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(Over)training effects on quantitative electromyography and muscle enzyme activities in standardbred horses

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Wijnberg ID, van Dam KG, de Graaf-Roelfsema E, Keizer HA, van Ginneken MM, Barneveld A, van Breda E, van der Kolk JH. (Over)training effects on quantitative electromyography and muscle enzyme activities in standardbred horses. *J Appl Physiol* 105: 1746–1753, 2008. First published October 2, 2008; doi:10.1152/jappphysiol.01272.2007.—Too intensive training may lead to overreaching or overtraining. To study whether quantitative needle electromyography (QEMG) is more sensitive to detect training (mal)adaptation than muscle enzyme activities, 12 standardbred geldings trained for 32 wk in age-, breed-, and sex-matched fixed pairs. After a habituation and normal training (NT) phase (*phases 1* and *2*, 4 and 18 wk, respectively), with increasing intensity and duration and frequency of training sessions, an intensified training (IT) group (*phase 3*, 6 wk) and a control group (which continued training as in the last week of *phase 2*) were formed. Thereafter, all horses entered a reduced training phase (*phase 4*, 4 wk). One hour before a standardized exercise test (SET; treadmill), QEMG analysis and biochemical enzyme activity were performed in muscle or in biopsies from vastus lateralis and pectoralis descendens muscle in order to identify causes of changes in exercise performance and eventual (mal)adaptation in skeletal muscle. NT resulted in a significant adaptation of QEMG parameters, whereas in muscle biopsies hexokinase activity was significantly decreased. Compared with NT controls, IT induced a stronger adaptation (e.g., higher amplitude, shorter duration, and fewer turns) in QEMG variables resembling potentially synchronization of individual motor unit fiber action potentials. Despite a 19% decrease in performance of the SET after IT, enzyme activities of 3-hydroxyacyl dehydrogenase and citrate synthase displayed similar increases in control and IT animals. We conclude that 1) QEMG analysis is a more sensitive tool to monitor training adaptation than muscle enzyme activities but does not discriminate between overreaching and normal training adaptations at this training level and 2) the decreased performance as noted in this study after IT originates most likely from a central (brain) rather than peripheral level.

equine; motor unit action potential analysis; training adaptation; muscle metabolism; enzymology; overreaching

THE AIM OF PHYSICAL TRAINING is to increase performance capacity by gradually increasing both duration and intensity of the exercise bouts. Each exercise session induces, dependent on the total training load, disturbances in cellular homeostasis. If the training load is balanced with the individual's exercise tolerance, regenerative processes will restore or increase athletic performance within a couple of days (35, 36, 39, 40). In

this situation adaptive processes result in, among others, increments in aerobic key enzymes in skeletal muscle (32) or excitability after endurance, strength, or sprint training (7, 23, 25, 28, 29). However, sustained vigorous training, often coupled with other stressors, may result in incomplete recovery, and decrement rather than improvement or maintenance of performance will occur. This process is termed overreaching, which might lead to the more severe chronic situation called overtraining (33, 40).

Since overtraining and, to a lesser extent, overreaching are severe conditions leading to decreases in performance capacity for several weeks to several months, this obviously necessitates descriptions of objective symptoms and/or finding objective parameters preceding those conditions. However, despite a host of overtraining research papers (5, 22, 26, 46), there has been little improvement in usable tools for the early detection of overreaching or overtraining (30, 54).

In human athletes, it has been shown that psychological changes (i.e., changes in mood and cognitive speed) occur as the first symptoms of unremittingly heavy training (20, 46), which implies an early involvement of the central nervous system.

In addition, there is ample evidence that contractile performance of working muscles is highly dependent on the ability of the muscle fibers to perform active Na^+/K^+ transport (8, 18, 45). Several studies have shown that muscle excitation and neuromuscular parameters change according to the training load (24, 25, 28) as well. An increase in muscle strength without noticeable hypertrophy is considered the result of neural involvement in acquisition of muscular strength (24, 25). Neural adaptations in skeletal muscle seem to play a greater role in the early phase of strength gain due to training than hypertrophy (29). One mechanism for increase in muscle force without hypertrophy is motor unit synchronization (24).

Previous electromyography (EMG) studies in horses indicated that the general principles of motor unit alterations as described in humans are applicable to horses (15, 44, 58, 62). Therefore, it is reasonable to assume that training effects as described in EMG in humans are also applicable to the horse. Motor unit action potential (MUP) amplitude is positively influenced by both hypertrophy and increased neuromuscular excitability, whereas MUP variables such as duration, phase, and turns are influenced by synchronization of the motor unit.

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An early sign of overreaching due to training is a deterioration of neuromuscular excitation, leading to low-amplitude EMG (38, 41). Therefore, quantitative EMG analysis is a potential tool for the early identification of stress due to an overload of training (overreaching). Additionally, quantitative EMG analysis is used to detect pathology in muscle or nerve (14, 59) in an early, subclinical stage enabling detection of pathology potentially relevant to elucidation of neuromuscular causes of decreased performance other than overreaching.

As a result of training-induced adaptation of neuromuscular excitability, measurement of biochemical adaptations in aerobic or anaerobic key enzymes in skeletal muscle can be expected, as well. Endurance training has been shown to increase maximal enzyme activities of carbohydrate and fatty acid metabolism, both in human (9–12, 32) and horse (13, 16, 27, 48). In addition, a recent study of Zoppi and Macedo (63) in rats showed a leveling off, rather than a further increase, after a 3-wk very high training load, probably due to increased oxidative stress.

The aim of the present study was to elucidate neuromuscular and biochemical adaptations in skeletal muscle induced by training and intensified training intended to induce overreaching. We hypothesize that quantitative EMG is a more sensitive or useful tool to detect changes in training status or overreaching than the activity of muscle enzymes.

To the best of our knowledge there are no previous published studies in which these two ways of adaptations are combined.

MATERIALS AND METHODS

Horses

Twelve standardbred geldings were used in this study. They were trained in two groups of six for logistical reasons. Per year, six horses were trained in three couples of two horses. Each test horse had its own matched control, and these formed a couple (pair) during the whole session. These individual couples underwent identical daily routines. Horses were aged 20 ± 2 mo at the beginning of the experiment and were owned by the Faculty of Veterinary Medicine of the University of Utrecht. The horses were individually housed, and their diet consisted of grass silage supplemented with concentrated feed and vitamin supplements and met nutrient requirements for maintenance and performance [58 MJ net energy (range 54–66)]. Salt blocks and water were available ad libitum. Weight and height were measured before each EMG and standardized exercise test (SET). All procedures were approved by the Institutional Animal Care and Medical Ethical Committee of Utrecht University and complied with the principles of laboratory animal care.

Training

All training sessions and exercise tests were performed on a high-speed treadmill (Mustang 2000, Graber). The training period consisted of a total of 32 wk divided into four phases. Each training session was preceded by a 30-min warm-up at the walking machine followed by an 8-min warm-up at the treadmill, which consisted of 4 min of walking at a speed of 1.6 m/s and 4 min of slow trotting at a speed of 3.0–4.0 m/s (no incline). Each training session ended with a cooldown that consisted of a 5-min walk at the treadmill followed by a 30-min walk at the walking machine. On the resting days the horses walked for 60 min at the walking machine.

To standardize training to the individual exercise capacity of the horses, training and exercise intensity were adapted to the maximal individual heart frequency. However, an incremental exercise test was

difficult to perform for the young, relatively untrained horses. In a previous study with 2- to 3-yr-old standardbred stallions (4), an average maximal heart frequency (HF_{max}) of 240 beats/min was obtained. This HF_{max} was used in this study as an estimated maximum ($HF_{est-max}$) to guide training intensity (speed and inclination) on the treadmill. Training intensity was adjusted on a weekly basis to the measured peak HFs (Polar S610i, Polar Electro, Kempele, Finland) during training sessions. The study was divided into four phases after accommodation to exercise. The training program was constructed as follows.

Phase 1 (habituation phase, 4 wk). Week 1 of phase 1 consisted of 3 times/wk exercise at 30% $HF_{est-max}$ for 20–30 min/session, week 2 of 4 times/wk exercise at 30% $HF_{est-max}$ for 25–45 min/session, week 3 of 4 times/wk exercise at 40% $HF_{est-max}$ for 30–45 min/session, and week 4 of 4 times/wk exercise at 50% $HF_{est-max}$ for 35–45 min/session.

Phase 2 (normal training phase, 18 wk). Training in phase 2 consisted of mixed endurance training (ET) and high-intensity interval training (HIT). Days of ET were alternated with HIT. An ET session consisted of continuous trotting for 20–24 min at 60% $HF_{est-max}$ or trotting for 16–18 min at 75% $HF_{est-max}$. Each HIT session contained either three 3-min bouts or four 2-min bouts of exercise at 80%–85% $HF_{est-max}$ interspersed by 3- or 2-min recovery bouts at 60% $HF_{est-max}$. Each training session ended with a cooldown consisting of a 5-min walk at the treadmill followed by a 30-min walk at the walking machine. The horses exercised 4 days/wk throughout this training period. We considered that in this phase training could be performed without excessive effort by the horses; therefore, we called this the normal training (NT) phase.

Phase 3 (intensified training phase, 6 wk). In phase 3, one horse of each couple was randomly selected and subjected to an intensified training (IT) program, whereas the other horse continued training at the volume, intensity, and frequency it received in phase 2. The IT regimen consisted of alternating days of HIT and ET as described in phase 2 for 6 days/wk during the first 3 wk. For the last 3 wk, horses were trained 7 days a week, HIT four times and ET three times. Exercise intensity during ET was gradually increased to 24–35 min at 60–75% $HF_{est-max}$. High-intensity exercise gradually increased to five 3-min bouts at 80–85% $HF_{est-max}$ interspersed with 2-min periods at 60% $HF_{est-max}$ or six 2-min bouts at 80–85% $HF_{est-max}$ interspersed with 1-min or 2-min periods at 60% $HF_{est-max}$. We anticipated that this training schedule was heavy for the animals.

Phase 4 (reduced training phase, 4 wk). In phase 4, all horses performed endurance training for 20 min at 60% $HF_{est-max}$ for 3 days and 70% $HF_{est-max}$ for 1 day a week. On rest days horses walked in a horse walker for 60 min.

Standardized Exercise Test

Four standardized exercise tests (SETs 1–4) were performed on the final day of each phase to monitor performance. The SET started with a 4-min warm-up period of walking at 1.5 m/s followed by 4 min of trotting at 4.5 m/s. Next, after 1 min of additional walking at 1.5 m/s, horses trotted for 20 min at $\sim 80\%$ $HF_{est-max}$. Finally, horses were allowed to cool down for 5 min at 1.5 m/s. Heart rate was measured with a Polar S610i heart rate meter and continuous ECG monitoring (Cardio Perfect Stress 4.0; Cardio Perfect, Atlanta, GA). In addition, during phases 1 and 2 similar time trials were repeated every 2 wk in order to adapt the training load.

Muscle Biopsies

Muscle biopsies were taken ~ 60 min before SETs 1, 2, 3, and 4. Left and right muscles were alternated, and at SETs 3 and 4 the former biopsy site was avoided. A 5-cm-deep biopsy of the vastus lateralis (VL) and a 4-cm-deep biopsy of the pectoralis descendens (PD) muscle were taken under local anesthesia [lidocaine hydrochloride (2%)] with a modified Barnstorm biopsy needle, specially developed

to obtain a greater tissue yield (Maastricht Instruments, Maastricht, The Netherlands) with a diameter of 7 mm. Blood and fat tissue were removed as fast as possible from the biopsy, and the biopsy was thereafter immediately frozen in liquid nitrogen for biochemical analysis. Frozen muscle tissue was stored at -80°C .

EMG Examination

At the end of each training phase, ~ 12 h before performance of the SET, quantitative EMG analysis was performed in the VL, PD, and subclavian (SC) muscles. Measuring left and right muscles was alternated and corresponded with the biopsy site. The depth of EMG sampling was 5 cm deep into the VL (i.e., on the same depth as the muscle biopsy was taken), 4 cm into the PD, and 2–3 cm into the SC. Details on definitions, materials, and methods of EMG examination can be found in former publications (58, 60). In brief, EMG signals were recorded with a portable apparatus (Nicolet Meridian, Nicolet Biomedical, Madison, WI) and concentric needle electrodes (Nicolet Biomedical). Band pass was between 5 and 10 kHz. Sweep speed was 10–20 ms/division. Amplifier gain was 50–100 μV for spontaneous activity and 10–500 μV for MUP recording.

A portable EMG apparatus (Nicolet Meridian, Nicolet Biomedical) was connected to a portable computer to record and store signals. A portable recorder was connected to the console to enable recording and storage of the complete EMG signal. Activity in resting muscle was recorded with an amplifier gain of 50 μV /division and a sweep speed of 20 ms/division. Filter settings were 5 and 10 kHz. For recording of MUP, amplifier gain was 100–500 μV /division, depending on the size of obtained MUP, and sweep speed was set at 20 ms/division.

Recordings were made with a disposable concentric 26-gauge EMG needle (length 50 mm, diameter 0.45 mm, sampling area 0.068 mm^2) for SC and PD muscles (Nicolet Biomedical). A concentric needle (length 100 mm, diameter 0.8 mm, sampling area 0.068 mm^2) was used for measurements of the larger VL muscle. A surgical pad attached to the horse with a girdle and connected to the preamplifier served as the ground electrode.

Insertional activity, pathological spontaneous activity (PSA), MUPs, and satellite potentials were recorded. PSA was assessed outside the end plate region in the same regions in which MUPs were obtained and included fibrillation potentials, positive sharp waves, complex repetitive discharges, and (neuro)myotonia. It was considered indicative of pathology if present in two or more locations. At least three insertions and three directions per insertion were made per investigated muscle at a level corresponding with muscle biopsy sites. EMG examination preceded taking biopsies. Former biopsies sites were avoided. The needle was redirected several times, and by selecting sharp-sounding MUPs while the needle was withdrawn with 3-mm increments sampling was performed throughout the muscle. MUPs were selected partly in a semiautomatic way, with a trigger line that selects identical MUPs above a chosen amplitude. Additional MUPs were selected manually off-line. From 20 to 30 MUPs per muscle, with a maximal rise time of 0.8 ms and identically firing at least four times amplitude, duration, number of phases, and number of turns were measured.

Biochemical Assays

For assessment of glycogen concentration, ~ 50 mg of frozen muscle tissue was freeze-dried overnight (16 h). Dry tissue was boiled in 1 N HCl for 3 h and equilibrated with 1 N NaOH. Samples were centrifuged and stored at -20°C . Glucose concentration in the supernatant was assayed with a commercially available glucose assay [Glucose (Hexokinase) Assay, Radiometer Nederland, Zoetermeer, The Netherlands] on an automated analyzer (Cobas Fara automated analyzer, Roche Diagnostics, Basel, Switzerland).

For enzymatic analysis, 50 mg of muscle tissue was homogenized in 1 ml of SET buffer (in mM: 250 sucrose, 2 EDTA, 10 Tris·HCl)

with an Ultraturrax homogenizer. Homogenates were subsequently sonicated three times and centrifuged at 15,000 g for 10 min. Supernatants were stored at -80°C until being analyzed. All chemicals used were of analytical grade.

Hexokinase (HK) was assayed according to a modified protocol from Bergmeyer (3). The assay mixture contained (in mM) 50 Tris, 1 glucose, 5 MgCl_2 , and 0.65 NADP, with 2 M ATP and 600 U/ml glucose-6-phosphate dehydrogenase. Appearance of NADPH was measured photometrically at 340 nm and 37°C on an automated analyzer.

Citrate synthase (CS) was assayed according to Shepherd and Garland (49). The assay mixture contained 100 μM 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), 50 μM acetyl-CoA, and 50 μM oxaloacetic acid. Appearance of DTNB-CoA was measured spectrophotometrically at 412 nm and 37°C on an automated analyzer.

Activity of 3-hydroxyacyl dehydrogenase (HAD) was assayed according to a modified protocol of Bergmeyer (3). Briefly, the amount of NAD^+ that was formed in the reaction acetoacetyl-CoA + $\text{NADH} \rightarrow 3\text{-hydroxybutyryl-CoA} + \text{NAD}^+$ was measured on an automated analyzer. The reagent we used was 10 mM NADH solution that was diluted 50 times in a tetrasodiumpyrophosphate (100 mM) buffer. Addition of 2 mM acetoacetyl-CoA started the reaction, and the formation of NAD^+ was measured spectrophotometrically at 340 nm and 37°C .

Statistical Analysis

The EMG data were statistically analyzed as described previously (58) with SPSS (SPSS, Chicago, IL) as the statistical program. Each horse had its own matched (age, breed, sex) control, and these were defined as a couple. The positively skewed data on MUP variables were transformed into natural logarithm (ln) to enable statistical analysis. To facilitate the interpretation, the mean ln transformed data are represented as geometric means (gmeans) that were derived from back-transformation of the ln values. Descriptive statistics were used to calculate gmean, SD, and 95% confidence interval (CI).

Univariate analysis was performed to determine differences between control and IT horses (group difference), horse couples, muscle, and time points (SET) and to determine whether group differences were the result of the (intensified) training (interactions). Contrast and post hoc testing according to Bonferroni of the transformed data were used to localize differences. For statistical analysis of all biochemical assays and physiological characteristics, one-way ANOVA tests were used. Post hoc tests according to Bonferroni were performed. Significance was set at 0.05, two-tailed.

RESULTS

Because of injury, two horses (1 control and 1 IT) were not able to complete the training. Analysis is therefore based on the remaining 10 horses. Body weight (392 ± 40 to 427 ± 44 kg) and height at withers (150.5 ± 7.4 to 154.6 ± 5.1 cm) increased significantly during the training period. There were no significant differences between the two training groups. Body temperatures were not different at the four SETs, between measurements of different muscles, or between the training groups.

SET

Adaptations of the SET were needed during the experimental period. In SET 1, it was observed that the horses were unable to trot at speeds and inclination corresponding to 80% $\text{HF}_{\text{est-max}}$. They had already started to gallop before they reached this frequency. Therefore, we decided to perform the time trials at the highest possible heart rate at which the horses were still able to trot. The average velocities, slopes, durations,

and heart rates during the SETs and all time trials are depicted in Fig. 1.

Regarding the IT group, horses maintained trotting at high speeds during SET 3 for 16.1 ± 2.3 min only, compared with 19.8 ± 0.4 min ($P = 0.012$) in control horses. As a consequence, the mean duration of trotting during SET 3 was decreased significantly by 19% in this group compared with control horses. These differences persisted during SET 4.

EMG Analysis

The insertional activity was not prolonged in both groups. All IT horses showed various types of PSA (fibrillation potentials, positive sharp waves, doublets of neuromyotonia) at some point during the training. At SET 2 PSA was present in two of five horses; at SET 3 and 4 PSA was present in three of five horses. PSA was present in four of five IT horses in the VL, in two of these five in the PD, and in no horses in the SC. Satellite potentials were present in both groups, but no significant differences were found between groups and the four SETs.

Natural logarithm of duration of MUP. The ln duration in the IT horses was higher than in the control horses ($P < 0.004$) during the training period (group effect) (Table 1). The ln duration at SET 1 was higher than at SETs 3 and 4 ($P < 0.0019$) in all muscles. The ln duration in the VL muscle was lower than in the SC muscle ($P < 0.004$). The effect of training was highest for the VL muscle ($P < 0.006$). The muscles showed different changes in time ($P < 0.006$). The effect of difference in training (interaction) was the highest at SETs 3 and 4 ($P < 0.03$). The ln duration was lowest at SET 3 in all muscles and increased at SET 4 in SC and VL.

Natural logarithm of amplitude of MUP. The group effect was significant, with lower amplitude for the IT group at SETs 1 and 2 and higher at SETs 3 and 4 ($P < 0.001$) (Table 1). At SET 1 the ln amplitude was lowest. The ln amplitude at SET 2 was higher than at SET 1 ($P < 0.019$) and higher than at SET 4 ($P < 0.002$). The ln amplitude was significantly highest ($P < 0.034$) at SETs 2 and 3 and decreased at SET 4 but was still higher at SET 4 than at SET 1 ($P < 0.034$). This effect was most obvious in VL and PD muscles ($P < 0.001$). The effect of the difference in training (interaction) was present in all

Table 1. Results of univariate analysis of turns: gmean, SD, and 95% CI of ln duration, amplitude, number of phases, and number of turns

Group	Variable	gmean	SD	95% CI gmean	
Control	Duration, ms	4.8	1.01	4.7–5.1	
	Amplitude, μV	217	1.01	210–225	
	No. of phases	2.2	1.01	2.2–2.3	
	No. of turns	2.5	1.01	2.5–2.6	
	Duration, ms*	5.0	1.01	4.9–5.2	
Test	Amplitude, μV^*	219	1.01	213–228	
	No. of phases*	2.3	1.01	2.3–2.4	
	No. of turns*	2.6	1.01	2.6–2.7	
	Duration, ms	5.1	1.01	5.0–5.3	
Time	1	Duration, ms	5.1	1.01	5.0–5.3
		Amplitude, μV	214	1.03	203–223
		No. of phases*	2.4	1.01	2.3–2.4
	2	No. of turns	2.7	1.01	2.6–2.7
		Duration, ms	5.1	1.01	4.9–5.3
		Amplitude, μV^*	230	1.03	219–242
	3	No. of phases	2.3	1.01	2.2–2.3
		No. of turns*	2.6	1.01	2.5–2.6
		Duration, ms*	4.6	1.01	4.5–5.2
	4	Amplitude, μV^*	218	1.02	208–229
		No. of phases	2.2	1.01	2.2–2.3
		No. of turns*	2.5	1.01	2.4–2.5
Duration, ms*		4.8	1.01	4.7–4.9	
Muscle	SC	Amplitude, μV	214	1.02	204–224
		No. of phases*	2.2	1.01	2.2–2.3
		No. of turns	2.6	1.01	2.5–2.6
		Duration, ms	5.0	1.01	4.9–5.2
	PD	Amplitude, μV^*	192	1.02	184–200
		No. of phases*	2.2	1.01	2.2–2.3
		No. of turns*	2.5	1.01	2.4–2.5
		Duration, ms	4.9	1.01	4.9–5.0
	VL	Amplitude, μV^*	238	1.02	226–248
		No. of phases	2.3	1.01	2.3–2.4
		No. of turns	2.6	1.01	2.5–2.7
		Duration, ms*	4.8	1.01	4.6–4.9
VL	Amplitude, μV^*	230	1.02	221–240	
	No. of phases	2.3	1.01	2.3–2.4	
	No. of turns	2.7	1.01	2.6–2.7	
	Duration, ms	4.8	1.01	4.6–4.9	

Five couples of 2 horses were divided into 2 groups (control and test horses). Per couple, 24–28 motor unit action potentials were included in the analysis. Test, intensified training group; gmean, geometric mean; CI, confidence interval; ln, natural logarithm. SC, subclavialis muscle; PD, pectoralis descendens muscle; VL, vastus lateralis muscle. *Significant differences were identified at this level, see text.

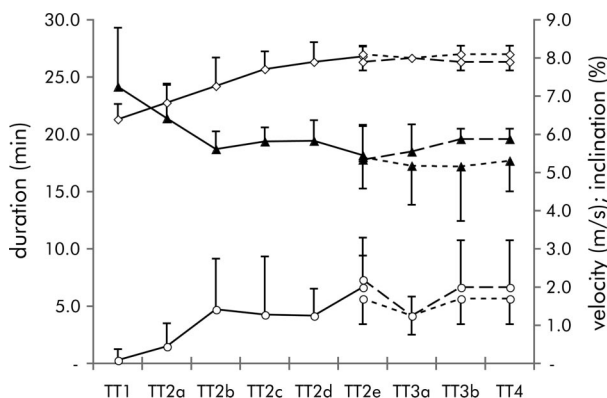


Fig. 1. Velocity, slope duration, and heart rate during time trials. Velocity, inclination, duration, and heart rate of consecutive time trials are presented as means \pm SD; a–e indicate time trials (TT) at certain time points within the 4 training phases (phases 1–4). Standardized exercise test (SETs) 1–4 are represented by TT1, TT2e, TT3b, and TT4. Solid lines represent phases 1 and 2; long-dashed lines represent normal training group in phases 3 and 4; short-dashed lines represent intensified training group in phases 3 and 4.

three muscles ($P < 0.02$) and not the same for all three muscles ($P < 0.002$). The ln amplitude was significantly highest at SET 2 ($P < 0.002$), with a difference between the control and IT groups. In the IT group, the ln amplitude was not only higher at SET 2 but also increased further at SET 3 and decreased at SET 4 in PD and VL muscle, whereas in the control group the amplitude at SET 3 decreased.

Natural logarithm of phase of MUP. The group effect was significant, with a higher ln phase in the IT group ($P < 0.007$) during the training period (Table 1). The ln phase at SET 1 was higher than at SET 2 ($P < 0.025$), SET 3 ($P < 0.001$), and SET 4 ($P < 0.035$). The ln phase in the SC muscle was lower than in the PD ($P < 0.002$) and VL ($P < 0.0015$) muscles. An effect of difference in training (interaction) was not present.

Natural logarithm of number of turns of MUP. The ln number of turns (ln turn) was significantly higher in the IT

group ($P < 0.006$) during the whole training period (group effect) (Table 1). The ln turn in the SC muscle was lower than in the PD ($P < 0.001$) and VL ($P < 0.0001$) muscles, with the lowest value at SET 2. There was an effect of difference in training during the whole period, with a lower number of turns at SET 3 in VL and PD muscles ($P < 0.019$) in the IT group.

Semiquantitative and quantitative EMG analysis indicated abnormally high MUP variables in both horses in one of five couples. The values of MUP variables had characteristics corresponding with neuropathic MUPs according to definitions determined in former studies in warmblood horses (2, 59, 60).

Biochemical Assays

A significant decrease in HK activity ($P < 0.05$) after 18 wk of combined interval and endurance training was observed in VL muscle (differences between SET 1 and SET 2) (Fig. 2). After *phase 3* (SET 3), however, both in the IT group and the control group, HK was significantly ($P < 0.05$) increased compared with SET 2, with no differences among groups. No changes were observed compared with SET 1. In PD muscle, no training or growth effects were observed.

IT, as applied in *phase 3* of this study, resulted in a significant ($P < 0.05$) increase in CS activity compared with control training in VL muscle (Fig. 3). After SET 4, however, CS activity was significantly ($P < 0.05$) increased in control animals and not in the IT group. In PD muscle, no changes in CS activity were observed.

In the IT group, HAD activity was significantly ($P < 0.05$) increased at the end of *phases 3* and *4* in both VL and PD muscles (Fig. 4). In the control group, HAD activity also increased ($P < 0.05$) in VL and PD muscles after SET 4 as well.

DISCUSSION

The aim of the present study was to elucidate neuromuscular and biochemical adaptations in skeletal muscle to training and intensified training aimed to induce a state of overreaching. Each test horse (the IT horse) had its own matched control, and they therefore formed a couple (pair) in order to minimize potential variations by, for example, growth, season of the year, and climate.

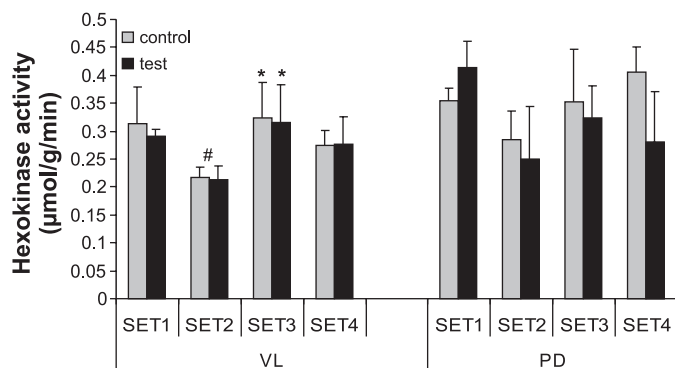


Fig. 2. Hexokinase activity at the different SETs. Hexokinase activity was significantly decreased in vastus lateralis (VL) muscle after normal training ($\#P < 0.05$). A significant increase ($*P < 0.05$) in hexokinase activity was observed between SET 2 and SET 3 in intensified training (test) and control groups. No group differences were observed. PD, pectoralis descendens.

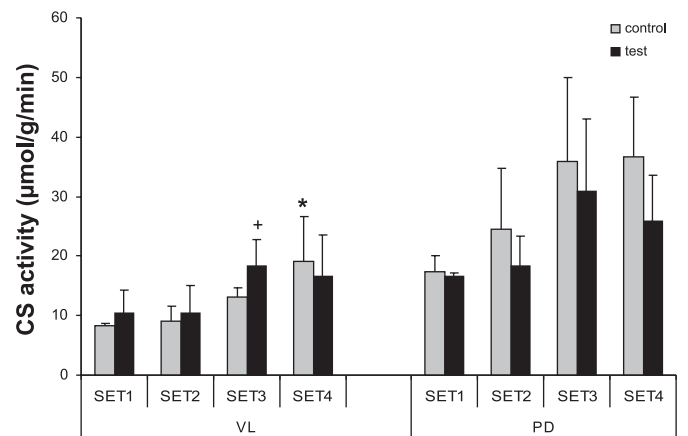


Fig. 3. Citrate synthase (CS) activity at different SETs. CS activity in VL muscle was significantly ($*P < 0.05$) increased at SET 4 compared with SET 2 in the control group. A significant ($+P < 0.05$) difference was apparent between control and intensified training groups in VL muscle at SET 3. No significant differences were observed in PD muscle.

The results of the present study showed stagnation in the development of performance in the IT group compared with the control group (difference 19%). From these results, we concluded that the IT group was overreached. In contrast, enzyme activities in VL and PD muscles showed more or less expected training responses, albeit that unaltered CS and HAD and decreased HK activities (the latter only in VL muscle) after *phase 2* (NT) were an unexpected finding. After *phase 3*, however, CS and HAD activity were only increased in the IT group, whereas HK activity returned to pretraining levels in both groups.

Our data corroborate those of Tyler et al. (52, 53) and McGowan et al. (43). These investigators found a decrease in performance after overload training but a normal increase in muscle CS and HAD activity (43) and unaffected mitochondrial volume (53). Other evidence for an absence of a relation between performance and aerobic muscle enzyme activity was reported by Roneus and Essen-Gustavsson (47). These authors biopsied 2-yr-old standardbred trotters before and after a

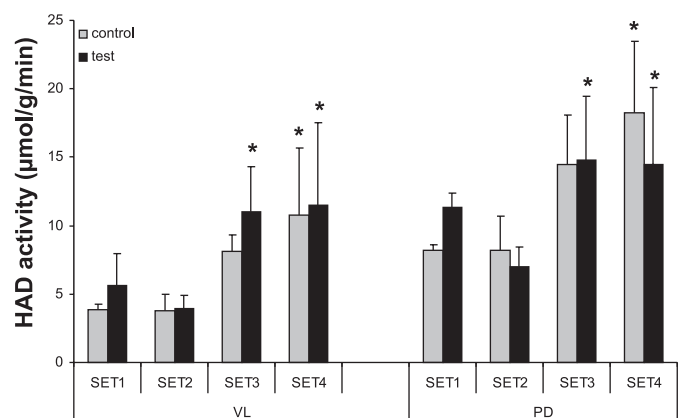


Fig. 4. 3-Hydroxyacyl dehydrogenase (HAD) activity in VL and PD muscles at SETs 1, 2, 3 and 4. No effects of training were observed between SET 1 and SET 2. In the VL of the intensified training group, HAD activity was significantly ($*P < 0.05$) increased after the intensified training period and was also increased at SET 4 in the control group. A similar image was observed in PD muscle.

1,600-m run and found a positive correlation between trotting speed and intramuscular glucose-6-phosphate but not HK and HAD activities. These findings, together with those of the present study, exclude an aerobic metabolic cause of decreased performance.

Other possible metabolic causes of decreased performance include a decreased muscle glycogen level. Lacombe et al. (37) reported that decreased performance was related to lower muscle glycogen levels, whereas maximal oxygen uptake was unaffected by the preceding training. However, in the present study the decrease in performance after IT could not be addressed to lower muscle glycogen levels (data not shown).

The training protocol used in this study resulted in increased MUP amplitude indicative of recruitment of larger motor units and synchronization due to training. The significant lower duration and lower number of phases and number of turns recorded in the trained horses reflect synchronization of the individual muscle fiber action potentials contributing to the MUP (14, 51). A period of IT resulted in an increase in training-associated adaptations in skeletal muscles. No evidence of adverse adaptation could be observed after the period of IT (SET 3). The potentially neuropathic abnormalities found in one couple might have contributed to small disturbances in locomotion observed in these horses, which could not be explained by orthopedic abnormalities.

An increase of muscle force as a result of training can be, among others, the result of increased hypertrophy of muscle fibers and/or motor unit synchronization (25, 41). Thus the significant increase in MUP amplitude at SET 2 compared with SET 1 in the VL and PD muscles is most likely the result of an increase in muscle fiber diameter, recruitment of larger (type II) motor units, and increased synchronization of the muscle fiber action potentials. However, fiber diameters were not increased according to the results of the six horses trained in the first year of the study (56). Therefore, the higher amplitude can be interpreted as recruitment of larger motor units and synchronization due to training (25, 28, 41).

Studies concerning the use of neuromuscular excitability to recognize overtraining in athletes reported that an increase in neural stimulus was required to produce a single contraction of reference muscle in overtrained athletes (38). The significant lower duration and lower number of phases and turns at SET 3 compared with SET 2 in both control and IT horses can be interpreted as synchronization of the individual muscle fiber action potentials contributing to the MUP. Only the duration of the MUP was significantly lower in IT compared with control horses, thereby making overreaching due to training less obvious since an early sign of overreaching due to training is a deterioration of neuromuscular excitation leading to low-amplitude EMG. Studies concerning the use of neuromuscular excitability to recognize overtraining in athletes reported that an increase in neural stimulus was required to produce a single contraction of reference muscle in overtrained athletes (38, 41). A reduction in MUP duration can be the result of motor unit synchronization. Motor unit synchronization is a possible mechanism for increase in muscle strength due to training (23). As an alternative explanation, myopathy can lead to a reduction in duration in MUPs (14, 60). The increased presence of neuropathic and myopathic PSA did indicate presence of increased membrane instability in the IT horses, but without evidence of decrease in amplitude myopathy it is a less likely

explanation. Whether increased membrane instability in IT horses that showed decreased performance is a result of this level of too much training or overreaching is not clear. Factors such as body temperature, intramuscular temperature, and growth in general can also influence MUP variables (6, 19). However, these variables were not different among the groups, especially not since the test horses formed fixed pairs with their age-matched controls. Therefore seasonal temperature and growth do not account for the differences found between the groups at SET 3.

In addition, it is interesting to see that the 95% CI of the amplitude and duration in standardbred horses are slightly higher than those measured in ~18-mo-old nonexercised Dutch warmblood horses in a former study (60). This might be explained by the potential effect of the higher workload of the standardbred horses on muscle fiber characteristics such as diameter or, alternatively, breed difference.

Increased motor unit synchronization in this period was accompanied by increases in CS, HAD, and HK activity, as observed previously after endurance training (17, 27, 31). Only CS activity was significantly increased in the IT horses compared with control horses. These increases in enzymatic activity can be explained by the fact that the IT horses recruited more muscle force as a training adaptation according to the EMG. Therefore, both EMG and biochemical results in this study indicate an effect of training, in control and IT horses, rather than evidence of overreaching or overtraining. The limited number of biochemical adaptations, in combination with the observed changes in MUP parameters, indicates that needle EMG analysis is potentially a sensitive tool in monitoring effects of training in equine skeletal muscle.

In this study we analyzed different muscles (EMG: VL, PD, and SC; biochemistry: VL and PD) because EMG values and biochemical enzyme activities can vary significantly between muscles (6, 16, 34, 61). Muscle biopsies were not taken from the SC because of its rather difficult access and small size for needle biopsies. The differences between muscles observed in this study might be a function of differences in muscle fiber type. Muscle fiber type influences all MUP variables (19, 51, 58), and recruitment of larger type II motor units results in high-amplitude MUPs (14, 58). Additionally, type I and IIa fibers usually have higher mitochondrial density and higher oxidative metabolism compared with type IIb muscle fibers.

Quantitative EMG data indicate that the training effect is lowest for the SC muscle and highest for the VL muscle. This is to be expected in light of the different role these muscles play in propulsion, with the most important role for the VL muscle. The fact that the SC muscle had the lowest values of the majority of MUP variables is in agreement with former studies on MUP analysis in horses and is explained by its higher percentage of type I fibers. The values of the VL and PD muscles were less different from each other. This might reflect the fact that the VL muscle contained an unexpectedly high percentage of type I fibers in the horses used in the present study (55, 57). Previous studies in different horse breeds showed a high abundance of type II muscle fibers in VL muscles (1, 42, 50). Differences in muscle function and fiber type distribution may also explain the differences in biochemical properties between VL and PD muscles (1, 42). The effects of training on CS and HK in the PD muscle follow the same

trend as in the VL muscle; however, in the PD muscle these activities showed a large SD, resulting in a lack of significance.

In conclusion, in this study we used quantitative EMG and biochemical analysis of skeletal muscle enzymes to evaluate changes induced by training and IT. Only the EMG analysis showed clear signs of training adaptation after 18 wk of NT, whereas biochemical assays were less sensitive for training-induced adaptations. Although its suitability for detecting early signs of too much training stress remains to be established, EMG analysis provides a useful tool for the identification of small improvements in fitness in horses. The increased presence of PSA in some animals of the IT group suggests that this might be the most sensitive tool to detect early changes. However, the results of the present study also indicate that overreaching does not originate at the peripheral level. We hypothesize that the decreased performance after the IT phase is at first caused by central fatigue, as has been reported recently in human athletes (20, 21, 46). This hypothesis remains to be proven in future behavioral and neuroendocrine studies in horses.

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