 0.325$	$P = 0.05$	$P = 0.785$

Stress parameters

As cortisol levels are known to follow circadian rhythm (Hoffsis *et al* 1970[a],[b]; Medica *et al* 2011), all blood samples were taken in the morning during the horses' normal race training. As expected, exercise led to highly significant increases in cortisol concentrations in SBs and TBs, which is in accordance with previous studies (Golland *et al* 1999; Horohov *et al* 1999; Cayado *et al* 2006; Cravana *et al* 2010). Surprisingly, TT application did not influence cortisol concentrations prior to the start of high intensity exercise in neither SBs nor TBs. The mean values before the application of TTs were already above published reference values (Cayado *et al* 2006). Anticipation of racing and preparation of race training itself may have increased cortisol levels before the application of TTs. In addition, horses' individual temperaments may play a role (Fazio *et al* 2013). To our surprise, whether or not horses had been previously accustomed to TT application, also had no influence on stress parameters and may be attributable to the same mechanisms. The same was true for age and sex. Possible multiple influences of equipment and rider/driver and character may have obscured the effect of TTs on stress parameters, in particular cortisol. Other studies have shown the influence of a rider (Becker-Birck *et al* 2013; Zebisch *et al* 2014). Therefore, further studies should focus on further common equipment used in horse-racing and the influence of trainer, rider or driver.

In contrast to previous findings (Evans 1971), the glucose concentrations increased significantly after racing in TBs, which may be a result of hypothalamic-pituitary-adrenal axis activation in situations of physical and mental stress stimulating gluconeogenesis and a reduction of circulating insulin. A concept supported by further studies (eg Martínez *et al* 1988). After application of the TT, no difference in glucose concentration was found in TBs or SBs, suggesting this parameter may be not sensitive enough to evaluate stress in correlation with TTs.

As expected, race training led to lactate acidosis. All horses reached the anaerobic threshold of 4 mmol l^{-1} used in human medicine (Kindermann *et al* 1979). Fourteen horses (seven SBs, seven TBs) even exceeded 20 mmol l^{-1}, which is in accordance with other studies following high-intensity exercise (Krzywanek *et al* 1972; Hodgson *et al* 1987; Rainger *et al* 1994). As for glucose, TT application had no significant influence on lactate concentrations at rest and was not sensitive enough to evaluate stress in correlation with TTs at rest or after racing.

Analysis of HRV

Important differences in HRV parameters were found between SBs and TBs. In SBs, dominant parasympatholytic activity was evident at rest, but sympathetic activity increased with the application of TTs and after race training, which is a strong stressor by itself as shown by the increases in cortisol level and HRV parameters in horses exercised without TTs. In young horses up to three years of age, increased sympathetic activity was already found before the application of TTs showing the excitement or stress going along with the preparation of race training as described by other authors (Schmidt *et al* 2010a). Sex might also play a role, as SB mares showed a more pronounced stress reaction, evident as higher LF values, in comparison to geldings. Mares also show a predisposition for recurrent exertional rhabdomyolysis (RER; Vervuert 2011), although this may not necessarily be the consequence of greater stress. In TBs, dominant sympathetic activity was found over all time-points even before TT application, but these horses were at a younger mean age. It might be useful to include older TBs in further studies to look for differences concerning TT use in older horses more familiar with the training practice. In 20 young warmbloods, HRV parameters decreased less after five months of training (Visser *et al* 2002).

Nevertheless, HRV parameters were found useful to evaluate a sympatho-vagal imbalance in SB and TB race-horses in contrast to former studies (van Breda 2006; Becker-Birck *et al* 2013; Zebisch *et al* 2014; Latimer-Marsh *et al* 2017). As the effect of the stressor TT on HRV might have been obscured by other factors, including age, time in race training, sex, breed and training intensity (Thayer *et al* 1997; Kuwahara *et al* 1999; Physick-Sheard *et al* 2000; Ohmura *et al* 2002), further studies should include higher numbers of horses to increase the statistical power. This might allow the evaluation of groups of greater homogeneity regarding these factors.

Behaviour

Our results are in unison with previous findings on the behavioural stress response to TT application in horses at rest (Latimer-Marsh *et al* 2017). It may be a study limitation that observers were obviously not blinded to whether a TT had been applied or not, which could perhaps have biased the results more than in objective measurements of HRV or cortisol concentrations. Unfortunately, four-times more horses were familiar with TT application than not, perhaps biasing the outcome towards more adverse reactions, as previously described. Nevertheless, this may indicate that repeated application of this device does not lead to the development of tolerance but may aggravate behavioural defence in expectance of pain.

Animal welfare implications and conclusion

The application of TTs was banned by the Fédération Equestre Internationale (FEI) in most disciplines, including dressage, showjumping and eventing. It is still very common in SB and TB racing (Chalmers *et al* 2013) and is documented by most racing authorities. In Germany, the use of TTs was outlawed in June 2018 for TB racing (DVR 2018).

Although it was hard to distinguish the influence of TTs on stress parameters and HRV parameters during racing, the results of this study do not support the use of TTs, for which the adverse behavioural reactions documented is reason enough. In SBs, HRV decreased after TT, an effect even more visible after high-intensity exercise, a potent stressor itself as shown by increasing stress parameters and decreasing HRV. In young horses, especially TBs, this effect might have been obscured by the excitement of race

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training. Mares seem to be predisposed to be stressed by the application of TTs. In addition, the majority of SBs, most of them familiar with TT application, showed mild-severe adverse behaviour. The results of our study might provide objective evidence for future decisions by equine sports organisations regarding regulations on TTs.

Declaration of interest

None.

References

Alexander SL and Irvine CH 1998 The effect of social stress on adrenal axis activity in horses: the importance of monitoring corticosteroid-binding globulin capacity. *The Journal of Endocrinology 157*: 425-432. https://doi.org/10.1677/joe.0.1570425

Alexander SL, Irvine CH and Donald RA 1996 Dynamics of the regulation of the hypothalamo-pituitary-adrenal (HPA) axis determined using a nonsurgical method for collecting pituitary venous blood from horses. *Frontiers in Neuroendocrinology 17*: 1-50. https://doi.org/10.1006/frne.1996.0001

Bachmann I, Bernasconi P, Herrmann R, Weishaupt MA and Stauffacher M 2003 Behavioural and physiological responses to an acute stressor in crib-biting and control horses. *Applied Animal Behaviour Science 82*: 297-311. https://doi.org/10.1016/S0168-1591(03)00086-8

Barakzai SZ and Dixon PM 2005 Conservative treatment for thoroughbred racehorses with intermittent dorsal displacement of the soft palate. *The Veterinary Record 157*: 337-340. https://doi.org/10.1136/vr.157.12.337

Barakzai SZ, Finnegan C and Boden LA 2009a Effect of 'tongue tie' use on racing performance of thoroughbreds in the United Kingdom. *Equine Veterinary Journal 41*: 812-816. https://doi.org/10.2746/042516409X434134

Barakzai SZ, Finnegan C, Dixon PM, Hillyer MH and Boden LA 2009b Use of tongue ties in thoroughbred racehorses in the United Kingdom, and its association with surgery for dorsal displacement of the soft palate. *The Veterinary Record 165*: 278-281. https://doi.org/10.1136/vr.165.10.278

Barton A, Lindenberg I, Klaus D, Blohm KO and Gehlen H 2019 Casuistic evaluation of tongue tie use in Standardbred and Thoroughbred racehorses in Germany – a questionnaire study. *Equine Medicine 35*: 1-7. https://doi.org/10.21836/PEM20190503

Becker-Birck M, Schmidt A, Wulf M, Aurich J, von der Wense A, Möstl E, Berz R and Aurich C 2013 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. *Journal of Animal Physiology and Animal Nutrition 97*: 322-330. https://doi.org/10.1111/j.1439-0396.2012.01274.x

Berntson GG, Bigger JT, Eckberg DL, Grossmann P, Kaufmann PG, Malik M, Nagaraja HN, Porges SW, Saul JP, Stone PH and van der Molen MW 1997 Heart rate variability: origins, methods, and interpretive caveats. *Psychophysiology 34*: 623-648. https://doi.org/10.1111/j.1469-8986.1997.tb02140.x

Bertin FR, Taylor SD, Bianco AW and Sojka-Kritchevsky JE 2016 The effect of fasting duration on baseline blood glucose concentration, blood insulin concentration, glucose/insulin ratio, oral sugar test, and insulin response test results in horses. *Journal of Veterinary Internal Medicine 30*: 1726-1731. https://doi.org/10.1111/jvim.14529

Brüggemann T 1995 Spektralanalyse zur Beurteilung der Herzfrequenzvariabilität. *Herzschrittmachertherapie & Elektrophysiologie 5*: 19-24. [Title translation: Spectral analysis for evaluation of heart rate variability]

Cayado P, Munoz-Escassi B, Dominguez C, Manley W, Olabarri B, Sanchez de la Muela M, Castejon F, Maranon G and Vara E 2006 Hormone response to training and competition in athletic horses. *Equine Veterinary Journal Supplements 36*: 274-278. https://doi.org/10.1111/j.2042-3306.2006.tb05552.x

Chalmers HJ, Farberman A, Bermingham A, Sears W and Viel L 2013 The use of a tongue tie alters laryngohyoid position in the standing horse. *Equine Veterinary Journal 45*: 711-714. https://doi.org/10.1111/evj.12056

Cottin F, Barrey E, Lopes P and Billat V 2006 Effect of repeated exercise and recovery on heart rate variability in elite trotting horses during high intensity interval training. *Equine Veterinary Journal Supplements 38*: 204-209. https://doi.org/10.1111/j.2042-3306.2006.tb05540.x

Cottin F, Medique C, Lopes P, Petit E, Papelier Y and Billat VL 2005 Effect of exercise intensity and repetition on heart rate variability during training in elite trotting horse. *International Journal of Sports Medicine 26*: 859-867. https://doi.org/10.1055/s-2005-837462

Cravana C, Medica P, Prestopino M, Fazio E and Ferlazzo A 2010 Effects of competitive and noncompetitive showjumping on total and free iodothyronines, beta-endorphin, ACTH and cortisol levels of horses. *Equine Veterinary Journal Supplements 38*: 179-184. https://doi.org/10.1111/j.2042-3306.2010.00264.x

Direktorium für Vollblutzucht und Rennen eV 2018 *Der Einsatz von Zungenbändern ist ab 1 Juni verboten. Pressemeldung.* https://www.german-racing.com/gr/aktuelles/meldungen/20180531-310518-news-zunge.php. [Title translation: Use of tongue ties is forbidden from June 1st]

Eckberg DL 1997 Sympathovagal balance: a critical appraisal. *Circulation 96*: 3224-3232. https://doi.org/10.1161/01.CIR.96.9.3224

Evans JW 1971 Effect of fasting, gestation, lactation and exercise on glucose turnover in horses. *Journal of Animal Science 33*: 1001-1004. https://doi.org/10.2527/jas1971.3351001x

Fazio E, Medica P, Aronica V, Grasso L and Ferlazzo A 2008 Circulating beta-endorphin, adrenocorticotrophic hormone and cortisol levels of stallions before and after short road transport: stress effect of different distances. *Acta Veterinaria Scandinavica 50*: 6. https://doi.org/10.1186/1751-0147-50-6

Fazio E, Medica P, Cravana C and Ferlazzo A 2013 Cortisol response to road transport stress in calm in and nervous stallions. *Journal of Veterinary Behaviour 8*: 231-237. https://doi.org/10.1016/j.jveb.2012.10.010

Fenner K, Yoon S, White P, Starling M and McGreevy P 2016 The effect of noseband tightening on horses' behavior, eye temperature, and cardiac responses. *PLoS One 11(5)*: e0154179. https://doi.org/10.1371/journal.pone.0154179

Ferlazzo A, Medica P, Cravana C and Fazio E 2009 Endocrine changes after experimental showjumping. *Comparative Exercise Physiology 6*: 59-66. https://doi.org/10.1017/S1755254009990110

Findley JA, Sealy H and Franklin SH 2016 Factors associated with tongue tie use in Australian Standardbred racehorses. *Equine Veterinary Journal 48*: 5-30. https://doi.org/10.1111/evj.32_12612

Golland LC, Evans DL, Stone GM, Tyler-McGowan CM, Hodgson DR and Rose RJ 1999 Plasma cortisol and beta-endorphin concentrations in trained and over-trained standardbred racehorses. *Pflugers Archiv: European Journal of Physiology 439*: 11-17. https://doi.org/10.1007/s004249900111

Harbuz MS and Lightman SL 1992 Stress and the hypothalamo-pituitary-adrenal axis: acute, chronic and immunological activation. *Journal of Endocrinology 134(3)*: 327-339. https://doi.org/10.1677/joe.0.1340327

Harris P and Snow DH 1988 The effects of high intensity exercise on the plasma concentration of lactate, potassium and other electrolytes. *Equine Veterinary Journal 20*: 109-113. https://doi.org/10.1111/j.2042-3306.1988.tb01470.x

Hodgson DR, Kelso IB, Bayly WM and Gollnick PD 1987 Responses to repeated high intensity exercise: influence on muscle metabolism. *Equine Veterinary Journal* 302-311

Hoffsis GF and Murdick PW 1970b The plasma concentrations of corticosteroids in normal and diseased horses. *Journal of the American Veterinary Medical Association 157*: 1590-1594

Hoffsis GF, Murdick PW, Tharp VL and Ault K 1970a Plasma concentrations of cortisol and corticosterone in the normal horse. *American Journal of Veterinary Research 31*: 1379-1387

Horohov DW, Dimock A, Guirnalda P, Folsom RW, McKeever KH and Malinowski K 1999 Effect of exercise on the immune response of young and old horses. *American Journal of Veterinary Research 60*: 643-647. https://doi.org/10.21836/PEM19960410

Hottenrott K 2002 *Grundlagen zur Herzfrequenzvariabilität und Anwendungs-möglichkeiten im Sport. Herzfrequenzvariabilität im Sport - Prevention, Rehabilitation and Training*. K Czwalina Verlag: Hottenrott Hamburg, Germany. [Title translation: Basics of heart rate variability and applications in sports medicine. Heart rate variability in sports - prevention, rehabilitation and training]

Houle MS and Billman GE 1999 Low-frequency component of the heart rate variability spectrum: a poor marker of sympathetic activity. *American Journal of Physiology 276*: 215-223. https://doi.org/10.1152/ajpheart.1999.276.1.H215

Hydbring E, Nyman S and Dahlborn K 1996 Changes in plasma cortisol, plasma beta-endorphin, heart rate, haematocrit and plasma protein concentration in horses during restraint and use of naso-gastric tube. *Equine Medicine 12*: 423-427.

Kindermann W, Simon G and Keul J 1979 The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *European Journal of Applied Physiology and Occupied Physiology 42*: 25-34. https://doi.org/10.1007/BF00421101

Krzywanek H, Schulze A and Wittke G 1972 Behavior of various blood parameters in trotters following defined exertion. *Berliner und Münchener Tierärztliche Wochenschrift 85*: 325-329

Kucera M 2006 *Herzfrequenzvariabilität.* 36001 Karlovy Vary: Czech Republic. [Title translation: Heart rate variability]

Kuwahara M, Hashimoto S, Ishii K, Yagi Y, Hada T, Hiraga A, Kai M, Kubo K, Oki H, Tsubone H and Sugano S 1996 Assessment of autonomic nervous function by power spectral analysis of heart rate variability in the horse. *Journal of the Autonomic Nervous System 60*: 43-48. https://doi.org/10.1016/0165-1838(96)00028-8

Kuwahara M, Hiraga A, Kai M, Tsubone H and Sugano S 1999 Influence of training on autonomic nervous function in horses: evaluation by power spectral analysis of heart rate variability. *Equine Veterinary Journal Supplements 30:* 178-180. https://doi.org/10.1111/j.2042-3306.1999.tb05213.x

Latimer-Marsh L, Hazel S, Santos L and McGreevy PD 2017 The effect of tongue-tie application on stress responses in resting horses. *World Equine Airways Symposium*. 13-15 July 2017, Copenhagen, Denmark

Lindner A, von Wittke P, Schmald M, Kusserov J and Sommer H 1992 Maximal lactate concentrations in horses after exercise of different duration and intensity. *Journal of Equine Veterinary Science 12*: 30-33. https://doi.org/10.1016/S0737-0806(06)81384-5

Luna SP, Taylor PM and Wheeler MJ 1996 Cardiorespiratory, endocrine and metabolic changes in ponies undergoing intravenous or inhalation anaesthesia. *Journal of Veterinary Pharmacology and Therapeutics 19*: 251-258. https://doi.org/10.1111/j.1365-2885.1996.tb00046.x

Malik MD 1996 Heart rate variability. Standards of measurement, physiological interpretation, and clinical use: Task Force of the European Society of Cardiology and the North American Society for Pacing and Electrophysiology. *European Heart Journal 17*: 354-381

Malinowski K, Shock EJ, Rochelle P, Kearns CF, Guirnalda PD and McKeever KH 2006 Plasma beta-endorphin, cortisol and immune responses to acute exercise are altered by age and exercise training in horses. *Equine Veterinary Journal Supplements 30*: 267-273. https://doi.org/10.1111/j.2042-3306.2006.tb05551.x

Malliani A, Pagani M, Lombardi F and Cerutti F 1991 Cardiovascular neural regulation explored in the frequency domain. *Circulation 84(2)*: 482-492. https://doi.org/10.1161/01.CIR.84.2.482

Martínez R, Godoy A, Naretto E and White A 1988 Neuroendocrine changes produced by competition stress on the Thoroughbred race horse. *Comparative Biochemistry and Physiology: A Comparative Physiology 91*: 599-602. https://doi.org/10.1016/0300-9629(88)90640-8

May A 2007 *Evaluierung von Stressparametern beim Pferd im Zusammenhang mit dem Klinikaufenthalt.* Doctoral Thesis, Veterinary Faculty of Ludwig-Maximilian-University Munich, Germany. [Title translation: Evaluation of stress parameters in horses during hospital stay]

Medica P, Bruschetta G, Cravana C, Ferlazzo A and Fazio E 2019 Effect of transportation on the sympatho-adrenal system responses in horses *Research in Veterinary Science 125*: 401-404. https://doi.org/10.1016/j.rvsc.2017.10.001

Medica P, Cravana C, Fazio E and Ferlazzo A 2011 24 hour endocrine profiles of quarter horses under resting conditions *Equine Veterinary Science 31*: 35-40. https://doi.org/10.1016/j.jevs.2010.11.015

Möstl E and Palme R 2002 Hormones as indicators of stress. *Domestic Animal Endocrinology 23*: 67-74. https://doi.org/10.1016/S0739-7240(02)00146-7

Ohmura H, Hiraga A, Aida H, Kuwahara M and Tsubone H 2002 Effects of initial handling and training on autonomic nervous function in young Thoroughbreds. *American Journal of Veterinary Research 63*: 1488-1491. https://doi.org/10.2460/ajvr.2002.63.1488

https://doi.org/10.7120/09627286.31.2.007 Published online by Cambridge University Press

Ohmura H, Hobo S, Hiraga A and Jones JH 2012 Changes in heart rate and heart rate variability during transportation of horses by road and air. *American Journal of Veterinary Research 73:* 515-521. https://doi.org/10.2460/ajvr.73.4.515

Pagani M, Lombardi F, Guzzetti S, Rimoldi O, Furlan R, Pizzinelli P, Sandrone G, Malfatto G, Dell'Orto S and Piccaluga E 1986 Power spectral analysis of heart rate and arterial pressure variabilities as a marker of sympatho-vagal interaction in man and conscious dog. *Circulation Research 59*: 178-193. https://doi.org/10.1161/01.RES.59.2.178

Physick-Sheard PW, Marlin DJ, Thornhill R and Schroter RC 2000 Frequency domain analysis of heart rate variability in horses at rest and during exercise. *Equine Veterinary Journal 32*: 253-262. https://doi.org/10.2746/042516400776563572

Pomeranz B, Macaulay RJ, Caudill MA, Kutz I, Adam D, Gordon D, Kilborn KM, Barger AC, Shannon DC and Cohen RJ 1985 Assessment of autonomic function in humans by heart rate spectral analysis. *American Journal of Physiology 248*: 151-153. https://doi.org/10.1152/ajpheart.1985.248.1.H151

Quick JS and Warren-Smith AK 2009 Preliminary investigations of horses' (*Equus caballus*) responses to different bridles during foundation training. *Journal of Veterinary Behaviour 4*: 169-176. https://doi.org/10.1016/j.jveb.2008.12.001

Rainger JE, Evans DL, Hodgson DR and Rose RJ 1994 Blood lactate disappearance after maximal exercise in trained and detrained horses. *Research in Veterinary Science 57*: 325-331. https://doi.org/10.1016/0034-5288(94)90125-2

Ralston SL 2002 Insulin and glucose regulation. *The Veterinary Clinics of North America: Equine Practice 18*: 295-304. https://doi.org/10.1016/S0749-0739(02)00014-7.

Roberts CA, Marlin DJ and Lekeux P 1999 The effects of training on ventilation and blood gases in exercising thoroughbreds. *Equine Veterinary Journal Supplements 30*: 57-61. https://doi.org/10.1111/j.2042-3306.1999.tb05189.x

Rompelman O, Coenen AJ and Kitney RI 1977 Measurement of heart-rate variability: Part 1-Comparative study of heart-rate variability analysis methods. *Medical and Biological Engineering and Computing 15*: 233-239. https://doi.org/10.1007/BF02441043

Rose RJ, Hodgson DR, Kelso TB, McCutcheon LJ, Reid TA, Bayly WM and Gollnick PD 1988 Maximum O_2 uptake, O_2 debt and deficit, and muscle metabolites in Thoroughbred horses. *Journal of Applied Physiology 64*: 781-788. https://doi.org/10.1152/jappl.1988.64.2.781

Sammito S and Bockelmann I 2015 Analysis of heart rate variability. Mathematical description and practical application. *Herz 40 Suppl 1*: 76-84. https://doi.org/10.1007/s00059-014-4145-7

Schmidt A, Aurich J, Möstl E, Müller J and Aurich C 2010a Changes in cortisol release and heart rate and heart rate variability during the initial training of 3-year-old sport horses. *Hormones and Behaviour 58*: 628-636. https://doi.org/10.1016/j.yhbeh.2010.06.011

Schmidt A, Biau S, Möstl E, Becker-Birck M, Morillon B, Aurich J, Faure JM and Aurich C 2010b Changes in cortisol release and heart rate variability in sport horses during long-distance road transport. *Domestic Animal Endocrinology 38*: 179-189. https://doi.org/10.1016/j.domaniend.2009.10.002

Schmidt A, Hödl S, Möstl E, Aurich J, Müller J and Aurich C 2010c Cortisol release, heart rate, and heart rate variability in transport-naive horses during repeated road transport. *Domestic Animal Endocrinology 39*: 205-213. https://doi.org/10.1016/j.domaniend.2010.06.002

Schmidt A, Möstl E, Wehnert C, Aurich J, Müller J and Aurich C 2010d Cortisol release and heart rate variability in horses during road transport. *Hormones and Behaviour 57*: 209-215. https://doi.org/10.1016/j.yhbeh.2009.11.003

Stull CL and Rodiek AV 2002 Effects of cross-tying horses during 24 h of road transport. *Equine Veterinary Journal 34*: 550-555. https://doi.org/10.2746/042516402776180214

Tarvainen MP, Niskanen JP, Lipponen JA, Ranta-Aho PO and Karjalainen PA 2014 Kubios HRV-heart rate variability analysis software. *Computer Methods and Programs in Biomedicine 113*: 210-220. https://doi.org/10.1016/j.cmpb.2013.07.024

Thayer JF, Hahn AW, Pearson MA, Sollers JJ III, Johnson PJ and Loch WE 1997 Heart rate variability during exercise in the horse. *Biomedical Sciences Instrumentation 34*: 246-251

van Breda E 2006 A nonnatural head-neck position (Rollkur) during training results in less acute stress in elite, trained, dressage horses. *Journal of Applied Animal Welfare Science 9*: 59-64. https://doi.org/10.1207/s15327604jaws0901_5

Vervuert I 2011 Fütterungsempfehlungen bei Muskelerkrankungen des Pferdes: Stand der Forschung versus Supplementiasis. *Der Praktische Tierarzt 11*: 988-992. [Title translation: The vet in practice]

Visser EK, CG van Reenen, van der Werf JTN, Schilder MBH, Knaap JH, Barneveld A and Blokhuis HJ 2002 Heart rate and heart rate variability during a novel object test and a handling test in young horses. *Physiology and Behaviour 76*: 289-296. https://doi.org/10.1016/S0031-9384(02)00698-4

Wagner AE 2010 Effects of stress on pain in horses and incorporating pain scales for equine practice. *The Veterinary Clinics of North America: Equine Practice 26*: 481-492. https://doi.org/10.1016/j.cveq.2010.07.001

Walther M 2017 *Untersuchung zur Tötung von Pferden*. Doctoral Thesis, Veterinary Department, Freie Universitaet Berlin, Germany. [Title translation: Evaluation on the euthanasia of horses]

Zebisch A, May A, Reese S and Gehlen H 2014 Effect of different head-neck positions on physical and psychological stress parameters in the ridden horse. *Journal of Animal Physiology and Animal Nutrition 98*: 901-907. https://doi.org/10.1111/jpn.12155