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Effects of Aqua-Treadmill Exercise on Selected Blood Parameters and on Heart-Rate Variability of Horses

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With 6 figures and 1 table

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Summary

The objectives of the present study were to investigate the effects of Aquatraining of horses (aqua-treadmill exercise; treadmill manufactured by Equitech – L.u.S. Equipment, Warendorf, Germany) on selected blood parameters [lactic acid concentration (mmol/l), haemoglobin content (g/l)] and on heart-rate variability (HRV) [heart rate (beats per min; b.p.m.), standard deviation of all NN-intervals (SDNN; ms), normalized power of the low and high frequency band (LF_{norm}, Hf_{norm}; au), % recurrence, % determinism and $ratio_{corr}$]. Seven horses performed six exercise tests with different work loads (walking ($\bar{x} = 1.56 \pm 0.08$ m/s) and trotting ($\bar{x} = 2.9 \pm 0.13$ m/s); dry, water above the carpus and water above the elbow). The standardized test-protocol was: 5 min warm-up at walk while the water was pumped in, followed by the 20-min exercise period at walk or trot, followed by a 5-min walk while pumping out the water. Blood samples were taken prior to each test at rest in the stable, as well as exactly 5 min after the end of the 20-min exercise period. Electrocardiograms were recorded during rest and the 20-min exercise period. Compared to rest, neither the chosen velocities, the two water levels, nor the dry tests led to a significant increase of the lactic acid concentration in any horse. The haemoglobin content showed a significant increase as a result of exercise. Significant differences could be found between the heart rates at rest and the six exercise tests and between the mean of the levels ‘walking’ and the mean of the levels ‘trotting’. An exercise-induced change of HRV was characterized by a decreasing SDNN, a significantly higher LF_{norm} (sympathetic influence) combined with a significantly lower HF_{norm} power (parasympathetic activity) and a rising degree of order (significantly higher % determinism and nearly unchanged % recurrence) and stability (significantly rising $ratio_{corr}$) of the recurrence plot. In conclusion, the used training-protocol for aqua-treadmill exercises only represents a medium-sized aerobic work load for horses, but the different levels of burden were indicated especially by changes in HRV.

Introduction

Aquatraining, also known as Aquawalking or Aquajogging, is a well-established training and rehabilitation method in human medicine. The advantages are an easing of the burden on joints, muscles and tendons, and simultaneous activation of numerous muscles which are combined with nearly identical motions compared to dry ground (Froboese, 1994).

To make use of the benefits of this method for horses, special treadmills have been developed which can be flooded with water allowing the horses to be exercised at velocities and water levels which can be varied individually. It has to be pointed out, that the most important feature of this kind of Aquatraining is the fact that the animals always maintain contact with the ground. In contrast to swimming the effect of this training is based on the movement of the limbs against the resistance of the water without weightlessness of the body.

Up to now little has been known about the metabolic consequences of this method during exercise. By examining selected physiological parameters, the aim of the present investigation is to determine the degree of burden for horses in Aquatraining. Two different approaches were used to reach this goal: on the one hand metabolic parameters like lactate and haemoglobin concentration – known as reliable markers to indicate exercise-induced changes (Persson, 1969) – were investigated. On the other hand, parameters describing heart-rate variability (HRV) were used to characterize changes in the central control mechanism(s) during exercise. Because it is well known that linear as well as non-linear mechanisms are involved in central control (Signori et al., 1994; Hagerman et al., 1996), both established time- and frequency-domain parameters (Kuwahara et al., 1996) and parameters describing non-linear chaotic dynamics (Webber and Zbilut, 1994) were used.

Materials and Methods

The investigations were carried out between December 1998 and February 1999 in the ‘Trainings- und Rehabilitationszentrum für Sportpferde’ at Reichertsheim, Bayern.

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Seven horses (three mares of the Hannover breed, two geldings of the Bayern breed, one Holstein gelding and one Hannover stallion) aged between 4 and 15 years with different states of fitness were available. The daily training regimes usually applied ranged from pasturing alone to 1-h riding (gymnastics, dressage, jumping, condition) combined with pasturing. Housing and feeding conditions were almost identical.

Each horse was recorded at rest and performed in six exercise tests:

1 RR, at rest;
2 WD, walking on a dry treadmill;
3 WC, walking in water above the carpus;
4 WE, walking in water above the elbow;
5 TD, trotting on a dry treadmill;
6 TC, trotting in water above the carpus;
7 TE, trotting in water above the elbow.

The treadmill (Equitech – L.u.S. Equipment, Warendorf, Germany) was formed as a box with watertight doors at each short margin, equipped with protective upholstery. The dimensions were $4 \times 1.5 \times 2$ m (length × width × height). Minimal times for flooding and pumping out (water level approximately 120 cm) were each 5 min. The average velocities of the treadmill were 1.56 ± 0.08 m/s and 2.9 ± 0.13 m/s during walking and trotting, respectively.

Blood samples were drawn from the V. jugularis with a vacuum blood-sampling system ('Venoject', Terumo) in heparinized tubes (10 ml), ethylenediaminetetraacetic acid (EDTA) tubes (4 ml) and in silicon-coated tubes (10 ml). Among other blood parameters, special importance was attached to haemoglobin content and lactic acid concentration.

The standardized test protocol was: 5-min warm-up at walk while the water was pumped in, followed by the 20-min exercise period at walk or trot, followed by a 5-min walk while pumping out the water (Fig. 1).

Blood samples were taken prior to each exercise test at rest in the stable (basic values) as well as exactly 5 min (after pumping off the water) and 30 min (relaxing period; calm standing under the solarium) after the end of the 20-min exercise period, each. A fourth blood sample was taken to determine serum enzymes and total protein after a recovery period of 24 h.

Lactic acid concentration in blood (Dr Lange, ESAT 6661, Eppendorf, Germany) and pH value (Radiometer, Copenhagen, Denmark) were determined immediately in the exercise room.

Blood in the silicon-coated tubes was centrifuged and the remaining plasma was pipetted and chilled. This plasma and the chilled blood samples in the EDTA tubes were sent to a commercial laboratory for analysis.

An electrocardiogram (ECG) was recorded during rest and during the 20-min exercise periods using a transportable datalogger (Par-Port/M).

HRV was calculated from interbeat intervals [IBI (ms): normal-to-normal intervals: all intervals between two adjacent QRS complexes which result from a sinus node depolarization (Malik, 1996)], which were derived from the recorded ECG by measuring the time of R-R intervals. Based on these time series of IBIs, the linear parameters of the time domain and the frequency domain were calculated, following the criteria of the Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology (Malik, 1996). After transforming the IBI series into equidistant time series (instantaneous heart rate), frequency domain parameters of 5-min intervals were calculated by Fourier analysis. To describe the non-linear component in (HRV) recurrence plot parameters of the same 5-min intervals were calculated with the aid of the program RQA 5.2. (Webber and Zbilut, 1994).

Heart-rate variability

The linear parameters for time domain parameters were as follows: heart rate (beats per minute, b.p.m.); IBI; and the standard deviation of all normal (NN) intervals (SDNN).

The linear parameters for the frequency domain parameters were as follows: LF_{norm}, calculated as the power in the low-frequency band × 100/(LF + HF); and HF_{norm}, calculated as the power in the high-frequency band × 100/(LF + HF).

Non-linear parameters were the following recurrence plot parameters: % recurrence, the percentage of the plot occupied by recurrent points; % determinism, the percentage of recurrent points that form upward diagonal line segments; and $ratio_{corr}$, calculated as (% determinism)/(% recurrence) and addressing non-stationary characteristics in recurrence plots.

The influences on metabolism and on (HRV) are discussed comparing rest and exercise values (for blood parameters: 1. BS and 2. BS) using the following parameters: lactic acid concentration (mmol/l), haemoglobin content (g/l), heart rate (b.p.m.), SDNN (ms), LF_{norm} (au), HF_{norm} (au), % recurrence, % determinism and $ratio_{corr}$.

The statistical calculations and diagram representations were carried out with the SigmaStat® and SigmaPlot® computer programs (Jandel Scientific Corp.). All statistical evaluations were made in two steps: first, using analysis of variance (ANOVA) on ranks (because of not normally distributed values), second, doing *post hoc* tests with the Student–Newman–Keul's method (equal size of groups) or Dunn's method (unequal size of groups).

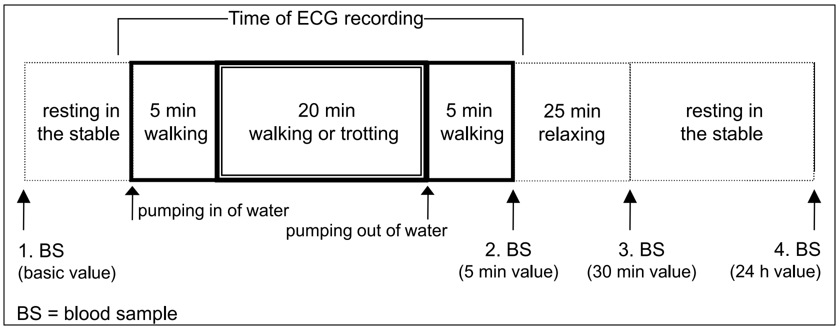

Fig. 1. Exercise test protocol.

All results are presented as median ± MAD (median deviation) (Sachs, 1984) and significance differences with $P < 0.01$.

Results

All figures show the results at rest and for the six different levels of exercise. Individual traces of each of the seven horses (Vt.1–Vt.7) as well as the summarized results (box plots) are presented.

The lactic acid concentrations are demonstrated in Fig. 2. The values varied only in small boundaries from 0.59 mmol/l (Vt.5 at TD) to 1.24 mmol/l (Vt.2 at TC) and for the medians from 0.83 ± 0.09 mmol/l (RR) to 1.01 ± 0.17 mmol/l (TC). No significant differences were found between the medians of lactate at different levels of testing. It could be noted that medians of lactic acid concentrations were only floating in narrow bounds.

Figure 3 shows medians and MAD of the haemoglobin content. There is a statistically significant difference between the basic physiological value (134.0 ± 11.0 g/l) and the values of the four exercise tests in water. It is interesting that no significant differences could be found within the six test levels. For Vt.5 higher values could be found after all walking tests in comparison to the three trotting tests.

The medians and MAD of heart rate are given in Fig. 4. The heart rates ascended from 32 ± 1.6 b.p.m. at rest to significantly higher values during all of the six exercise tests; they ranged between 69 ± 15.4 b.p.m. (WD) and 96 ± 11.8 b.p.m. (TE).

Within the two gaits (walking and trotting) no significant difference could be found with reference to the different water levels. Comparing the mean values of trotting medians to those of walking revealed a significant difference of 18 b.p.m. between these two paces. In general, a heart rate increase could be found while changing from easier work loads (WD) to the heavier ones (TC and TE).

The analysis of the SDNN showed a significant difference between resting values (341.37 ± 84.01 ms) and the six exercise tests (Fig. 5). There were also significant differences between walking on the dry treadmill (WD) and WE, TC and TE. The high variance of the different resting values was marked.

In contrast to the trend that heart rates rose while going from easier work loads (WD) to heavier ones (TC and TE), the

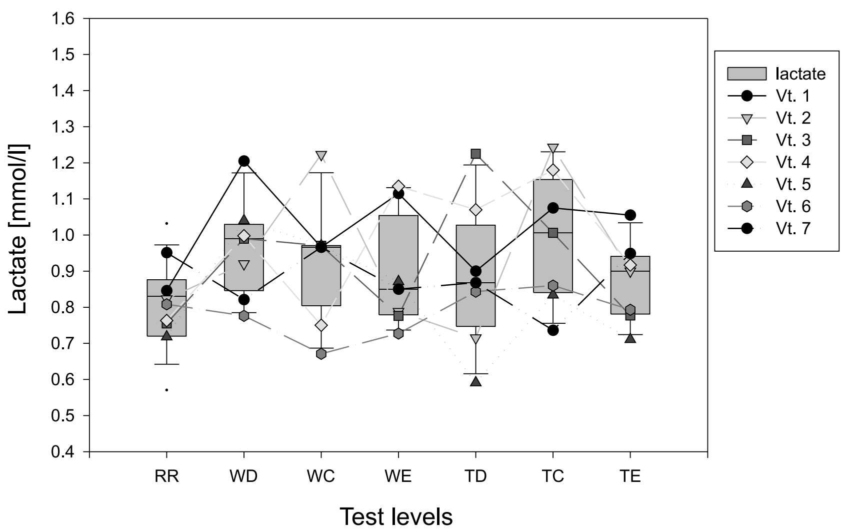

Fig. 2. Median and MAD of lactic acid concentrations of the seven horses at rest (RR, basic value) and exactly 5 min after the 20-min exercise period at different water levels and velocities (WD to TE are the different exercise tests).

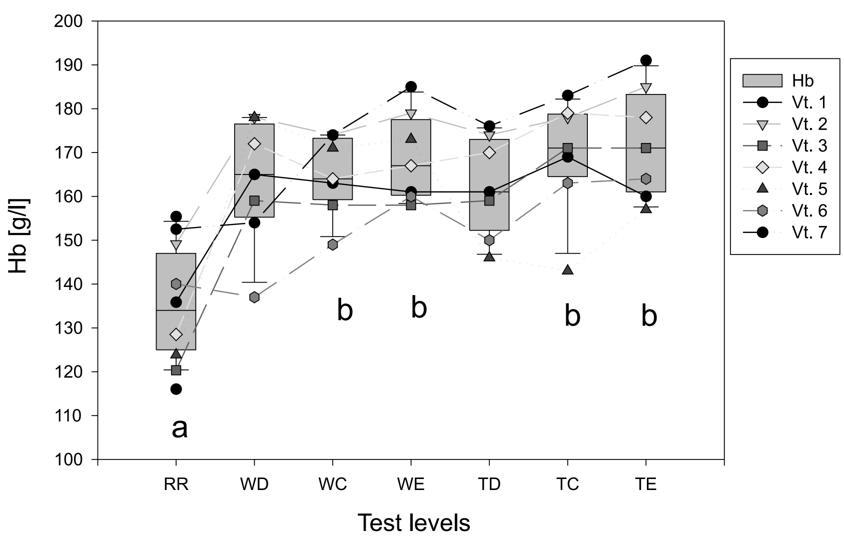

Fig. 3. Haemoglobin content of all animals at rest (RR, basic value) and exactly 5 min after the 20-min exercise tests at different water levels and velocities (WD to TE are the different exercise tests); different characters indicate significant differences ($P < 0.01$).

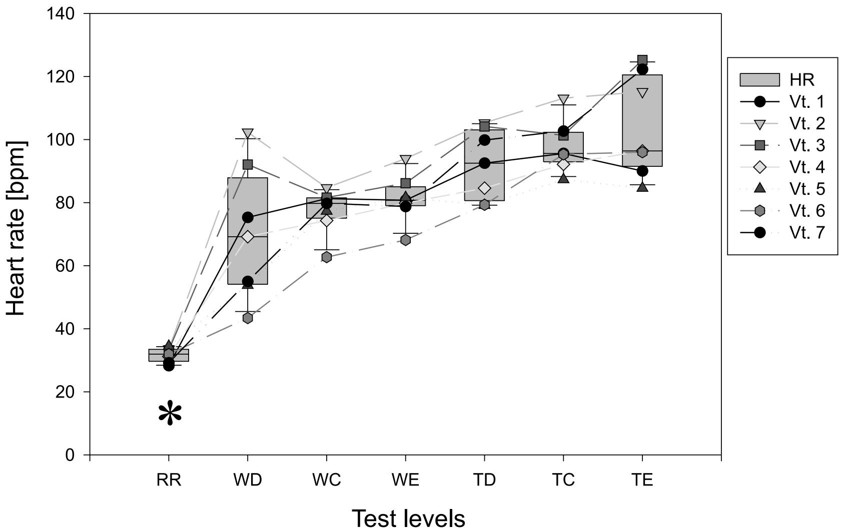

Fig. 4. Median and MAD of the heart rate of each horse at rest and during the 20-min exercise period of the six tests; asterisk indicates a significant difference at $P < 0.01$ to all levels of exercise.

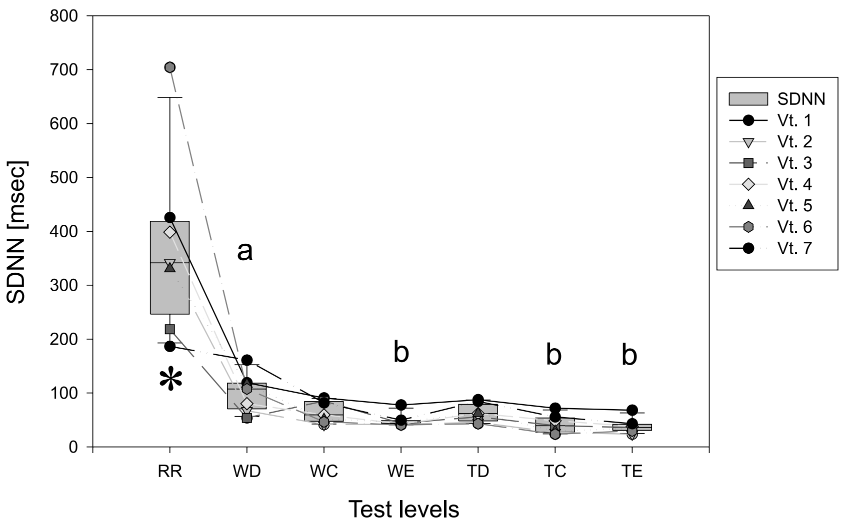

Fig. 5. Median and MAD of SDNN of each horse at rest and during the 20-min exercise period of the six tests; asterisk indicates a significant difference at $P < 0.01$ to all levels of exercise; different characters indicate significant differences ($P < 0.01$).

medians of the SDNN decreased (except for TD). There was a decrease of values from WD (107.22 ± 26.99 ms) to WC (59.27 ± 17.91 ms) to WE (43.46 ± 2.55 ms). While trotting on a dry treadmill (TD) the SDNN (61.85 ± 16.36 ms) was lower than during WD, but higher than during WC and WE. Trotting in a water-level above the carpus (TC) led to a lower SDNN (39.30 ± 12.75 ms) than walking without water (WD) or in water of different levels (WC, WE) or trotting on a dry treadmill (TD). During the last exercise test, trotting in water above the elbow (TE), the SDNN was lowest (35.86 ± 6.47 ms).

Figure 6 demonstrates the opposite development of LF_{norm} and HF_{norm} values. For both parameters the exercise values were significantly different in comparison to the basic values (rest: LF_{norm}, 63.26 ± 6.48; HF_{norm}, 36.74 ± 6.48). Also, walking on a dry treadmill (WD) was significantly different to trotting on a dry treadmill (TD).

The comparison of both graphs clarifies the opposite influence of the two components of the vegetative nervous system. While the LF_{norm} parameter, which shows the modulation by the sympathetic tone on heart rate, increased with work load, the HF_{norm} parameter, the expression of the parasympathetic tone, decreased to the same degree and at the same time.

The non-linear recurrence plot parameters % determinism, % recurrence and $ratio_{corr}$, during rest and the six test levels are listed in Table 1.

There were pronounced changes for % determinism but only small ones for % recurrence. For the $ratio_{corr}$ there was an increasing tendency from resting to exercising and from the easier work loads to the heavier ones.

There was a significant difference between the resting value of % determinism in comparison to the six exercise tests. In addition, trotting in water above the carpus (TC) was significantly different to WD, WE and TD. For % recurrence we found significant differences between WD and the six other test conditions. According to this trotting in water above the elbow was significantly different from RR, WC and TD. The calculation of their ratio (% determinism/% recurrence) resulted in significant differences between the resting value and the exercise tests, except for WD. The median of this test was significantly different from WE, TD, TC and TE. The median for TE was also significantly different from that for WC.

The $ratio_{corr}$, with the exception of test WD, increased significantly as a result of exercise. This was caused by a

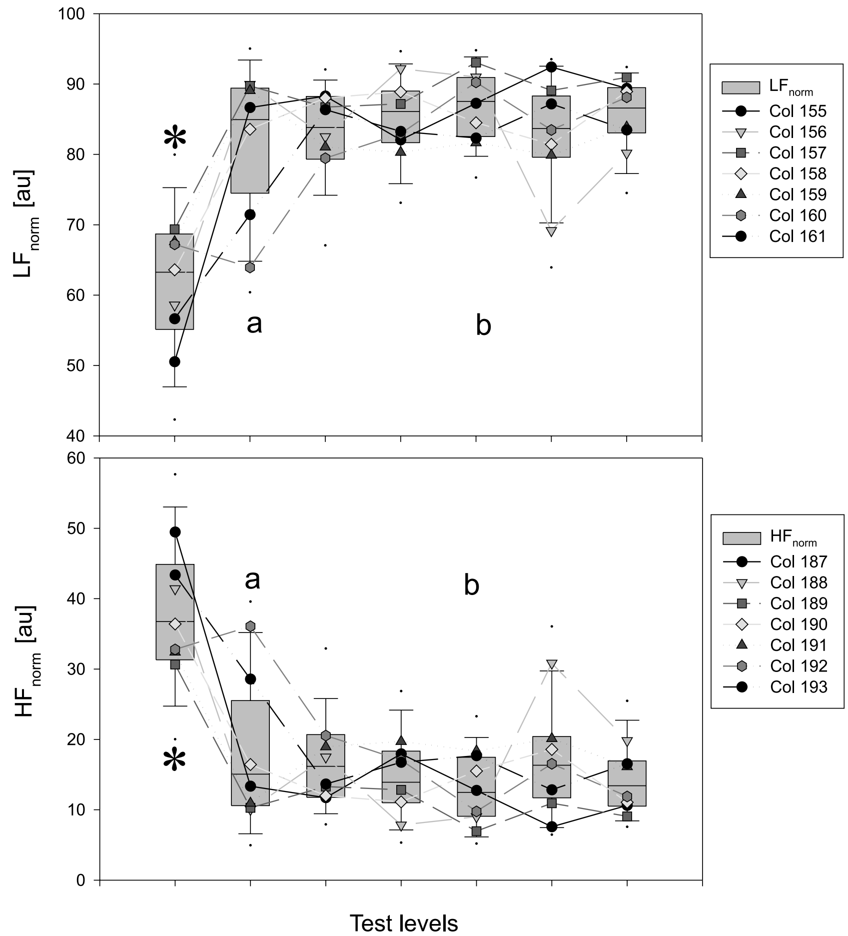

Fig. 6. Medians and MAD of LF_{norm} and HF_{norm} of each horse at rest and during the 20-min exercise period of the six tests; asterisk indicates a significant difference at $P < 0.01$ to all levels of exercise; different characters indicate significant differences ($P < 0.01$). Notice the fact that LF_{norm} rose in the same degree that HF_{norm} decreased.

Table 1. Medians and MAD of % determinism, % recurrence and $ratio_{corr}$ at rest and in the 20-min exercise period

	RR	WD	WC	WE	TD	TC	TE
% determinism	21.47* ± 14.26	39.14^{a} ± 7.79	38.45 ± 6.78	40.69^{a} ± 8.86	40.54^{a} ± 6.00	34.25^{b} ± 5.85	37.58 ± 4.93
% recurrence	$5.50^{b,d}$ ± 4.00	9.09^{a} ± 4.13	$5.20^{b,d}$ ± 2.67	5.71^{b} ± 2.42	$5.79^{b,d}$ ± 2.92	3.95^{b} ± 2.78	$2.88^{b,c}$ ± 1.39
$Ratio_{corr}$	3.02^{a} ± 2.49	4.56^{c} ± 2.54	$6.95^{b,e}$ ± 4.34	$7.85^{b,d}$ ± 4.43	$7.01^{b,d}$ ± 2.80	$8.90^{b,d}$ ± 5.44	$12.97^{b,d,f}$ ± 6.70

*Significant difference, $P < 0.01$, to all levels of exercise; different characters (^{a} compared to ^{b,c} compared to ^{d} and ^{e} compared to ^{f}) indicate significant differences ($P < 0.01$). Notice the pronounced changes for % determinism and the small ones for % recurrence. For the $ratio_{corr}$ there was an increasing tendency from resting to exercising and from the easier work loads to the heavier ones.

significant increase of % determinism, while % recurrence remained almost unchanged.

As for heart rate and SDNN, the $ratio_{corr}$, with exception of TD, tended to increase from WD up to the heaviest exercise stage (TE).

Discussion

Lactate

Our investigation showed that none of the exercising tests produced lactacidaemia.

We conclude that the horses were able to compensate the higher oxygen demand during exercise via respiration and also via an increasing capacity for O_2 transport through mobilization of the splenic blood-cell reservoir. Furthermore, because of solely low lactate values we presume that mainly the oxidative working muscle fibres type I and/or type IIA are recruited. Therefore, aerobic work can be achieved for this kind of exercise.

Hemoglobin

It is well known that the haemoglobin concentration increases as a result of the mobilization of the erythrocyte reservoir by physical exertion (Barcroft, 1925; Persson, 1969; Krzywanek et al., 1972; Rose et al., 1983) and this is confirmed by our results. Apart from physical work, the level of haemoglobin is also influenced by the individual excitement of the horse (Revington, 1983; Krzywanek et al., 1972). The horses were stressed differently by work, surroundings, the events in the Aquatrainer and by the taking of blood samples (invasive method). This might be the explanation for the divergence of the individual values at some of the testing levels. For example Vt.5 was the youngest horse and was less accustomed to being handled than the others. Despite some days of adaptation, this horse was very excited during the three walking levels and showed higher results in all of them compared to the trotting levels, but then calmed down remarkably.

Heart rate

The heart rates of all horses at rest were less than 35 b.p.m., which is within the physiological range of 25–40 b.p.m. (Evans, 1994), and increased during the working tests without reaching the maximum values of 210–240 b.p.m. described in literature (Asheim et al., 1970; Krzywanek and Wittke, 1970). The highest recorded heart rate was 125 b.p.m. (Vt.3 at TE). Persson (1983) found a linear relationship of heart rate and work load over a range of approximately 120–210 b.p.m.. The non-linearity below a rate of 120 b.p.m. is ascribed to the influence of psychogenic factors. During submaximal work this psychogenic influence on the exercise heart rate is more pronounced if the relative work load (velocity or water level) is low (Persson, 1983) or the horse is near to a state of rest (Krzywanek and Wittke, 1970).

The fact that heart rate did not reach a level of 210 b.p.m. explains the low blood-lactate concentrations (close to resting level) after all of our testing procedures.

According to these results, two observations can be made. Firstly, for WD, which was the first and easiest work load, some of the horses were more excited than during the following exercise programs, and therefore there were relatively large individual variations between the heart rates of the horses. Secondly, despite the fact that there were no significant differences within the three walking or trotting exercises, we found, as expected, a tendency to increasing heart-rate medians in the course of easier work loads (WD) to heavier ones (TC and TE), depending on gait and water level. There was a small significant differences of 18 b.p.m of the mean values of the medians between the two paces walking and trotting as different work loads.

This small difference, the relatively low levels of heart rates and blood lactate concentrations near resting values makes clear that the applied training protocol only implied a medium-sized work load.

SDNN

If heart rate (b.p.m.) rises, the time intervals between the single beats have to become shorter. To evaluate the influence of this rise on HRV, SDNN, the standard deviation of all NN-intervals (square root of variance), can be used. The overall variability of the heart rate in a defined period, can be described by this parameter (Malik, 1996).

Our investigation showed that the SDNN of all horses at rest was significantly higher than during exercise. This means, that the length of the intervals between the single beats varied more around a mean value at rest compared to during exercise. In addition, we found relatively large individual variations between the single horses at rest. The reasons for this high variance at rest were the physiological sinus dysrhythmias, which appeared in each horse with different incidence. They occur because of the very high vagal tone which leads to temporary blockade of the sinus and/or atrioventricular node. Because of the decrease in vagal tone, these dysrhythmias disappeared as a result of exercise.

Within the different work-load tests, WD was significantly different from WE, TC and TE conditions. SDNN during trotting in water above the elbow was somewhat smaller than during trotting in water above the carpus or all other experimental conditions. In general, trotting in water showed lower variations than walking in water, and walking in water above the elbow varied less than walking in water above the carpus. Summarizing, it can be concluded that a rise of heart rate is accompanied by a decrease of overall variability.

LF_{norm} and HF_{norm}

Another method by which to characterize HRV is the calculation of the frequency domain parameters by power spectral density analysis. This method shows the distribution of power (variance) as a function of frequency (Malik, 1996). Two spectral components can be distinguished in short-term recordings (5 min). Firstly, a low-frequency component in the range > 0.04–0.116 Hz as a marker of sympathetic modulation (Lombardi et al., 1996) and secondly, a high-frequency component in the range of > 0.116–0.4 Hz as a marker of parasympathetic (vagal) modulation (Pomeranz et al., 1985). By calculation of LF_{norm} and HF_{norm} (normalization to exclude interindividual differences) it is possible to describe the influence of the autonomic nerve system at different experimental conditions on the pacemaker tissues (sinoatrial node) of the heart.

Our investigation showed the significant change of the influences of the two branches of the autonomic nerve system, comparing rest to exercise. During all exercise tests a significantly higher influence of the sympathetic nervous system (LF_{norm}) could be found and, in the same degree, a significant decrease in parasympathetic activity (HF_{norm}).

In the literature different responses of the two branches of the autonomic nervous system during exercise are descsribed. Perini et al. (1990) found no change of HF power, whereas the LF power decreased. The results of Nakamura et al. (1993), Warren et al. (1997) and Thayer et al. (1997) showed an exercise-induced increase of sympathetic and a decrease of parasympathetic influences.

In our investigation all of the chosen work loads led to a rising sympathetic and, to the same degree, a decreasing parasympathetic activity during exercise compared with rest.

% determinism, % recurrence and $ratio_{corr}$

The recurrence plot calculation is used to analyse non-linear components in heart rate. In our tests, when comparing rest to exercise, we found that the % recurrence, the percentage of the plot occupied by recurrent points (Webber and Zbilut, 1994) (quantitative measure) remained almost the same. In contrast to this, the % determinism (qualitative measure) clearly rose, which means that more recurrent points formed upward diagonal line segments (Webber and Zbilut, 1994). In other words, the degree of order in the recurrence plot increased with exercise.

The calculation of the ratio of these two parameters showed that the stationary state or stability of the heart rate rose with exercise. When walking or trotting on a dry treadmill (WD, TD) HRV was less stable (lower $ratio_{corr}$ value) compared to working at the same gait in water. According to this, walking in water above the elbow (WE) had a higher degree of stability than trotting on the dry treadmill (TD).

In conclusion, Aquatraining, following our training protocol represents a medium-sized aerobic work load for horses. The almost unchanged lactate level and the detected changes of the haemoglobin content, as a result of erythrocyte distribution out of the spleen, and the increased heart rate show that this kind of training does not lead to an overload of metabolism. Although the measured heart rate was not maximal, a marked change in HRV was found, demonstrated via an exercise-induced lower variance of NN-intervals around a mean value, rising sympathetic and decreasing parasympathetic activity and a higher degree of order and stability. However, it is probable that the observed changes in HRV could also be obtained by working on dry treadmills. Therefore, these changes must be considered as an expression of a higher demand on the performance of the organism rather than as a specialized outcome of Aquatraining.

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