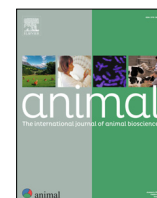




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Stress response of 18-, 24- and 30-month-old sport horse stallions to a pretraining programme

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ABSTRACT

Warmblood sires traditionally have been presented for stallion licencing at 2 years of age, but the age at which horses are mentally fit for training is a point of controversy. We have therefore investigated the stress response of young stallions to pretraining for licencing. Salivary cortisol concentration, heart rate and heart rate variability (HRV) were determined repeatedly over 12 weeks. Stallions were 24 and 30 months old and either housed in groups or individual boxes (Group 24, $n = 9$; Box 24, $n = 10$; Box 30, $n = 10$). Pretraining included free movement in an indoor arena, training in a horse walker, lunging and jumping of obstacles without rider. In addition, group-housed 18-month-old stallions (Group 18, $n = 10$) underwent an abbreviated programme with only free movement and horse walker exercise. We hypothesised that the stress response to pretraining is reduced by group housing but not affected by age. In stallions of all groups, cortisol concentration increased in response to pretraining events ($P < 0.001$). Cortisol release differed among events ($P < 0.001$) was most pronounced after free movement in weeks 1 and 2 and became less pronounced with event repetition ($P < 0.001$). When horses were in the horse walker for the first time, cortisol release was increased in group-housed in comparison to individually housed stallions (time $\times$ stable $P < 0.001$). An increase ($P < 0.001$) in heart rate differed among events ($P < 0.001$) and became smaller with repetition ($P < 0.001$). The HRV decreased transiently in response to pretraining events ($P < 0.001$) indicating sympathoadrenal activation. In conclusion, pretraining of young stallions is both a physical and a mental demand and induces a stress response. With the repetition of events, even stallions as young as 18 months adapted rapidly to these demands. Group housing had no stress-reducing effects. Our study does not provide evidence that the systematic pretraining of young Warmblood stallions raises animal welfare concerns.

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Implications

The age at which horses are mentally fit for training is a point of controversy. Based on the stress hormone cortisol in saliva and changes in heart rate and heart rate variability, the present study indicates that a non-ridden pretraining of 18-, 24- and 30-months old Warmblood stallions is a physical and mental challenge, inducing a stress response. Group housing of stallions had no stress-reducing effects. With repetition of events, even stallions as young as 18 months habituated rapidly to the demands of pretraining. Our study does not provide evidence that a systematic

pretraining of young Warmblood stallions raises animal welfare concerns.

Introduction

The minimal age at which young horses are fit to begin the initial training that will finally lead to a career in racing or equestrian sports is a point of controversy. Discussions are focused on Thoroughbred racehorses entering training as yearlings and competing in races when they are 2 years old (Armitage, 2024). In Germany, Guidelines on Welfare in Equine Sports (Federal Ministry for Food and Agriculture, 2020) exclude training of horses before 30 months of age, but at present exemptions exist for Thoroughbred racehorses and trotters. The training of Warmblood sport horses usually starts when the animals are 3 years old. Warmblood

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stallions preselected for breeding, however, are often prepared for presentation at stallions licencing assessments before they are 30 months old and then begin their career before they are 3 years old. Although preparation for stallion licencing does not include strenuous physical exercise and therefore should be classified as pretraining rather than training, stallions younger than 30 months must be excluded on the assumption that they could be mentally overstrained (Federal Ministry for Food and Agriculture, 2020).

In contrast to public opinion, physiological, clinical, and epidemiological data indicate that stimulation of the equine musculoskeletal systems at a young age supports physical health and longevity. Early athletic activity in horses enhanced bone remodelling with positive effects on bone strength and articular cartilage. Racehorses that enter training as 2-year-olds therefore have a reduced overall risk of musculoskeletal injuries (reviewed by Logan and Nielsen, 2021). While there is thus scientific evidence for the positive effects of physical activity in young horses, information on their mental development is virtually absent. Behaviour patterns of horses are influenced by positive interaction with humans, and horses handled at an early age scored higher on learning tests than those not handled (Heird et al., 1981).

When ridden, adult horses can exhibit hyperreactive behaviours, such as bucking or spooking, which is often interpreted as a sign of pain (Dyson et al., 2018) or confusion (McLean and Christensen, 2017) thus potentially indicating compromised welfare (Luke et al., 2023). Based on analysis of cortisol release, heart rate as a proxy measure for noradrenaline release and heart rate variability, it has been demonstrated that the initial training of young horses following the system of classical European equitation can be a stress challenge for the animals (Schmidt et al., 2010a; Kaps et al., 2022). It is, however, less stressful than other anthropogenic stressors such as road transport to which domestic horses are regularly exposed (Baucus et al., 1990; Clark et al., 1993; Schmidt et al., 2010b–d). A pronounced acute stress response occurred when Warmblood horses were mounted for the first time by a rider (Schmidt et al., 2010a) and a similar response has recently been described in Thoroughbred racehorses backed for the first time by a jockey (Holtby et al., 2023). These results strengthen the importance of a careful approach during the first steps of pretraining and training in young horses. When accepted equestrian protocols are followed, horses adapt to the demands of their riders and training and competitions are no longer perceived as stressful (Lange et al., 1997; Cayado et al., 2006; Becker-Birck et al., 2013; Ille et al., 2013; von Lewinski et al., 2013; Ille et al., 2014a). This adaptation may be enhanced when training is based on learning theory principles (McLean and Christensen, 2017). The studies on ridden horses, however, did not include animals younger than 3 years.

Young horses are usually raised in groups but are stabled individually before training. Although individual stabling of horses allows visual and olfactory contact between animals in adjacent boxes and thus does not represent total isolation, separation of horses previously kept in groups induced a transient stress response in some (Alexander and Irvine, 1998; Erber et al., 2013) but not all studies (Strand et al., 2002; Harewood and McGowan, 2005). Furthermore, it has been suggested that horses kept in social groups are easier to handle than horses housed alone (Christensen et al., 2002; Søndergaard and Ladewig, 2004).

To what extent young horses can successfully meet the mental challenges associated with their initial training and how transient or permanent behaviour problems can be avoided, to the best of our knowledge has not been addressed in controlled studies so far. Preparing young Warmblood stallions for licencing and studbook registration is very similar to pretraining of horses before they are ridden and trained either for equestrian sports or racing.

In fact, after studbook registration, Warmblood stallions will undergo equestrian training and participate in competitions. We have therefore investigated the response of young Warmblood sport horse stallions to a controlled pretraining programme by analysing physiological stress parameters. To complement the physiological data, behaviour evaluation, growth and development of the stallions, injury recordings and diurnal cortisol and heart rate profiles will be presented in a second manuscript. At the beginning of pretraining, stallions' age was between 17 and 31 months and they were either housed in a group stable or in individual boxes. We hypothesised that group housing of stallions during pretraining improves physical fitness and reduces the potential stress response compared to stallions kept in individual boxes and thus with less contact to other horses. In addition, we followed the hypothesis that the stress response to pretraining is not affected by stallion age.

Material and methods

Animals

For this study, 40 Warmblood stallions at the Brandenburg State Stud in Neustadt (Dosse), Germany, were available over a 2-year period. One stallion had to be withdrawn due to lameness in the 1st week of pretraining, leaving 39 stallions for data evaluation. All horses had been at the stud either since birth or since weaning. Before start of the study, stallions were kept in groups on pasture divided by age. Horses were allocated by age and housing system to four groups as outlined in Fig. 1 and Table 1. Groups represent the age at which Warmblood stallions currently enter pretraining (30 months), the age at which pretraining was initiated until recently (24 months) and, for comparison with the older stallions and with Thoroughbred racehorses in future studies, a group of 18 months-old stallions. All horses were kept on straw and were fed concentrates and hay twice daily, water was available at all times. Group-housed stallions were haltered and tethered for feeding of concentrates. All stallions had access to outdoor paddocks (60 × 60 m) for 2–3 h per day, either individually (Box 24 and Box 30) or as a group (Group 18 and Group 24). All horses were examined by a veterinarian with experience in equine orthopaedics and declared healthy and fit for pretraining.

Experimental design

Stallions of groups **Box 24** (24 months old, individual box), **Group 24** (24 months old, group stable) and **Box 30** (30 months old, individual box) were submitted to a 12-week pretraining programme for stallion licencing by a sport horse breed registry with increasing demands from week 1 to week 12. The pretraining included free movement in an indoor arena, showing at hand, exercise in an automated horse walker, lunging and jumping of obstacles without a rider (Table 2) but not riding. **Group-18** (18 months old, group stable) stallions were submitted to an abbreviated 5-week pretraining with free moving in an indoor arena once weekly and exercise in an automated horse walker once weekly. Salivary cortisol concentration, heart rate and heart rate variability (HRV) were determined at 12 different events during the pretraining programme in stallion groups Box 24, Group 24 and Box 30 as outlined in Table 3. Group 18 stallions were followed over 5 weeks, only, and in each week were exercised for a maximum of 10 min freely in an indoor arena and for 15 min in an automated horse walker in walk (weeks 1–3) and in walk and trot (weeks 4 and 5). For Group 18 stallions, data are shown for weeks 1, 3 and 5. All examined events in all four stallion groups were performed between 0800 and 1200 h. A standardised exercise test was performed in stallions

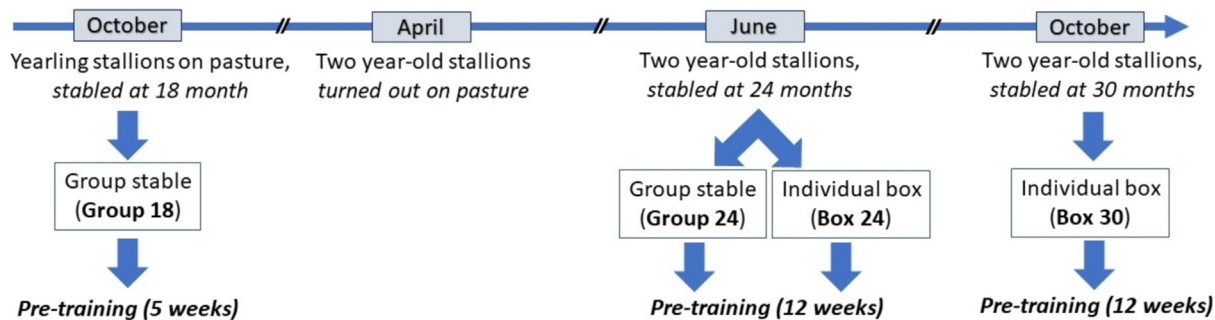


Fig. 1. Timeline of the experiment and allocation of stallions to four experimental groups.

Table 1
Stallion groups included into the study with housing system and age, BW, body condition score (BCS) and height at withers (mean ± SD and range) at the start of the pretraining programme.

Group	Housing system	Age (months)	BW (kg)	BCS	Height at withers (cm)
Group 18 (n = 10)	Group stable (26 × 6.50 m)	19 ± 2 (17–21)	424 ± 32 ^b (375–470)	25 ± 3 ^c (20–31)	158 ± 4 ^a (154–163)
Group 24 (n = 9)	Group stable (14 × 18 m)	26 ± 1 (24–27)	485 ± 39 ^a (449–578)	28 ± 3 ^a (21–30)	159 ± 3 ^a (155–165)
Box 24 (n = 10)	Single boxes (3.6 × 4.3 m)	25 ± 2 (24–27)	513 ± 21 ^a (483–553)	28 ± 2 ^a (24–32)	160 ± 3 ^{a,b} (158–166)
Box 30 (n = 10)	Single boxes (3.6 × 4.3 m)	30 ± 1 (29–31)	500 ± 19 ^a (469–524)	31 ± 3 ^b (25–35)	163 ± 3 ^b (161–169)

^{a,b,c} Different superscript letters indicate significant group differences ($P < 0.05$; BW and height at withers: ANOVA with subsequent Tukey test; BCS: Kruskal-Wallis-H-test with subsequent Mann-Whitney test).

Table 2
Training elements (events) included into the 12-week pretraining programme for 24- and 30-month-old stallions.

Events
• Free movement in indoor riding arena in walk, trot and canter, 15 min, once in week 1 • Lunging in walk, trot and canter, initially without and after week 2 with side reins, three times per week, starting with 15 min in week 1 and increasing to 30 min • Free movement in indoor riding arena in walk, trot and canter with jumping of obstacles (2 obstacles, maximum height 115 cm), 20 min, once weekly starting in week 6 • Training of showing at hand in walk and trot, 15 min, once during the pretraining programme • Movement in a horse walker in walk and trot once weekly, initially 15 min starting in week 4 and increasing to 30 min

Table 3
Training elements (events) analysed for salivary cortisol concentration, heart rate and heart rate variability at different times during a 12-week pretraining programme for stallion licencing.

Event no.	Week	Event
1	1	First free movement in indoor riding arena
2	1	Lunging without lunging girth and side reins
3	1	Lunging with lunging girth without side reins
4	2	First lunging with lunging girth and side reins
5	4	First movement in automated horse walker
6	6	First free jumping (jumping without rider)
7	7	Lunging with lunging girth and side reins
8	7	Movement in automated horse walker
9	9	Free jumping
10	11	Movement in automated horse walker
11	11	Lunging with lunging girth and side reins
12	12	Free jumping

of groups Box 24, Group 24 and Box 30 but not Group 18 in weeks 5 and 12 of the pretraining programme and changes in heart rate and blood lactate concentration in response to exercise were determined.

Saliva collection and cortisol analysis

For analysis of cortisol release in response to pretraining events, saliva samples were collected at 60 and 30 min before the event to

determine basal cortisol concentration. Further samples were taken immediately after the event and 15, 30, 45, 60, 75, 90 and 120 min thereafter. Saliva was collected as described previously (Schmidt et al., 2010d) with cotton swabs (Salivette, Sarstedt, Nümbrecht-Rommelsdorf, Germany) grasped with a surgical clamp and inserted gently into the mouth of the horse at the angle of the lips. The cotton roll was placed on the tongue of the horse for at least 1 min until it was well moistened. The cotton swab was then centrifuged for 10 min at 1 000 × g. At least 1 mL of saliva was obtained and frozen at −20 °C until analysis. All horses tolerated this procedure without resistance and saliva was always collected by a single person without any need for fixation of the horse. Furthermore, horses were habituated to saliva collection before start of the study. Concentrations of cortisol in saliva were determined with an enzyme immunoassay without extraction (Demedtec Diagnostics, Kiel-Wellsee, Germany) validated for equine saliva in our laboratory (Kuhl et al., 2016). The intra-assay CV was 4.8%, the interassay CV was 6.3%, and the minimal detectable concentration defined as two SD from zero binding was 0.01 ng/mL.

Heart rate and heart rate variability

For analysis of heart rate, cardiac beat-to-beat intervals were recorded with a mobile recording system (Polar V800, Kempele, Finland) as described previously (Schmidt et al., 2010b). In brief,

Table 4

Results of statistical comparisons among stallion ages, housing systems, events and repetitions for salivary cortisol, heart rate and heart rate variability parameter root mean square of successive beat-to-beat differences (RMSSD).

Effect	Three groups (Group 24, Box 24, Box 30) and four events (free movement/lunging without side reins, horse walker, lunging with side reins and free jumping)			Four groups (Group 18, Group 24, Box 24, Box 30) and two events (free movement/lunging without side reins and horse walker)		
	Cortisol	Heart rate	RMSSD	Cortisol	Heart rate	RMSSD
Event	$P < 0.001$ $F_3 = 29.9$	$P < 0.001$ $F_3 = 20.52$	$P = 0.016$ $F_3 = 3.90$	$P < 0.001$ $F_1 = 12.05$	$P < 0.001$ $F_1 = 62.81$	$P = 0.002$ $F_1 = 11.72$
Event $\times$ age	n.s. $F_3 = 1.8$	$P < 0.001$ $F_3 = 6.49$	n.s. $F_3 = 1.16$	$P < 0.001$ $F_2 = 11.42$	n.s. $F_2 = 2.01$	n.s. $F_2 = 0.23$
Event $\times$ stable	n.s. $F_3 = 0.45$	$P = 0.035$ $F_3 = 3.04$	n.s. $F_3 = 0.32$	n.s. $F_1 = 1.12$	n.s. $F_1 = 0.24$	n.s. $F_1 = 0.00$
Repetition	$P < 0.001$ $F_2 = 28.20$	$P < 0.001$ $F_2 = 16.58$	n.s. $F_2 = 0.49$	$P < 0.001$ $F_2 = 30.44$	$P < 0.001$ $F_2 = 62.32$	n.s. $F_2 = 0.76$
Repetition $\times$ age	n.s. $F_2 = 2.80$	n.s. $F_2 = 0.13$	n.s. $F_2 = 1.53$	$P = 0.021$ $F_4 = 3.10$	$P = 0.001$ $F_4 = 5.05$	n.s. $F_4 = 2.28$
Repetition $\times$ stable	n.s. $F_2 = 2.69$	$P < 0.001$ $F_2 = 8.71$	n.s. $F_2 = 0.38$	n.s. $F_2 = 2.74$	$P = 0.029$ $F_2 = 3.74$	n.s. $F_2 = 1.79$
Time	$P < 0.001$ $F_8 = 46.70$	$P < 0.001$ $F_{11} = 1178.11$	$P < 0.001$ $F_{11} = 10.15$	$P < 0.001$ $F_8 = 31.13$	$P < 0.001$ $F_1 = 474.12$	$P < 0.001$ $F_{11} = 10.08$
Time $\times$ age	n.s. $F_8 = 0.18$	n.s. $F_{11} = 0.56$	n.s. $F_{11} = 1.56$	$P = 0.022$ $F_{16} = 1.89$	$P = 0.23$ $F_{22} = 0.17$	n.s. $F_{22} = 0.92$
Time $\times$ stable	$P < 0.001$ $F_8 = 4.30$	n.s. $F_{11} = 0.45$	n.s. $F_{11} = 1.41$	$P < 0.001$ $F_8 = 6.55$	n.s. $F_{11} = 0.14$	n.s. $F_{11} = 0.69$
Event $\times$ repetition	$P < 0.001$ $F_6 = 6.57$	$P < 0.001$ $F_6 = 4.48$	n.s. $F_6 = 0.47$	$P < 0.001$ $F_2 = 8.94$	$P < 0.001$ $F_2 = 14.47$	n.s. $F_2 = 0.43$
Event $\times$ time	$P < 0.001$ $F_{24} = 4.27$	$P < 0.001$ $F_{33} = 14.80$	n.s. $F_{33} = 0.76$	$P < 0.001$ $F_8 = 3.89$	$P < 0.001$ $F_{11} = 37.31$	$P < 0.001$ $F_{11} = 3.02$
Repetition $\times$ time	$P < 0.001$ $F_{16} = 10.91$	$P < 0.001$ $F_{22} = 7.10$	n.s. $F_{22} = 2.45$	$P < 0.001$ $F_{16} = 12.69$	$P < 0.001$ $F_{22} = 11.29$	$P < 0.001$ $F_{22} = 1.92$
Age	n.s. $F_1 = 0.30$	n.s. $F_1 = 2.34$	n.s. $F_1 = 0.23$	$P = 0.034$ $F_2 = 3.72$	$P = 0.01$ $F_2 = 5.34$	n.s. $F_2 = 0.92$
Stable	n.s. $F_1 = 0.04$	n.s. $F_1 = 0.48$	$P = 0.043$ $F_1 = 5.01$	n.s. $F_1 = 0.73$	n.s. $F_1 = 2.41$	n.s. $F_1 = 4.10$

n.s. non-significant.

an elastic girth containing two electrodes was fixed around the horse's thorax, the positive electrode located on the left side of the withers and the negative on the left side of the thorax next to the heart base. Cardiac recordings were made from 70 min before to 130 min after the specific pretraining events. From the recordings, 5 min intervals beginning at 60 and 30 min before start of the specific event, at the start and the end of the event and at 15, 30, 45, 60, 75, 90, 105 and 120 min after the event were analysed as in previous studies on horses (Schmidt et al., 2010a–d). From the recorded beat-to-beat intervals, heart rate and the HRV parameter **RMSSD** (root mean square of successive beat-to-beat differences) was calculated using the Kubios software (Biomedical Signal Analysis Group, Department of Applied Physics, University of Kuopio, Finland) as described previously (Schmidt et al., 2010a–d).

Exercise tests

For the exercise tests, stallions of groups Box 24, Group 24 and Box 30 were lunged with a lunging girth and side reins in walk (10 min), trot (10 min) and canter (3 min at an approximate speed of 300 m/min) in an indoor riding arena; heart rate and pre- and postexercise blood lactate concentration were determined. Heart rate was recorded continuously as described in the previous paragraph with the modification that successive 2-min intervals were analysed. Basal heart rate was determined at 0730 and 0800 h and at 60 and 30 min before the exercise tests in the horse's stable, after 2, 4, 6, 8 and 10 min of walk, 2, 4, 6, 8 and 10 min of trot, one and 3 min of canter and at 3, 5, 7, 10, 15, 30, 45 and 60 min after the end of the test. Blood for analysis of lactate concentration was collected from one jugular vein at 60 min before the exercise test, directly after the walk, trot and canter phase and at 3, 5, 7, 10 and 15 min after the end of the exercise test. Lactate concentration

was determined with a hand-held lactate analyser (Lactate Pro 2 LT-1730; Praxisdienst, Longuich, Germany) validated for analysis in horse blood (Siegers et al., 2018).

Statistical analysis

Statistical analysis was performed with the SPSS statistics programme (Version 29.0, IBM, Armonk, NY, USA). Normal distribution of data was confirmed by Shapiro-Wilk test and homogeneity of variance by Levene test. Salivary cortisol concentration, heart rate and HRV in 24-month-old and 30-month-old stallions (Group 24, Box 24, Box 30) for all events and repetitions, were analysed by ANOVA using a GLM for repeated measures with event type, repetition, and time (–60 to +120 min) as within-subject factor and housing system (single box and group stable) and stallion age as between-subject factors. Furthermore, salivary cortisol concentration, heart rate and HRV in response to free movement and initial lunging without side reins (event 1) and to repeated horse walker exercise (event 2) were analysed for all four stallion groups (Group 18, Group 24, Box 24, Box 30) with event type, repetition, and time as within-subject factor and housing system and stallion age as between-subject factors. Data are given as mean and SEM. A P -value < 0.05 was considered significant. Exact P -values are given except for $P < 0.001$.

Results

Cortisol

Cortisol concentration increased in saliva of stallions in response to the different pretraining events ($P < 0.001$, $F_8 = 46.70$). Peak cortisol concentrations were mostly observed

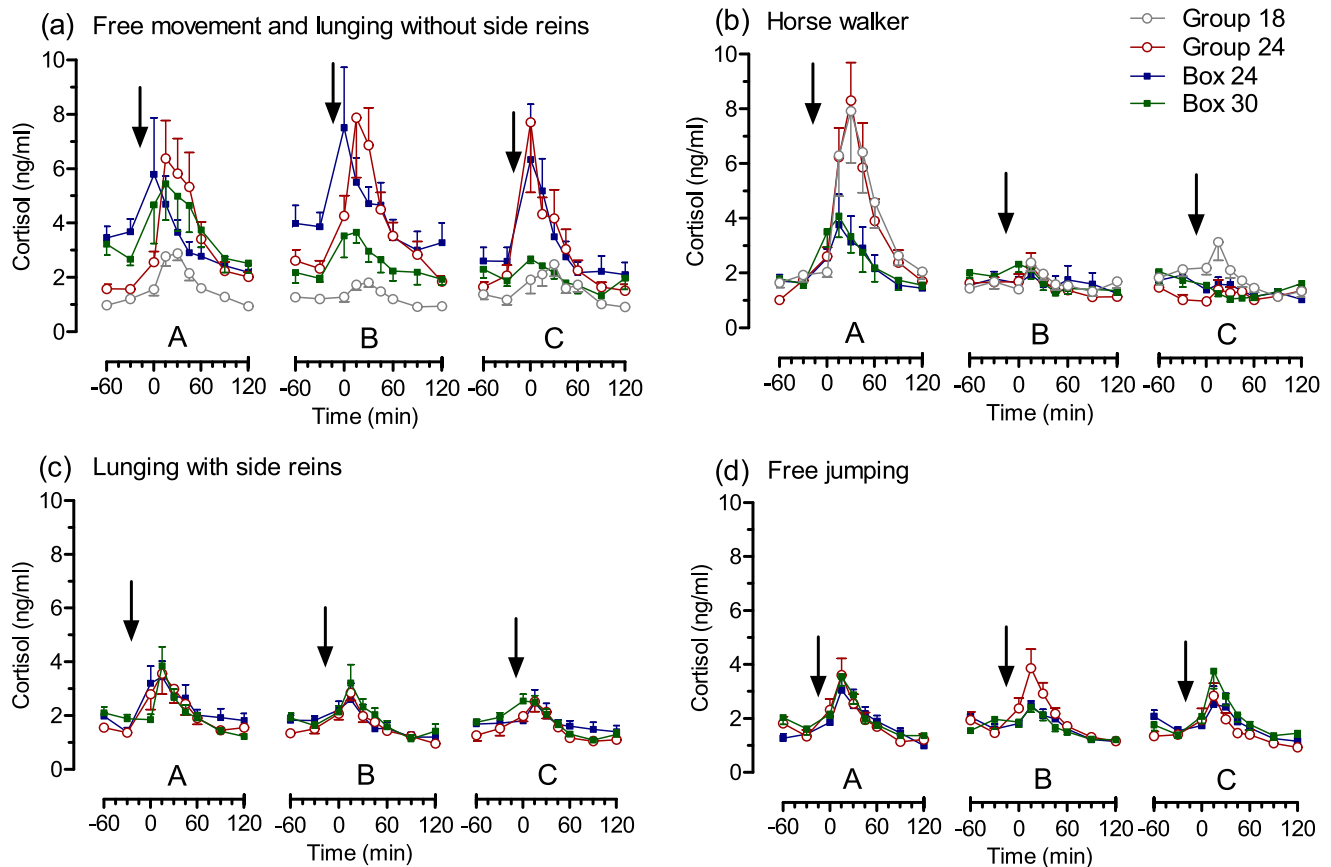


Fig. 2. Salivary cortisol concentration in 18-month-old stallions kept in a group stable (Group 18, $n = 10$), 24-month-old stallions kept in a group stable (Group 24, $n = 9$), 24-month-old stallions kept in single boxes (Box 24, $n = 10$) and 30-month-old stallions kept in single boxes (Box 30, $n = 10$) in response to (a) free movement in a riding arena and lunging without side reins, (b) movement in a horse walker, (c) lunging with side reins and lunging girth and (d) free jumping. Note that Group 18 stallions are included only in (a) and (b). The time of the different events is indicated by vertical arrows, A, B, or C indicates repeated exposure to similar events (see also Table 3). For results of statistical analysis, see Table 4.

directly or 15 min after the end of the respective events (Fig. 2). In stallions of groups Group 24, Box 24, and Box 30 which were exposed to four different events (free movement or lunging without side reins, automated horse walker, lunging with side reins, free jumping; Fig. 2a–d), the increase in salivary cortisol concentration differed among the event types ($P < 0.001$, $F_3 = 29.9$) with the most pronounced increase in response to free movement or free lunging in weeks 1 and 2 compared to repetitions in the horse walker, lunging with side reins and free jumping. The cortisol release became less pronounced with the repetition of an event ($P < 0.001$, $F_2 = 28.20$). The decrease over three repetitions was most pronounced for exercise in the automated horse walker (event $\times$ repetition $P < 0.001$, $F_6 = 6.57$; Fig. 2b). In the 18-month-old stallions (Group 18), the most pronounced cortisol release occurred when they were brought into the horse walker for the first time in week 1 of their pretraining. The cortisol response declined with repeated horse walker exercise (repetition $P < 0.001$, $F_2 = 30.44$; Fig. 2b).

A direct comparison of 18-month-old stallions (Group 18) with older stallions (Box 24, Group 24, Box 30) was made for free movement and lunging without side reins as well as for horse walker exercise (Fig. 2a,b). In response to free movement in the indoor riding arena but not in response to horse walker exercise, 18-month-old, group-stabled stallions and 30-month-old individually stabled stallions had a less pronounced cortisol release than Group 24 and Box 24 stallions (event $P < 0.001$, $F_1 = 12.05$, event $\times$ age $P < 0.001$, $F_2 = 11.42$). When salivary cortisol concentration was compared for repeated exercise in the horse walker in all four stallion groups,

there was an initial marked increase (time $P < 0.001$, $F_8 = 31.13$) which decreased with repetition (repetition $P < 0.001$, $F_2 = 30.44$, time $\times$ repetition $P < 0.001$, $F_{16} = 12.69$; Fig. 2b). When horses were exposed to the horse walker for the first time, cortisol release was more pronounced in stallions kept in groups (Group 18 and Group 24) than in stallions kept in individual boxes (Box 24 and Box 30; time $\times$ stable $P < 0.001$, $F_8 = 6.55$), but this group effect was no longer evident with repeated horse walker exercise.

Heart rate and heart rate variability

In all stallions, heart rate increased during all pretraining events (time $P < 0.001$, $F_{11} = 1178.11$), but the increase differed regarding the type of event ($P < 0.001$, $F_3 = 20.52$) and was reduced with repeated exposure to the same event type (repetition $P < 0.001$, $F_2 = 16.58$; Fig. 3a–d). The reduced rise in heart rate with repetition was most evident for horse walker exercise (event $\times$ repetition $P < 0.001$, $F_6 = 4.48$). When heart rate was compared for repeated exercise in the horse walker in all four stallion groups (Fig. 3b), there was an initial marked increase (time $P < 0.001$, $F_1 = 474.12$) which became less with repetition (repetition $P < 0.001$, $F_2 = 62.32$, time $\times$ repetition $P < 0.001$; $F_{22} = 11.29$). Heart rate at first horse walker exercise but not repetitions was increased in group-housed in comparison to individually housed stallions (repetition $\times$ stable $P < 0.001$, $F_2 = 14.47$; Fig. 3b).

In stallions of all groups, the HRV parameter RMSSD decreased transiently in response to the pretraining events (time $P < 0.001$, $F_{11} = 10.15$) and basal RMSSD was lower during free movement

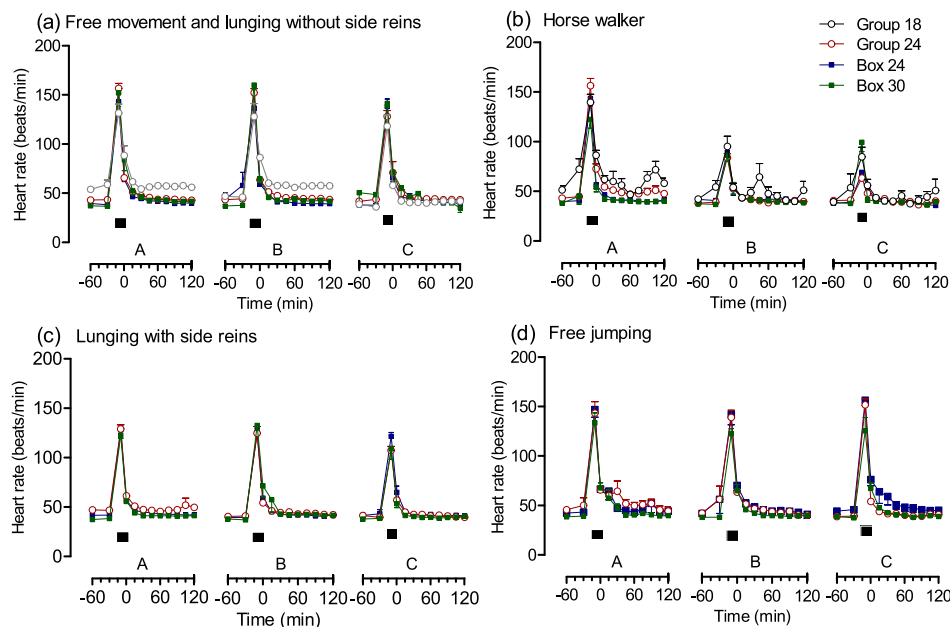


Fig. 3. Heart rate in 18-month-old stallions kept in a group stable (Group 18, $n = 10$), 24-month-old stallions kept in a group stable (Group 24, $n = 9$), 24-month-old stallions kept in single boxes (Box 24, $n = 10$) and 30-month-old stallions kept in single boxes (Box 30, $n = 10$) in response to (a) free movement in a riding arena and lunging without side reins, (b) movement in a horse walker, (c) lunging with side reins and lunging girth and (d) free jumping. Note that Group 18 stallions are included only in (a) and (b). The time of the different events is indicated by vertical arrows, A, B, or C indicates repeated exposure to similar events (see also Table 3). For results of statistical analysis, see Table 4.

and lunging (Fig. 4a,c) than in response to horse walker exercise and free jumping (event $P = 0.016$, $F_3 = 3.90$; Fig. 4b,d). When all four groups were compared for only first free movement, the decrease in RMSSD was more pronounced in group-housed than in single box-housed stallions (time $\times$ age $P = 0.037$, $F_{22} = 1.63$; Fig. 4a). For repeated exercise in a horse walker (Fig. 4b), the decrease in RMSSD (time $P < 0.001$, $F_{11} = 9.12$) became less evident with repetition (repetition $\times$ time $P < 0.001$, $F_{22} = 4.55$).

Exercise tests

In the exercise tests performed with Group 24, Box 24 and Box 30 stallions, heart rate increased as expected from baseline to lunging in walk, trot and canter (time $P < 0.001$, $F_{22} = 228.70$; Fig. 5a,c) with a slightly less pronounced increase in 30-month compared 24-month-old stallions in the second test in week 12 (repetition $P = 0.015$, $F_1 = 7.23$, time $\times$ age $P < 0.001$, $F_{22} = 2.27$, repetition $\times$ time $P = 0.009$, $F_{22} = 1.90$). Blood lactate concentration was increased after the canter phase ($P < 0.001$, $F_8 = 21.38$), but there were no effects of repetition (weeks 5 and 12), age and housing system (Fig. 5c,d).

Discussion

Habituating a young horse to accepting a rider on its back and to follow the rider's cues or aids is increasingly preceded by systematic pretraining before mounting the horse. In the present study, a pretraining programme for young stallions with free movement, exercise in an automated horse walker, lunging and free jumping but not being ridden was investigated. All pretraining events induced a stress response, as indicated by an increase in salivary cortisol concentration and heart rate and a transient decrease in HRV. The degree of the induced changes decreased with repetition of the same event, suggesting rapid adaptation of the young stallions to these challenges.

Cortisol concentration in saliva is a surrogate parameter for non-protein-bound cortisol concentration in plasma (Riad-Fahmy

et al., 1983) and has been validated for horses (Peeters et al., 2010). In agreement with previous studies (Peeters et al., 2010; Schmidt et al., 2010a; Kaps et al., 2022; Holtby et al., 2023), cortisol concentration in saliva peaked between 15 and 30 min after the respective events. The most pronounced cortisol release was evident in response to free movement and lunging without side-reins in the first weeks of pretraining and particularly to first exposure to an automated horse walker. With repeated exposure to the horse walker, the cortisol release became less evident, demonstrating adaptation of the young horses. The concomitant reduction in the heart rate increase with repeated exposure to the horse walker most likely indicates more a mental response to an unfamiliar situation than a physical strain. The cortisol and heart rate response to repeated exercise in the horse walker was, however, not affected by stallion age and, contrary to our hypothesis, 18-month-old stallions did not show a more pronounced response than older stallions. Interestingly, first exposure to a starting gate in Arab racehorses was not associated with increased cortisol release (Witkowska-Piłaszewicz et al., 2021). This may be because starting gates are placed in the open and allow the horse full visual perception of the environment which is not the case in a horse walker.

A similar decrease in the stress response of horses to anthropogenic challenges has been demonstrated previously when transport-naïve horses were transported by road repeatedly (Schmidt et al., 2010c). Road transport is a mental stressor except for the need to balance on a moving vehicle is without physical demands for horses. Despite an initial cortisol release and increase in heart rate, horse walker exercise does not necessarily have to be interpreted as a harmful event but may represent a eustress response. As in humans and laboratory animals (reviewed by McEwen and Sapolsky, 1995), moderate increases in cortisol concentration and heart rate as occurred during pretraining may enhance learning in horses. It has been proposed that these effects are associated with the influence of cortisol and neurotransmitters on certain brain regions. In contrast, exposure to uncontrollable stress causing pronounced cortisol release should be avoided because learning may be impaired (Henshall et al., 2022).

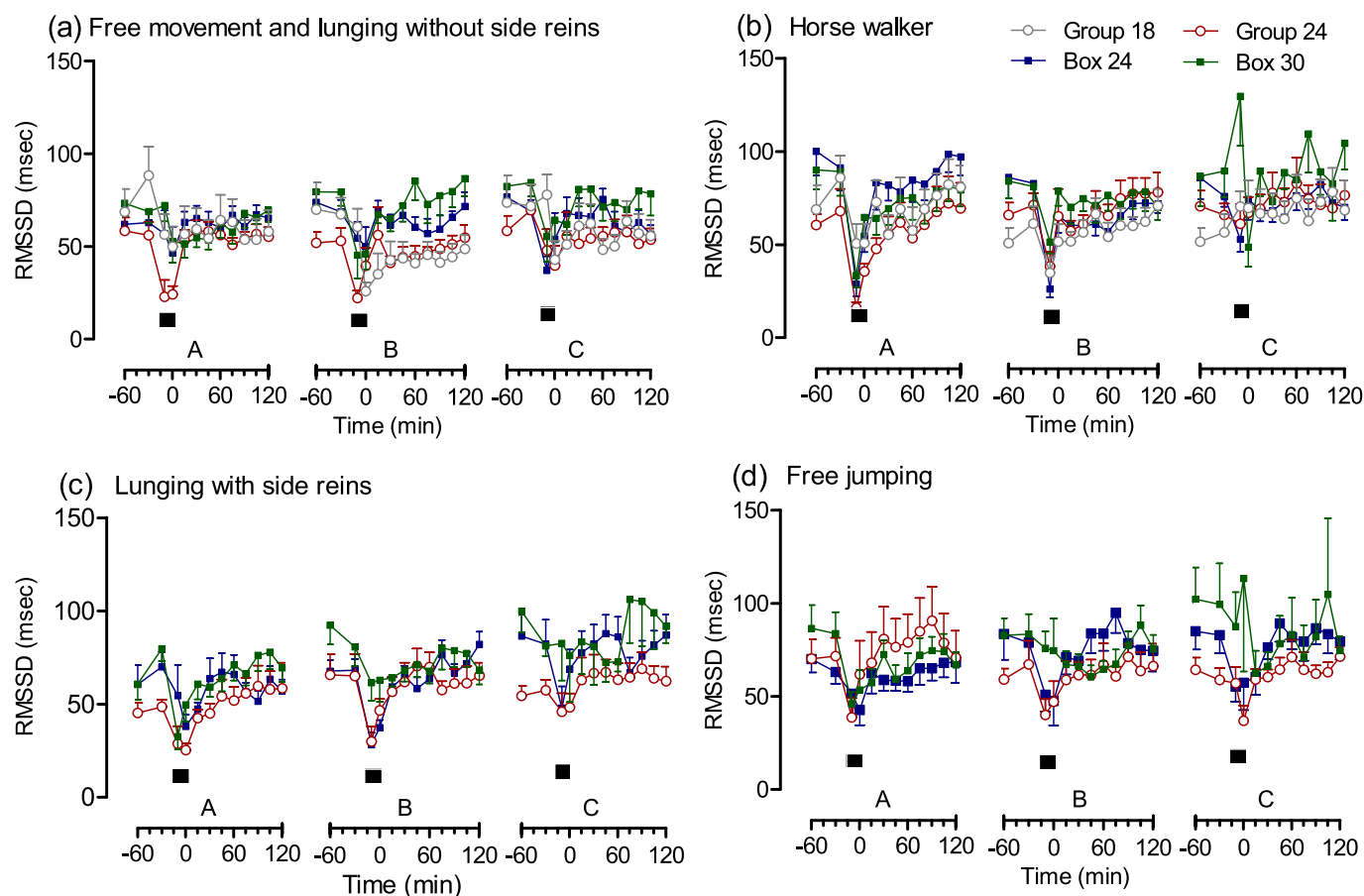


Fig. 4. Heart variability parameter RMSSD (root mean square of successive beat-to-beat differences) in 18-month-old stallions kept in a group stable (Group 18, $n = 10$), 24-month-old stallions kept in a group stable (Group 24, $n = 9$), 24-month-old stallions kept in single boxes (Box 24, $n = 10$) and 30-month-old stallions kept in single boxes (Box 30, $n = 10$) in response to (a) free movement in a riding arena and lunging without side reins, (b) movement in a horse walker, (c) lunging with side reins and lunging girth and (d) free jumping. Note that Group 18 stallions are included only in (a) and (b). The time of the different events is indicated by vertical arrows, A, B, or C indicates repeated exposure to similar events (see also Table 3). For results of statistical analysis, see Table 4.

Irrespective of stallion age, the cortisol and heart rate response to first exposure to the horse walker was clearly affected by the housing system of the stallions. In 24- and 30-month-old stallions kept in single boxes, cortisol release was reduced in comparison to 18- and 24-month-old stallions kept in groups. This suggests that stallions housed in individual boxes had been more accustomed to a restricted space than their group-housed conspecifics. Regarding cortisol release, 24-month-old stallions kept in single boxes or in groups differed only for the first exercise in the horse walker but not for any other event studied. The increased stress response of group-housed stallions suggests more arousal in response to the challenges of our study than in stallions kept in single boxes. This may be caused by the fact that group-housed stallions had to be newly separated from their herd mates for each individual pretraining event.

Except for the initial free movement, lunging and horse walker events of our pretraining programme, the relative increase in cortisol concentration in the 18- to 30-month-old stallions remained below or was similar to the increase induced by first mounting of a rider (Schmidt et al., 2010a; Kędzierski et al., 2014; Holtby et al., 2023). A marked increase in cortisol release in young Thoroughbred racehorses occurred also when they were 'driven' for the first time, i.e., controlled in walk and trot by a person on the ground with two long reins (Holtby et al., 2023). Although technically slightly different, this compares to initial lunging without side reins in our study and induced a similar cortisol release. Together, data from the present study and the 2-year-old Thoroughbreds

(Holtby et al., 2023) suggest that the initial events of pretraining induce a similar cortisol release as first mounting of a rider (Schmidt et al., 2010a). Our data do, however, not indicate that cortisol release in response to the pretraining events was affected by stallion age, at least not within the age range from 18 to 30 months. A mental immaturity in the younger stallions that would transmit into a more evident stress response is thus unlikely. Furthermore, stallions of all age groups adapted rapidly to the pretraining challenges with a consistent but only moderate increase in salivary cortisol concentration, i.e., to lunging with side reins and free jumping to which the horses were exposed with progression of the pretraining.

Given that the young stallions were mentally able to cope with the demands of our study, it could be speculated that early pretraining may even be beneficial not only for musculoskeletal development as shown in Thoroughbred racehorses (reviewed by Logan and Nielsen, 2021) but also for long-term interactions of such horses with humans. In a recent study on 24- to 36-month-old sport horses during initial training proceeding from lunging to riding, no major effects of social contact with conspecifics on the animals' behaviour during training was evident, but horses kept without social contact showed reduced signs of discomfort when ridden (Flamand et al., 2024). This agrees with a reduced stress response of the single box-housed stallions of our study when exercised for the first time in a horse walker. Horses with social contact, however, expressed less abnormal behaviour in the stable (Flamand et al., 2024). Whereas age and breed of horses in both

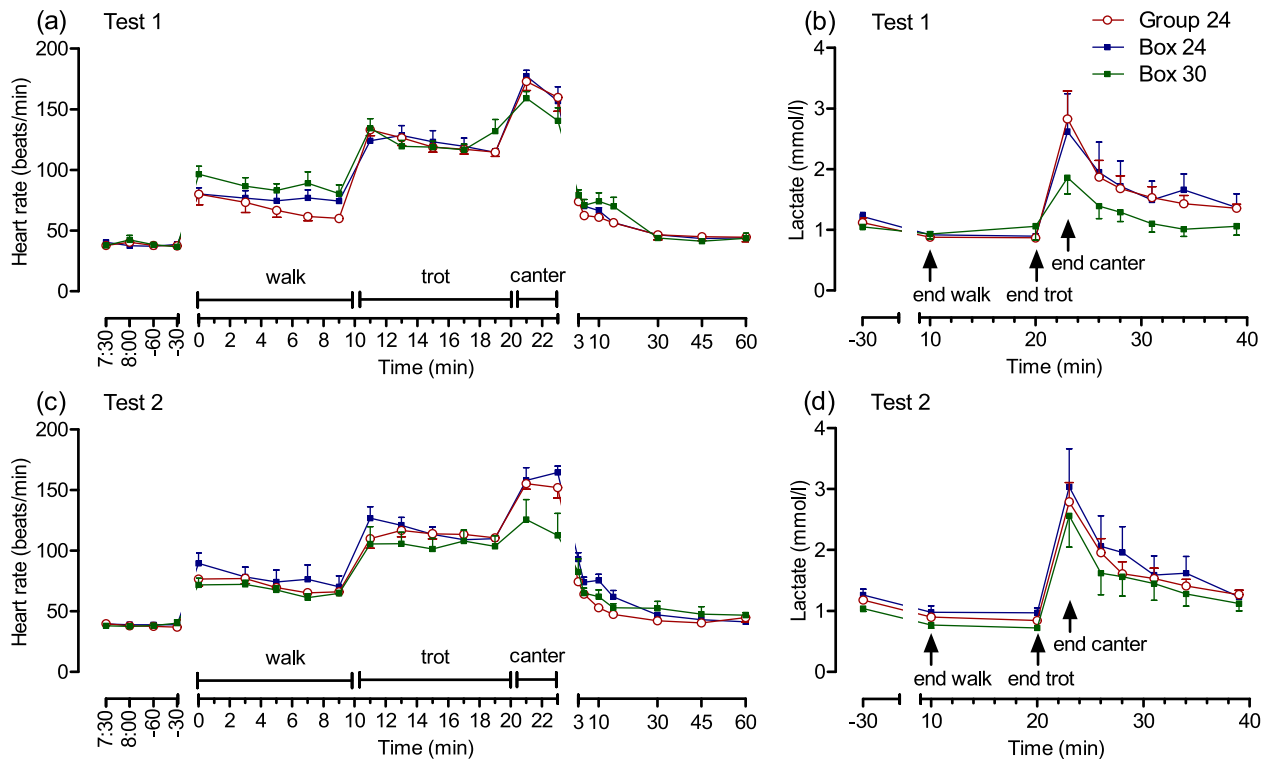


Fig. 5. (a,c) Heart rate and (b,d) postexercise blood lactate concentration in 24-month-old stallions kept in single boxes (Box 24, $n = 10$), 24-month-old stallions kept in a group stable (Group 24, $n = 9$) and 30-month-old stallions kept in single boxes (Box 30, $n = 10$) in response to an exercise test in (a,b) week 5 and (c,d) week 12 of a 12-week pretraining programme. Statistical differences: for heart rate: time $P < 0.001$ ($F_{22} = 228.70$), repetition $P = 0.015$ ($F_1 = 7.23$), time $\times$ age $P < 0.001$ ($F_{22} = 2.27$), repetition $\times$ time $P = 0.009$ ($F_{22} = 1.90$), for lactate concentration: time $P < 0.001$ ($F_8 = 21.38$).

studies are close to similar, the housing systems are not. In contrast to the study by [Flamand et al. \(2024\)](#), our study did not include completely isolated horses whereas their study did not include horses in group stables. In the study by [Flamand et al. \(2024\)](#), all horses were kept in individual boxes with one group allowed contact to the horse in the neighbouring box through an opening in the partition for 2 h per day. Given that the horses in individual boxes of our study were allowed constant visual, auditory, olfactory and – although restricted – tactile contact through a bar partition with two neighbours, the box-housed stallions in our study thus had more social contact to neighbouring horses than the social contact group in the study by [Flamand et al. \(2024\)](#).

When young horses were divided into group-housed and single box-housed at the early age of five months and were followed over 2 years, box-housed horses showed more aversive behaviour, such as biting their trainer than did group-housed horses. Their behavioural response thus demonstrates the benefits of raising young horses in groups ([Søndergaard and Ladewig, 2004](#)). Furthermore, when 2-year-old stallions were housed either individually or in groups for nine months and were subsequently transferred to group pastures, previously singly stabled stallions were more aggressive to their conspecifics than group-stabled stallions. On the other hand, stallions coming from individual boxes spent more time with social grooming and play behaviour when subsequently interacting freely with other horses ([Christensen et al., 2002](#)). In the present study, we did not find positive effects of group housing versus single housing on the stress response of stallions to the different pretraining events. This may be attributed to the fact that all stallions in our study had been kept in groups of 20–40 horses on pasture until 1 week before the study. As suggested previously ([Søndergaard and Ladewig, 2004](#)), young horses raised in groups will adapt more easily to a subsequent training than young horses raised with less contact to conspecifics. The pretraining programme in our study lasted for three months and included fre-

quent horse-handler interactions. The finding that single-box housing for three months accompanied by frequent handling did not negatively affect the animals' stress response to the pretraining events thus does not exclude negative effects when horses are isolated over a longer time, with less handling or without visual, auditory and olfactory contact to horses in directly adjacent boxes.

The interpretation of changes in salivary cortisol concentration is corroborated by changes in HRV. The HRV, i.e., short-term fluctuations in the cardiac beat-to-beat interval, reflects the balance of sympathetic and parasympathetic tone and provides information on the stress response of the autonomic nervous system. An increase in time-domain HRV variables such as RMSSD reflects a shift towards parasympathetic dominance, while reduced HRV indicates a shift towards more sympathetic dominance (reviewed by [von Borell et al., 2007](#)). The transient decrease in HRV in response to all pretraining events – except to horse walker repetitions – indicates sympathoadrenal activation. A comparable HRV reduction but lasting for a longer time exists in horses transported by road and reflects a stress response ([Schmidt et al., 2010b,d](#)). In contrast, HRV was more reduced in ridden horses, either 3-year-old sport horse mares and stallions in the early phase of equestrian training ([Schmidt et al., 2010a](#)), older horses in training for show jumping ([Ille et al., 2013](#)) or sport horses participating in dressage and show jumping competitions ([Becker-Birck et al., 2013](#)). Based on the observed changes in HRV, the pretraining of 18- to 30-month-old young stallions is thus not perceived as particularly stressful by the animals. It is thus unlikely to raise animal welfare concerns regarding the acute stress response to the training events. Basal HRV was constant throughout repetitions of free movement and free jumping without a rider but increased with repeated lunging, suggesting a continuous increase in parasympathetic tone. This can be interpreted as a potential relaxation of the horse with adaptation to this type of event throughout the pretraining programme. The HRV is best determined in the standing horse and

is confounded by movement of the animal (Ille et al., 2014b). Movement not controlled by a rider or handler as occurred particularly in association with free jumping may thus have masked, although not completely, psychologically induced HRV decreases in the stallions of our study.

Heart rate increased during all pretraining events, but the extent of this increase differed among individual events. The reduced increase in heart rate with repeated horse walker exercise is most likely caused by decreasing emotional arousal of the animals. As for cortisol, group-housed stallions irrespective of their age perceived the horse walker as a greater challenge than stallions adapted to individual boxes. Heart rate reflects sympathoadrenal activation as part of a stress response, and this may be valid also for initial free movement and lunging without side reins. The heart rate response to repeated lunging with side reins and to free jumping was close to identical in all stallion groups and is suggested to represent predominantly physical activity. One might expect jumping more of a physical effort than lunging but on the other hand, the use of the side reins for lunging may affect the horse's ability to use its head and neck normally which in naïve horses could induce some physical strain until they adapt to the restrictions imposed by the side reins.

The current findings of our study are based on changes in physiological stress parameters in response to physical activity and increasing workload as well as psychosocial stressors due to exposure to new tasks and the use of novel equipment. The data thus provide detailed information on changes in the horses' state of arousal during pretraining and adaptation to the anthropogenic interventions of the study. Because training and the extent to which a horse is under stimulus-control can override inherent emotional responses, behavioural indicators may differ from a horse's ability to tolerate potentially stressful procedures (Squibb et al., 2018). Behaviour data from the stallions of our present study will complement the present physiological parameters in a subsequent manuscript.

Heart rate in the stallions during lunging, free jumping and during the exercise test peaked at 150–160 beats/min. This is still a moderate increase compared to maximum heart rates between 200 and 240 beats/min in Thoroughbred racehorses during a race (Krzywanek et al., 1970) or 180–200 beats/min in eventing horses during the cross-country phase (Armory et al., 1993; Serrano et al., 2002). Postexercise blood lactate concentration in competing horses during a cross-country course peaked at values approximately fivefold higher than in the exercise test of our study. Although the increase in heart rate during pretraining events and heart rate and blood lactate concentration during the exercise test remained below peak values in competing horses, they are in the same order of magnitude as in eventing horses training for advanced-level competitions (Serrano et al., 2002). This indicates that the peak cardiovascular demands of pretraining in our stallions are similar to the demands during training of older sport horses, but young stallions adapted rapidly to these demands. On the other hand, older horses trained for competitions are apparently not pushed to their performance limits during training. Heart rate does not only indicate the degree of physical activity but is also a measure of arousal. A reduced increase in heart rate during training will reflect both physical adaptation to the exercise and a reduction in psychosocial arousal as horses habituate to the training routines.

Conclusions

Pretraining of young stallions for stallion licencing is both a physical and a mental demand. During the initial pretraining events, a physiological stress response was evident. With repetition of these events, even stallions as young as 18 months habitu-

ated rapidly to these demands. Because glucocorticoids enhance energy mobilisation and affect animal behaviour during short-term stress, an initially increased cortisol release may enhance the adaptation of horses to these anthropogenic challenges. Our study does not provide any evidence that a moderate, systematic pretraining of 2-year-old Warmblood sport horse stallions raises animal welfare concerns. Although our data do not allow conclusions about long-time isolated housing of stallions, group housing of stallions had no beneficial or stress-reducing effects compared to housing stallions in single boxes during a 3-month pretraining programme.

Ethics approval

The study was approved by the competent authority for animal experimentation in Brandenburg State, Germany (*Landesamt für Arbeitsschutz, Verbraucherschutz und Gesundheit*, licence number 2347-05-2022-G).

Data and model availability statement

None of the data were deposited in an official repository. Data are available from the authors upon request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

None.

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