J Appl Physiol 102: 1871–1882, 2007.
First published January 25, 2007; doi:10.1152/japplphysiol.01093.2006.

Effects of intensity and duration of exercise on muscular responses to training of thoroughbred racehorses

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Submitted 29 September 2006; accepted in final form 18 January 2007

Rivero JL, Ruz A, Martí-Korff S, Estepa JC, Aguilera-Tejero E, Werkman J, Sobotta M, Lindner A. Effects of intensity and duration of exercise on muscular responses to training of thoroughbred racehorses. *J Appl Physiol* 102: 1871–1882, 2007. First published January 25, 2007; doi:10.1152/japplphysiol.01093.2006.—This study examined the effects of the intensity and duration of exercise on the nature and magnitude of training adaptations in muscle of adolescent (2–3 yr old) racehorses. Six thoroughbreds that had been pretrained for 2 mo performed six consecutive conditioning programs of varying lactate-guided intensities [velocities eliciting blood lactate concentrations of 2.5 mmol/l (v2.5) and 4 mmol/l (v4), respectively] and durations (5, 15, 25 min). Pre- and posttraining gluteus muscle biopsies were analyzed for myosin heavy chain content, fiber-type composition, fiber size, capillarization, and fiber histochemical oxidative and glycolytic capabilities. Although training adaptations were similar in nature, they varied greatly in magnitude among the different training protocols. Overall, the use of v4 as the exercise intensity for 25 min elicited the most consistent training adaptations in muscle, whereas the minimal training stimulus that evoked any significant change was identified with exercises of 15 min at v2.5. Within this range, muscular adaptations showed significant trends to be proportional to the exercise load of specific training programs. Taken together, these data suggest that muscular adaptations to training in horses occur on a continuum that is based on the exercise intensity and duration of training. The practical implications of this study are that exercises for 15 to 25 min/day at velocities between v2.5 and v4 can improve in the short term (3 wk) the muscular stamina in thoroughbreds. However, exercises of 5–15 min at v4 are necessary to enhance muscular features related to strength (hypertrophy).

skeletal muscle fibers; myosin heavy chain; muscle fiber types; blood lactate; equine muscle physiology

THE THOROUGHBRED RACEHORSE is an extraordinary (supreme) athlete that can run at high speeds (18 m/s, 65 km/h), maintained for ~1–2 min over distances of 800–1,500 m. This superior athletic ability is attributable to a number of physiological adaptations: its high maximal aerobic capacity, the ability to increase oxygen-carrying capacity of blood at the onset of exercise through splenic contraction, its increased muscle bulk, large intramuscular stores of energy substrates (glycogen in particular), high mitochondrial volume in muscle, high proportion of fast-twitch muscle fibers, and efficiency of gait (for recent review, see 11). Probably because of these unique physiological features, training thoroughbred racehorses is much more difficult than training endurance and other racehorses. In practice, a substantial part of the training of thoroughbreds would appear to involve short distance and submaximal exercises (~50–70% of top speed) because of serious concern for musculoskeletal system damage (7). However, this stimulus is rather low to recruit the glycolytic type IIAX and type IIX muscle fibers that have the fastest speed of contraction (33). In this breed, these fibers represent ~40–50% of the fiber-type composition in propulsive muscles (33; present results), and they are considered as a pool of reserve that is only recruited during maximal (or supramaximal) exercise intensities (21, 33).

Recent years have seen the publication of various experimental studies focused on muscle plasticity by using well-documented exercise training protocols in both thoroughbreds and other racehorses (6, 14, 17, 26, 31. 33). Despite most of these studies being carried out under noncomparable experimental conditions, it seems from comparative analysis of their results that high-intensity exercise [~80–110% of maximal oxygen uptake ($\dot{V}O_{2\,max}$)] maintained for ~5–10 min is essential to improve muscular features related to both stamina and strength. However, despite the extant literature describing muscular adaptations to training in thoroughbreds and other racehorses, little is still known about the durations and intensities of exercise that promote optimal response in skeletal muscles. For example, only a few studies have compared muscular adaptations that occur after different training programs with different exercise intensities (5, 29) or examined the combined effect of intensity and duration of the exercise (9). As a consequence, results from many of these experimental studies are of little, if any, application in practice.

The present study describes the relative contribution of exercise intensity and duration on the short-term muscular responses to training in adolescent (2–3 yr old) thoroughbreds after a brief period (2 mo) of basic training. The conventional approach to investigating the response to different doses of a treatment is to perform a repeated-measures study in which each subject receives all of the different doses. We have used this approach in the present study in which the same six horses performed, in a randomized six × six latin square design, six consecutive conditioning programs of varying lactate-guided intensities [i.e., exercise velocities that result in blood lactate concentrations of 2.5 (v2.5) and 4 mmol/l (v4), respectively] and durations (5, 15, and 25 min). Each conditioning program

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lasted 22 days and was followed by a 10-day resting period between each two consecutive programs to prevent (or reduce) the long-lasting effects of a given dose of training. A part of the present full project (data concerning myosin heavy chain composition) has preliminarily been reported as a contribution to the 7th International Conference on Equine Exercise Physiology (27), but the present statistical analyses represent a substantial extension of these previously published data.

METHODS

Experimental Design and Procedures

Animals. Six clinically healthy thoroughbred racehorses (4 mares and 2 geldings) were used. Four horses were 2 yr old, whereas the other two were 3 yr old at the beginning of the study. The two 3-yr-old horses had been racing before, but they were out of racing training for at least 4 months before the start the experiment. The 2-yr-old horses had not been in race training; two of them had not been ridden before and two had been broken in. All were pasture-kept horses before the study. The study was approved by the Ethical Committee of Baden-Württemberg (Germany). Horses were kept in 3 × 3 m boxes at night and during the day were turned out to pasture. They were fed daily with a concentrate of 4.5 kg plus 5 kg of silage (in a relation 2:1 grass to corn silage and split into 2 feedings). Hay, straw, and water were always available. The mean body weight of the horses was 454 ± 23 kg (mean ± SD) at the beginning of the study and 465 ± 29 kg at the end.

All the exercise tests and exercise workouts were done on a high-speed treadmill (Kagra AG Mustang 2200) at 6% incline. Horses were acclimated to exercise on the treadmill for the 2 mo before starting the specific trial (acclimatization plus basic training period). In the first of these 2 mo, horses were trained to canter on the treadmill 2–4 min/day every second day, while in the second month they were submitted to exercises at speeds up to 8 m/s at 6% incline for up to 25 min/day every second day to resemble as much as possible the experimental period thereafter.

Experimental period. The experiment consisted of a randomized six × six latin square design, in which horses performed six consecutive conditioning programs of varying lactate-guided intensities and durations (Fig. 1). Horses were exercised at their individual v2.5 or v4 [derived mathematically when run under well-defined conditions (34)] for 5, 15, or 25 min in each consecutive conditioning program. Each program lasted 22 days and consisted of 11 exercise sessions once a day every second day and followed by a 10-day resting period between consecutive programs. Thus the experimental period of the study lasted ~6 mo in total.

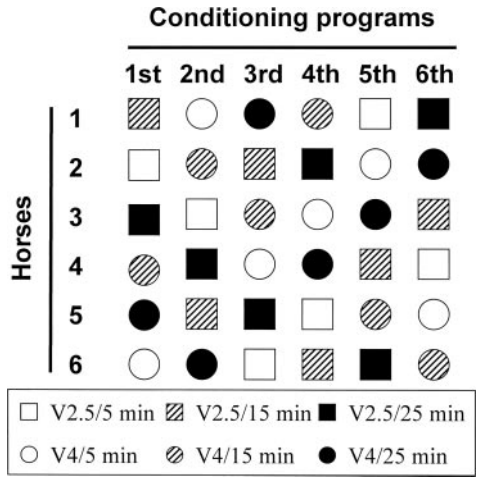

Fig. 1. Study design with the order in which each individual horse was assigned to each conditioning program with exercises of different intensities [velocities eliciting blood lactate concentrations of 2.5 mmol/l (v2.5) and 4 mmol/l (v4), respectively] and durations (5, 15, and 25 min).

Standardized exercise test. Before and after each conditioning program, horses performed a standardized exercise test to determine their individual v2.5 and v4. The test consisted of a warm-up of 10 min at between 1.5 and 4 m/s followed by several exercise workouts of 5-min duration each. Between two consecutive steps there was a resting period of 1 min. The velocity in the first step was 6 m/s, and each consecutive step was increased by 0.5 m/s. The test finished when the horses' blood lactate concentration was above 4 mmol/l. Blood lactate analysis was done in situ with test-strip BM-lactate (Accusport, Boehringer-Mannheim) on whole blood samples. At the beginning of the test, but after warm-up, and immediately after each step, blood samples were collected from the external jugular vein to measure blood lactate concentration. The parameters v2.5 and v4 were derived from the blood lactate-running speed relationship by exponential regression equation.

Muscle biopsy sampling procedure. Before and after the 2-mo acclimatization period and again before and after each conditioning program, two percutaneous needle biopsies were obtained at depths of 20 mm (superficial region) and 60 mm (deep region) below the gluteal fascia, through the same incision, from the gluteus medius muscle of each horse, according to Lindholm and Piehl (15). This muscle was selected as it has been shown to be active at all exercise intensities (15). To avoid excessive damage due to the impact of repeated biopsying on muscle tissue, muscle biopsies were taken alternatively from the right or the left muscle between each two consecutive conditioning programs. All muscle biopsies were collected at rest before each exercise test and always by the same investigator, experienced in equine biopsy studies, taking care to standardize the location and depth of the sample.

After collection, muscle samples weighing 75–100 mg were mounted on corks with the use of OCT embedding medium (Tissue-Tek II, Miles Laboratories, Naperville, IL) and oriented so that myofibers could be cut transversely. Specimens were systematically frozen by immersion in isopentane (30 s), kept at the freezing point in liquid nitrogen, and stored at −80°C until analyzed. A total of 168 muscle biopsies [7 experimental stages (6 conditioning programs plus the acclimatization and basic training period) × 2 time point (pre- and posttraining) × 2 sampling depths × 6 horses] was available for biochemical and morphological analyses.

Laboratory Analyses

Myosin heavy chain electrophoresis. Myosin heavy chain (MHC) electrophoresis was performed following the SDS-PAGE protocol previously described and validated for horse muscle (24). A standard sample of adult rat costal diaphragm muscle (Sigma, St. Louis, Mo) was included in gels and used as a control, which has been shown to contain four different MHC isoforms identified as types I, IIa, IIx, and IIb. Several cross-sections of 25 μm thickness were obtained from each biopsy sample on a cryostat and placed in precooled (−20°C) microcentrifuge tubes. This muscle fraction was homogenized in a 1:100 dilution in Tris buffer (62.5 mM Tris, pH 6.8). Polyacrylamide gels were comprised of 8% separating gel with 30% (wt/vol) glycerol and 4% stacking gel with 30% glycerol. Aliquots of diluted myofibrillar proteins, which contained 5 μg of protein, were electrophoresed for 22 h at 285 V and 4°C in a large-gel apparatus (SE600, 18 × 16 cm; Hoefer Pharmacia Biotech, San Francisco, CA). Subsequently, separating gels were stained with Coomassie blue stain (50% vol/vol methanol, 10% vol/vol glacial acetic acid, 0.06% wt/vol Coomassie blue R250) for 2 h at 45°C and destained in methanol (10% vol/vol) and glacial acetic acid (10% vol/vol) until defined bands could be detected. Three bands in the gels were identified as MHCs-I, -IIx, and -IIa going from the fastest to the slowest migrating band (Fig. 2), as described by Ref. 23. Gels were then scanned with a video-scanning

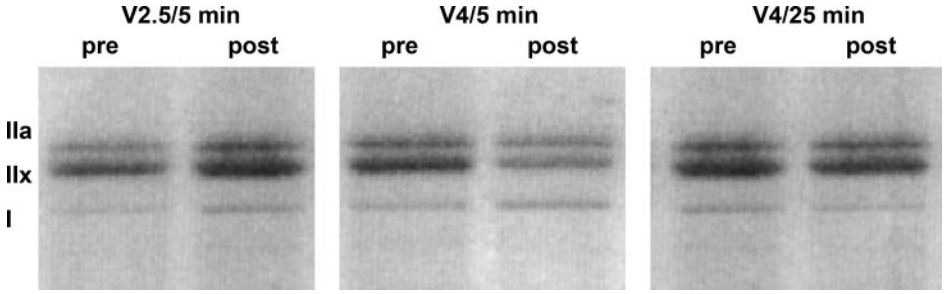

Fig. 2. SDS-PAGE gels of deep gluteus medius muscle biopsy samples from a representative subject in 3 different conditioning programs showing myosin heavy chain (MHC) isoform separation before (pre) and after (post) each exercise training. Note the decrease in the band corresponding to MHC-IIx from pre- to post-training for the 2 subjects exercised at v4.

densitometric system (GS-700, Bio-Rad, Hercules, CA), and a quantification of each MHC isoform was obtained in relative terms for each muscle biopsy specimen through the relative MHC protein isoform optical density (OD) derived with a specific imaging analysis software (Multi-Analyst version 1.0, Bio-Rad).

Immunocytochemistry. Muscle biopsy samples were cross-sectioned serially in a cryostat for immunocytochemistry, quantitative histochemistry, and histology (Fig. 3). Immunocytochemistry was performed with four monoclonal antibodies specific to MHC isoforms: BA-D5 (DMS, Braunschweig, Germany; anti-MHC-I), SC-71 (DMS; anti-MHC-IIa), BF-35 (DMS; anti-MHCs-I plus -IIa), and S5-8H2 (Biocytex Biotechnology, Marseille, France; anti-MHCs-I plus -IIx) (Fig. 3, *A–D*). The specificity of these antibodies for MHCs in horse skeletal muscle has previously been reported (23, 25). The immunoperoxidase staining protocol with avidin-biotin complex (ABC) protocol, as previously described in horse muscle (23), was used. Briefly, sections 10 μm in thickness were preincubated in a blocking solution of stock goat serum. The primary antibody was applied for 30 min at 37°C. The optimum working dilution of each primary antibody was determined to obtain a completely white background in negative fibers. After incubation, the sections were washed and then reacted for 30 min with a secondary antibody (biotinylated goat anti-mouse IgG; code no. E0433, Dako). Sections were washed again and reacted for 1 h in the absence of light with ABC reagent. The immunocomplexes were visualized by incubating the sections for ~8 min in a 0.05 M Tris·HCl buffer solution containing 0.7 mg/ml diaminobenzidine tetrahydrochloride and 0.01% H_2O_2. After being stained, slides were soaked for 10 min in distilled water, dehydrated in graded ethanol series, cleared in xylol, and coverslipped with DPX mountant for microscopy (BDH Laboratory Supplies, Poole, UK).

Quantitative histochemistry of individual muscle fibers. The activity of the enzyme succinate dehydrogenase (SDH, EC 1.3.5.1), used as an oxidative marker, was determined on 10-μm-thick sections by a quantitative histochemical method previously adjusted and validated in horse muscle (20) (Fig. 3*E*). Briefly, three consecutive sections were stained in a medium containing (in mM) 100 potassium phosphate buffer (pH 7.6), 1.0 methoxyphenazine methosulfate, 0.75 NaN_3, 1.5 nitroblue tetrazolium, 5 EDTA, and 10 succinic acid for 10 min at 25°C. Two other sections were incubated in the same medium without substrate (succinic acid) and served as reaction controls.

The histochemical activity of the enzyme glycerol-3-phosphate-dehydrogenase (formerly, α-glycerophosphate dehydrogenase, GPDH) was used as an indirect marker for glycolytic potential of myofibers, since GPDH activity correlates with the activities of other glycolytic enzymes (17). Nevertheless, it is not known whether this histochemical method stains for the activity of the NAD-dependent GPDH (EC 1.1.1.8) or the mitochondrial FAD-dependent GPDH (EC 1.1.99.5). Neither GPDH is directly involved in the glycolytic pathway; however, both are directly involved in the transfer of NADH from glycolysis in the cytosol into $FADH_2$ in the mitochondria of skeletal muscle. The same histochemical procedure has previously been used and validated in horse muscle (20) (Fig. 3*F*). Three 16-μm-thick sections were stained in a medium containing (in mM) 100 potassium buffer (pH 7.4), 0.02 methoxyphenazine methosulfate, 1 NaN_3, 1.2 nitroblue tetrazolium, and 6.3 α-glycerophosphate for 35 min at 37 °C,

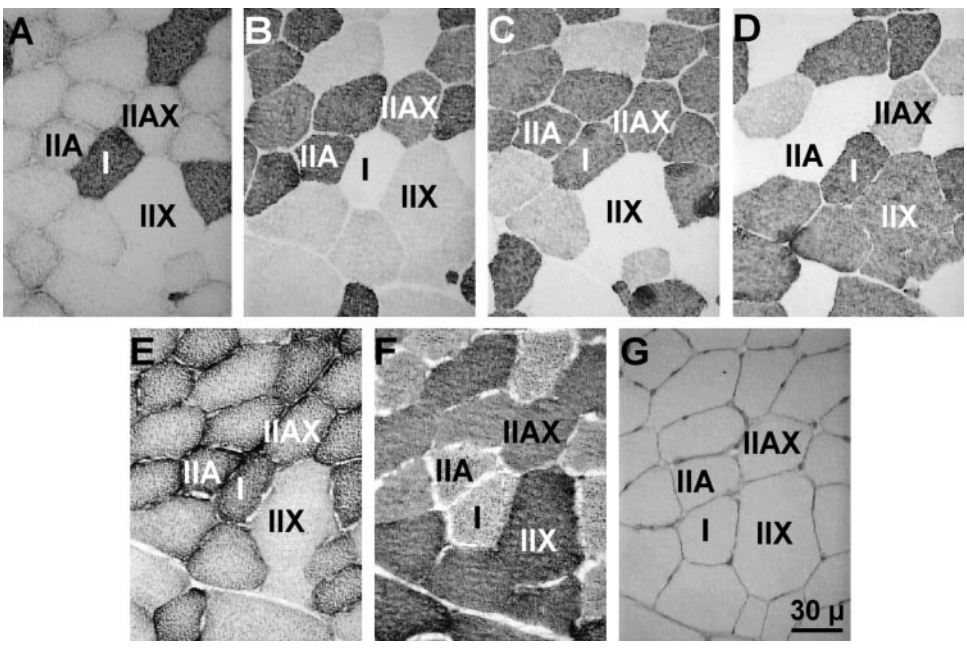

Fig. 3. Serial frozen sections of gluteus medius muscle from a representative horse stained for immunocytochemistry and enzyme histochemistry. *A–D*: sections were stained with monoclonal antibodies against specific MHC isoforms: BA-D5 (*A*; anti-MHC-I), SC-71 (*B*; anti-MHC-IIa), BF-35 (*C*; anti-MHCs-I and -IIa), and S5–8H2 (*D*; anti-MHCs-I and -IIx). Other sections were stained for quantitative histochemistry of succinate dehydrogenase (*E*) and glycerol-3-phosphate dehydrogenase (*F*). *G*: α-amylase periodic acid-Schiff for visualizing capillaries. The 4 MHC-based muscle fibers are labeled in all serial sections. Four main fiber types were then categorized: 3 of them as fibers expressing a unique MHC isoform (i.e., type I, IIA, and IIX fibers), and the 4th as a hybrid phenotype coexpressing MHCs-IIa and -IIx (i.e., type IIAX fibers). Bar, 30 μm.

and two sections were incubated in the same medium without substrate (α-glycerophosphate) and served as reaction controls.

An additional 14-μm-thick section was incubated for 60 min at 37°C in a 2.2% solution of α-amylase and then stained with a routine periodic-acid-Schiff technique by using a 1% solution of periodic-acid for 5 min at 37°C (1). This section was used to visualize and quantify capillaries surrounding each fiber type (Fig. 3*G*).

Image analysis and morphometry. All serial sections were visualized and digitized within 2–3 h after staining by using a Leica DMLS microscope (Leica Microsistemas, Barcelona, Spain), a Leica high-resolution (1 200 dpi) charge-coupled device camera (Leica Microsistemas), an eight-bit Matrox Meteor frame-grabber (Matrox Electronic Systems, Barcelona, Spain), and an image-analyzing software (Visilog 5, Noemi, Microptic, Barcelona, Spain). All serial sections were carefully surveyed to find regions free of artifacts, and a region containing ~40–80 fibers was selected for further analyses. Images were saved as digitized images at 256 gray levels. The gray levels were converted to OD units by using a calibrated set of OD filters. The digitized images of the fibers in both histochemical reactions (SDH and GPDH) within the selected region were traced manually and analyzed for fiber cross-sectional area (CSA) and the average OD for each histochemical reaction. The average fiber OD for each histochemical reaction was determined as the average OD for all pixels within the traced fiber from the section incubated with substrate minus the average OD for all pixels of the same fiber from the sections incubated without substrate. The mean OD measurement reflects the specific enzymatic activity per unit fiber volume (i.e., enzyme concentration) because each pixel represents a standardized fiber volume (pixel length × width × section thickness) (17). Based on a previous study (20), only coefficients of variation for triplicate measurements of OD below 5% were accepted in the present study; this demonstrates the high degree of analytical precision that can be achieved for the measurement of fiber OD on histochemical reactions. As the linearity of these quantitative histochemical reactions with respect to incubation time has already been verified in horse muscle (20), termination of the reactions before these points allowed the expression of enzyme activities as steady-state rates in OD per minute. Individual CSA and OD of myofibers were averaged according to the MHC fiber type (see below).

The number of capillaries around each fiber in the selected area of the sample was also obtained from the α-amylase-PAS staining and expressed in absolute terms of number of capillaries in contact with each specific fiber type.

The fibers in the selected area were classified according to their MHC content by means of visual examination (positive or negative) of immunostainings of the four serial sections stained with anti-MHC monoclonal antibodies (Fig. 3). Four main fiber types were then categorized, three of them as pure fibers expressing a unique MHC isoform (i.e., type I, IIA, and IIX fibers), and the fourth as a hybrid phenotype coexpressing MHCs-IIa and -IIx (i.e., type IIAX fibers) (25). To establish muscle fiber-type composition of each muscle biopsy specimen, at least 250 muscle fibers were identified in each muscle biopsy specimen.

Statistical Analysis

All statistics were run on Statistica 6.0 for windows (Statistical Solutions, Sugus, MA). All data throughout are expressed as means ± SD, with the exception of figures, where data are presented as means ± SE for clarity. Statistical analysis for each dependent variable was accomplished using a separate two-way ANOVA with repeated measures [7 × 2 design: 7 training conditions (1 basic and 6 specific training programs) × 2 time points (pre- and posttraining)]. Another 2 × 3 (2 exercise intensities × 3 exercise durations) ANOVA was run separately with the set of samples collected before and after the six conditioning programs to compare the fixed effects of these two factors on the dependent measures. Sampling depth was considered as a covariate in these analyses. When a significant F ratio was achieved ($P < 0.05$), post hoc comparisons were accomplished via a Fisher's least significant differences test. For clarity of difference between specific conditioning programs, all data were converted to a percent change between pre- and posttraining measurements, and the training program was converted to a variable that ranged from −1 for the shortest and lowest intensity exercise (i.e., 5 min at v2.5) to +1 for the longest and highest intensity protocol (i.e., 25 min at v4). The data were then modeled as polynomial functions of the rank-ordered volume (duration × intensity × no. of sessions) of the conditioning programs. Relationships between changes in variables and total volume of the training protocols were expressed as Pearson correlation coefficients.

RESULTS

Exercise Tests

All horses completed valid v4 tests before and after each specific training program from the beginning to the end of the study. When pretraining values were compared with posttraining values, all specific conditioning protocols induced no significant ($P > 0.05$) increase in v4, with the exception of a significant increase (4.8%, $P < 0.05$) after the training protocol with exercises of high intensity and long duration (v4 for 25 min; Fig. 4*A*). Nevertheless, improvements of v4 after training

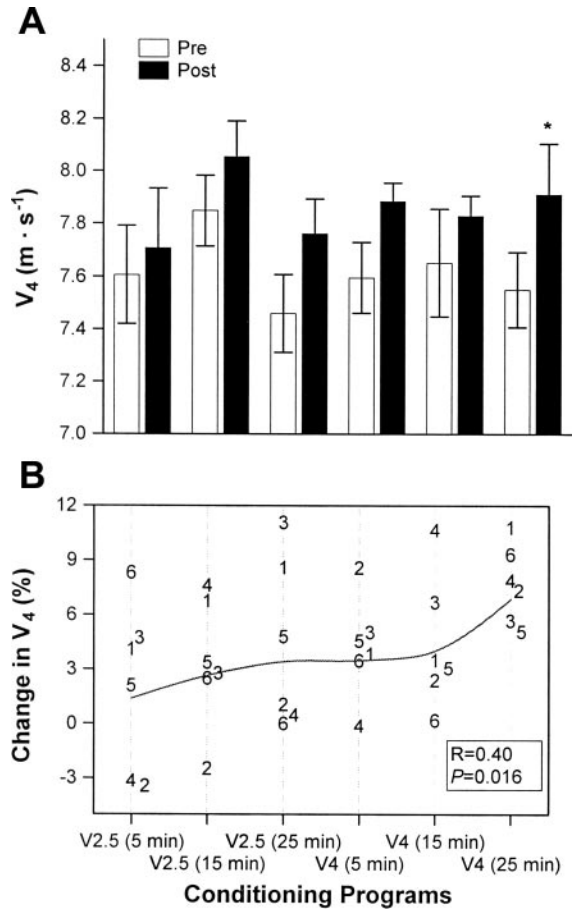

Fig. 4. *A*: effect of 6 conditioning programs of different intensity and duration of exercise training on v4; values are means ± SE; $n = 6$. *Significant difference between pre- and posttraining measurements ($P < 0.05$). *B*: individual percentage change in v4 after the 6 conditioning programs; horses are identified *1–6* according to Fig. 1; line denotes distance weighting of the data. *R*, Pearson correlation coefficient.

showed a substantive, statistically significant trend to increase as a function of the average volume of the total work accomplished (intensity × duration × no of sessions) for each specific conditioning program (Fig. 4*B*).

Effect of Sampling Depth on Muscle Variables

As expected, when superficial measurements were compared with deep measurements, significant differences were found in some muscle variables examined (results not shown). Overall, these variations were identical in pattern to those previously reported in the horses gluteus medius muscle (see, for example, Refs. 22, 28). Nevertheless, sampling depth did not influence significantly the degree of muscular responses to training since, when superficial sampling sites were compared with deep sampling sites, no significant differences ($P > 0.05$) were found in percent changes after training in any of the muscular traits examined in the present study. In consequence, data in Tables 1–6 are presented as pooled means for both sampling depths.

MHC Composition

No significant change was found in MHC content after the 2-mo basic training period (Table 1). Afterward, when pretraining values were compared collectively with posttraining values for all specific training groups, training increased significantly the fraction of MHC-IIa and decreased that of MHC-IIx (~5.6% in both) but did not affect the percentage of MHC-I (Table 1). In general, no significant differences in MHC composition were found either among the six specific training programs before training or between these groups and baseline values before the basic training period. In the set of posttraining samples, exercise intensity effect on MHC-IIa and MHC-IIx isoforms was highly significant, whereas exercise duration did not influence significantly the MHC composition. Thus horses that had been exercised at v4 had a higher fraction of MHC-IIa (6.9%, $P < 0.01$) and a lower percentage of MHC-IIx (8.0%, $P < 0.05$) than those that had been worked at v2.5. In consequence, when specific conditioning programs were compared, the greatest significant training effect on MHC composition was observed in the v4/25-min specific conditioning program (Table 1). Nevertheless, after all specific training programs, the IIa-to-IIx MHC isoform ratio showed a statistically significant trend to increase with increasing volume of the total work accomplished in each specific training program (Fig. 5).

MHC Muscle Fiber-Type Distribution

When pretraining values were compared with posttraining values, no significant change was found in muscle fiber-type distribution after 2 mo of basic training (Table 2). When the six specific conditioning programs were examined collectively, training increased significantly the percentage of type IIA (5.3%) and type IIAX (23.8%) fibers and decreased very significantly that of IIX muscle fibers (17.1%, Table 2). Both exercise intensity and exercise duration fixed effects were highly significant on the amplitude of the posttraining IIX muscle fiber decline. This variable was 11.8% lower ($P < 0.001$) after specific training programs with exercises at v4 than after exercise training protocols at v2.5 (Table 2). This myofiber percentage was also significantly lower after conditioning protocols with exercise sessions of 25 min than those with sessions of only 15-min (9.6%, $P < 0.05$) or 5-min (13.5%, $P < 0.01$) duration. The percent change in the relative frequency of IIX fibers was significantly higher in horses that had been conditioned with exercises at v4 for 25 min than in those exercise-trained at v2.5 for 5 and 15 min (Fig. 6*A*). More interestingly, the type IIA-to-IIX fiber ratio (frequently used as a global representation of training-linked myofiber transitions) showed in relative terms a significant trend to increase as a function of the combined effect of both intensity and duration of the exercise training (Fig. 6*B*). As a consequence, the longer the exercise duration and the higher the exercise intensity for a given specific conditioning program, the greater percent change there was in IIA-to-IIX muscle fiber ratio.

Muscle Fiber CSA

The 2-mo progressive basic training program caused an increase in the CSA of the four major fiber types by approxi-

Table 1. *Combined effect of intensity and duration of exercise training on percent MHC isoform expression*

Training Condition	MHC-I, %		MHC-IIa, %		MHC-IIx, %	
	Pre	Post	Pre	Post	Pre	Post
Basic training	8.5±3.0	7.6±2.7	38.6±3.8[a]	41.3±4.8[a]	52.8±6.2	51.1±6.8[a,b]
Specific training programs v2.5						
5 min	7.1±3.8	7.7±4.2	40.7±3.7[a,b]	40.9±4.4[a]	52.4±6.5	51.5±7.7[a]
15 min	8.4±3.1	9.8±3.8	40.6±4.3[a,b]	41.6±4.6[a]	51.0±7.0	48.7±6.4[a,b]
25 min	8.7±3.8	8.7±4.2	40.4±3.6[a,b]	43.2±5.6[a]	50.9±5.4	48.0±8.4[a,b]
Specific training programs v4						
5 min	10.0±4.9	8.8±4.7	40.7±2.9[a,b]	44.4±4.9[a,b,*]	49.3±6.3	46.8±7.4[a,b]
15 min	8.9±5.0	10.3±4.1	42.8±4.2[b]	44.2±4.3[a,b]	48.3±7.1	45.5±7.9[a,b]
25 min	9.0±4.1	9.8±4.4	41.5±4.0[a,b]	46.4±4.3[b,‡]	49.5±6.1	43.8±7.9[a,†]
All specific training groups (n = 72 samples)	8.7±4.1	9.2±4.2	41.1±3.8	43.4±4.9†	50.2±6.3	47.4±7.8*

Values are means ± SD; n = 12. MHC-I, -IIa, and -IIx, myosin heavy chain isoforms; v2.5 and v4, exercise velocities that result in blood lactate concentrations of 2.5 and 4 mmol/l, respectively; 5, 15, and 25 min are exercise durations; Pre and Post, before and after exercise training, respectively. Significantly different from corresponding pretraining value: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Within a column, means with different lowercase letters are statistically significant ($P < 0.05$).

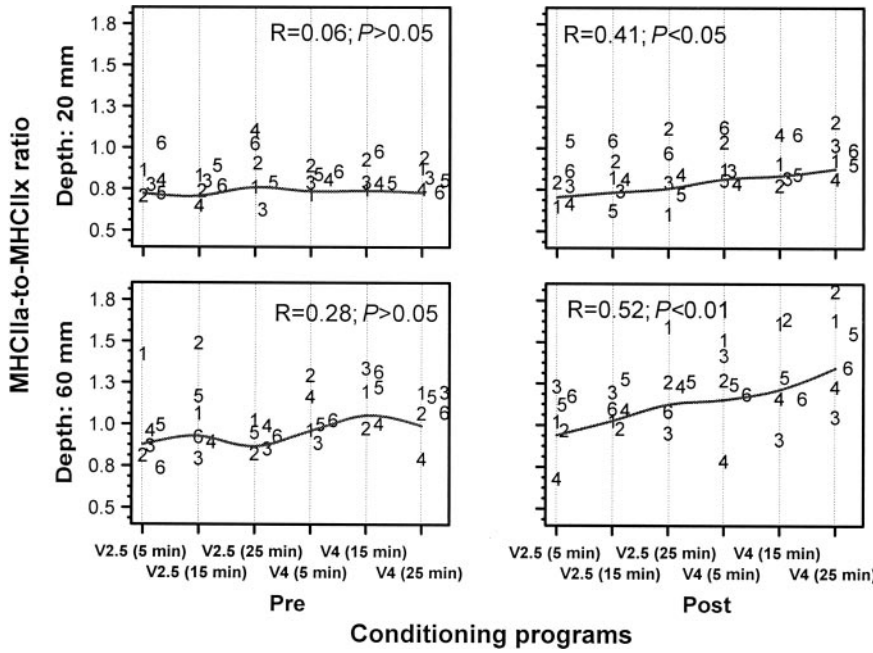

Fig. 5. Individual values in MHC-IIa-to-IIx ratio at the 2 muscle sampling depths before (Pre) and after (Post) the 6 conditioning programs of different intensities and durations; horses are identified *1–6* according to Fig. 1; lines denote trend of the data.

mately one-third of corresponding pretraining values (Table 3). For all of the six specific conditioning programs, the CSAs of all four muscle fiber types increased significantly after training by ~12% (Table 3). A selective, significant myofiber hypertrophic effect of either intensity or duration of the exercise training was not found in the present experiment. However, when the pretraining values were compared with the posttraining values for each conditioning program, a significant hypertrophic effect was only observed after all three training protocols completed with exercise sessions at v4, and for only the fast-glycolytic IIAX and IIX muscle fiber types (Table 3). In relative terms, the exercise training accomplished at high intensity (v4) for middle duration (15 min) caused the greatest hypertrophic response of these two muscle fiber types (Fig. 7*A*). Once more, improvements in mean CSAs of IIAX and IIX myofiber types showed a moderate significant trend to increase as a function of the combined influence of intensity and duration of the exercise training (Fig. 7*B*).

Muscle Fiber-Type Capillaries

A significant increase in the mean number of capillaries in contact with types I, IIA, and IIAX muscle fibers (range 11–15%) was observed after the basic training period (Table 4). When all the 6 specific conditioning programs were analyzed as a whole, there was a very significant increase in the mean number of capillaries in contact with all muscle fiber types (range 8.3–10.1%, Table 4). Exercise intensity fixed effect increased significantly the capillarization of type IIA fibers (by 6.4% from v2.5 to v4 exercise training protocols, $P < 0.05$). A selective fixed effect of exercise duration on capillarization was observed after specific training for only type I fibers (by increasing 8.2% from 15 to 25 min of exercise duration, $P < 0.05$; and 12.2% from 5 to 25 min, $P < 0.01$). Exercise intensity and duration fixed effects on capillaries surrounding IIX fibers were also statistically significant ($P < 0.01$), increasing ~10% from exercise training protocols at v2.5 to v4, as

Table 2. *Combined effect of intensity and duration of exercise training on muscle fiber-type composition*

	Type I, %		Type IIA, %		Type IIAX, %		Type IIX, %	
Training Condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Basic training	12.6±2.6	12.0±3.4[a,b]	40.1±5.1	41.3±4.2	12.6±5.2	15.3±3.9[a,b]	34.7±4.2	31.4±5.1[a]
Specific training programs								
v.2.5								
5 min	10.6±3.7	10.3±4.7[a]	41.8±4.2	42.5±5.3	13.4±4.5	15.1±6.3[a,b]	34.3±3.9	32.1±4.5[a]
15 min	12.1±3.5	13.3±4.1[a,b]	40.4±4.2	41.8±3.7	12.8±4.3	14.5±3.8[a]	34.7±5.6	30.4±3.9[a,*]
25 min	12.3±4.4	11.6±4.0[a,b]	39.4±5.4	43.0±6.7	13.9±5.8	16.7±8.4[a,b]	34.5±4.6	28.7±3.5[a,b,†]
v4								
5 min	11.7±4.6	11.8±5.3[a,b]	42.6±3.6	45.0±6.0	11.7±5.5	14.5±5.4[a,b]	34.1±3.5	28.7±4.1[a,b,†]
15 min	11.3±4.0	13.8±4.6[b]	42.0±4.3	44.5±5.2	12.6±5.6	13.9±6.2[a]	34.1±3.7	27.8±4.7[a,b,‡]
25 min	11.3±4.9	13.1±5.6[a,b]	41.7±5.8	44.3±6.0	11.5±6.3	18.9±7.4[b,†]	35.5±3.5	23.8±5.5[b,‡]
All specific training groups (n = 72 samples)	11.5±4.1	12.3±4.8	41.3±4.6	43.5±5.5*	12.6±5.4	15.6±6.4†	34.5±4.1	28.6±5.0‡

Values are means ± SD; n = 12. Type I, IIA, and IIAX are muscle fiber types. Significantly different from corresponding pretraining value: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Within a column, means with different lowercase letters are statistically significant ($P < 0.05$).

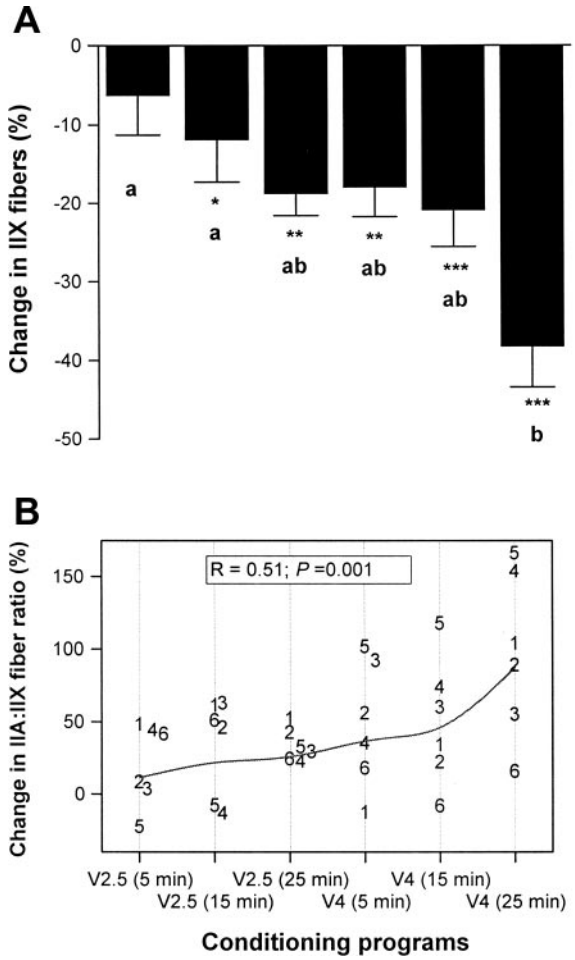

Fig. 6. *A*: percent change in type IIX muscle fibers after 6 conditioning programs of different intensity and duration of exercise training. Values are means ± SE; $n = 12$. Significant effect of the conditioning program: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; means with different lowercase letters are statistically different ($P < 0.05$ at least). *B*: individual percent change in type IIA-to-IIX muscle fiber ratio after the 6 conditioning programs; each data point denotes average of the 2 muscle sampling depths for each horse; horses are identified *1–6* according to Fig. 1. Line denotes trend of the data.

well as from protocols with exercise sessions of either 5-min or 15-min duration to training programs with exercises of 25 min. In consequence, the specific training program designed with exercise sessions at v4 for 25 min caused the greatest increases in capillarization of all muscle fiber types. In relative terms, the overall capillary-to-fiber ratio, used as a global representation of muscle capillary supply, increased significantly after all four conditioning regimens with exercises of either 15-min or 25-min duration but did not change significantly after the two protocols based on exercises of only 5-min duration (Fig. 8).

Muscle Fiber-Type SDH and GPDH Histochemical Activities

With the exception of a significant increase for SDH activity of type I fibers (~10.8%), no other statistically significant changes in SDH and GPDH histochemical activities of muscle fiber types were found after the basic training period (Tables 5 and 6). Overall, specific training protocols increased significantly mean SDH histochemical activities of types I, IIA, and IIAX fibers (range 4.2–7.1%) but did not affect that of pure IIX fibers (Table 5). A significant fixed effect of exercise duration ($P < 0.05$) on SDH activity of type I fibers was found, increasing approximately 8.5% from training programs with exercises of 15-min duration to protocols with sessions of 25-min duration. No other statistically significant global effects of either intensity or duration of exercise training on myofiber SDH activity were observed. Again, with the exception of a significant increase after training for SDH activity of type IIA fibers for the v2.5/25-min specific training program, the most substantive and significant posttraining increase for muscle fiber-type SDH activity occurred in the specific training protocol based on exercises accomplished at v4 for 25 min (Table 5).

Specific training programs increased significantly the GPDH histochemical activity of type IIX muscle fibers (~6% on average), but after each training protocol was analyzed separately, a significant increase was only recorded for the v4/25-min conditioning program (14%, Table 6). No other statistically significant effects of either intensity or duration of exercise training on this muscular feature were found.

Table 3. *Combined effect of intensity and duration of exercise training on muscle fiber-type CSA*

	CSA, μm²							
	Type I		Type IIA		Type IIAX		Type IIX	
Training Condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Basic training	1,512±346	1,944±438*	1,829±488	2,372±578*	2,251±649	3,140±707[b,‡]	3,593±844	4,242±613*
Specific training programs								
v2.5								
5 min	1,500±502	1,751±496	1,705±585	2,117±519	2,213±757	2,620±726[a]	3,555±997	3,669±637
15 min	1,775±617	1,956±617	2,042±589	2,308±740	2,713±662	2,819±708[a,b]	3,686±550	3,958±928
25 min	1,731±547	1,963±529	2,116±570	2,177±615	2,611±625	2,894±498[a,b]	3,710±863	3,838±738
v4								
5 min	1,892±562	1,872±361	2,003±312	2,235±572	2,211±325	2,763±521[a,b,*]	3,326±594	3,860±679*
15 min	1,743±483	2,109±434	2,151±417	2,537±500	2,383±503	3,149±520[b,†]	3,480±782	4,126±525*
25 min	1,762±365	2,033±691	2,119±665	2,323±560	2,243±606	2,905±815[a,b,*]	3,298±646	3,799±672*
All specific training groups ($n = 72$ samples)	1,734±514	1,947±526*	2,023±539	2,283±585†	2,396±608	2,858±641‡	3,509±746	3,875±696†

Values are means ± SD; $n = 12$. CSA, cross-sectional area. Significantly different from corresponding pretraining value: $*P < 0.05$, $†P < 0.01$, $‡P < 0.001$. Within a column, means with different lowercase letters are statistically significant ($P < 0.05$).

DISCUSSION

In the present study, we used for the first time in horses a new approach to the problem of studying the effect of different types of training on physiological variables relevant to physical performance by combining intensities and durations of exercises. With the experimental approach used, in which each horse received all the different doses (by combining exercise intensities and durations) of a treatment (specific training for 3 wk), the data can be best analyzed by modeling trends in the response to the different doses with appropriate mathematical functions of the dose (13). The resulting function of best fit allows prediction of a maximum and minimum response, the dose that produces these responses, the range of doses that give substantial or significant responses, and so on. A practical limitation of this approach (with the use of the same set of subjects) for studies of athletic training is that the long-lasting effects of a given dose of training could prevent subjects from receiving more than one dose of the treatment (30). The randomized latin square design used in the present study tried, however, to minimize this inconvenience from a triple perspective. First, in this design each subject serves as its own control (i.e., comparing pre- and posttraining measurements in each specific training program). Second, the order of specific conditioning programs for each subject was strictly randomized, and care was taken that not a single order occurred two consecutive times (Fig. 1). Third, the resting period inserted in between each two consecutive training programs results in partial or complete normalization of pretraining muscular features (28). In fact, with the exception of a few cases, most of the pretraining muscular values reported in the present study did not vary significantly among the different training groups, including here statistical comparisons against baseline values, before the basic training period (see Tables 1–6). Furthermore, when muscle characteristics were compared throughout the full experimental period (6 mo) for all groups, a statistically significant time or age effect was not found (results not shown). In consequence, it seems clear that carryover effects of previous training stimulus have not influenced significantly the muscular response to each specific training program accomplished in the present study.

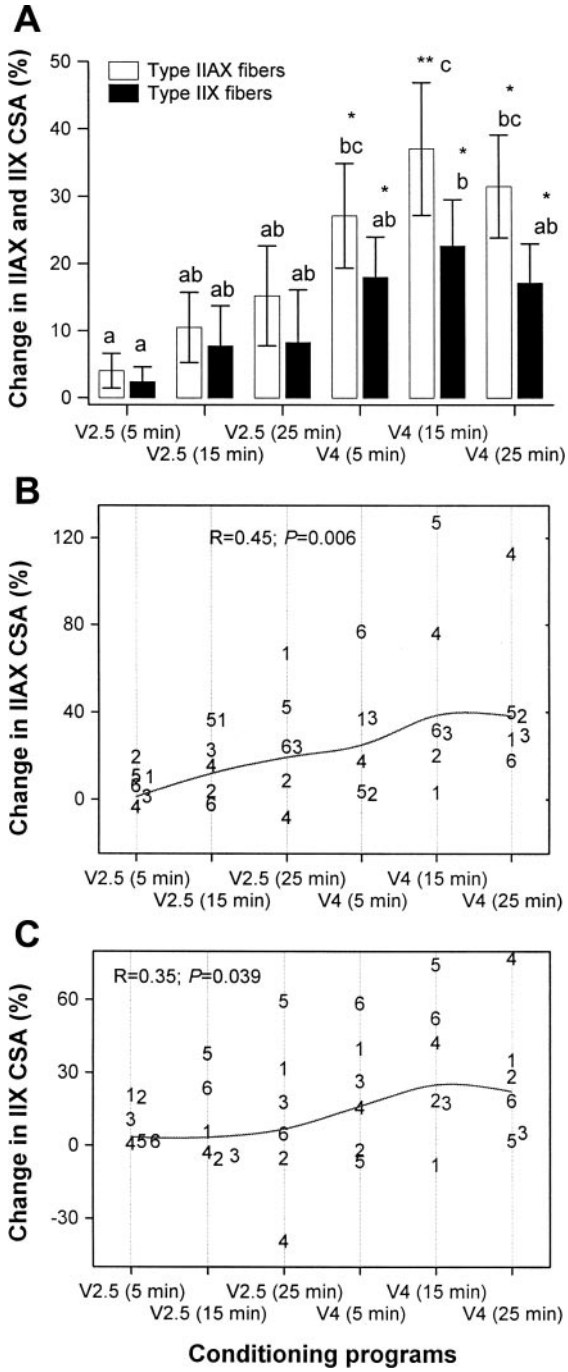

Fig. 7. *A*: percent change in mean cross-sectional area (CSA) of fast glycolytic type IIAX and IIX muscle fibers after 6 different conditioning programs of different intensity and duration of exercise training. Values are means ± SE; $n = 12$. Significant effect of this particular conditioning program on fiber size: $^{*}P < 0.05$, $^{**}P < 0.01$; means with different lowercase letters are statistically different ($P < 0.05$ at least). *B* and *C*: individual percent change in mean CSA of type IIAX (*B*) and type IIX (*C*) after the 6 conditioning programs; each data point denotes average of the 2 sampling depths for each particular horse. Horses are identified *1–6* according to Fig. 1; lines denote trends of the data.

An important finding of the present study was the significant relationship observed between training load and the subsequent improvement in v4 (Fig. 4*B*). This relationship predicted a maximum enhancement in performance after 11 work bouts (every second day) with an intensity equivalent to v4 and 25-min duration. The enhancement of ~5% observed in v4 after this specific training agrees well with previous findings of similar training studies in thoroughbreds (5, 8, 34). In agreement with two previous studies of horses exercising at either above or below the lactate threshold over a constant load training (5, 8), metabolic responses of the horses were independent of the exercise intensity during training. In contrast, in a study of human subjects, athletes exercising at the higher intensities (above the lactate threshold) demonstrated a greater improvement in indicators of metabolic capacity than subjects exercising at lower intensities (10). As training stimulus used in the present study might well have resulted differently from those used in previous studies (5, 8), the present results lend support to the idea that metabolic adaptations to training occur on a continuum that is based on the combined effect of intensity and duration of the exercise training. Thus duration of exercise at high speed seems to be a principal contributor for this metabolic adaptation of blood lactate concentration.

Similar to previous training studies in racehorses (see Ref. 21 for a review), the present study found significant training-

Table 4. *Combined effect of intensity and duration of exercise training on mean number of capillaries per fiber type*

Training Condition	Mean No. of Capillaries Per Fiber Type							
	Type I		Type IIA		Type IIAX		Type IIX	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Basic training	4.5±0.5[a,b]	5.1±0.6[a,*]	5.0±0.4[a,b]	5.5±0.4[a,b,*]	5.0±0.3[a,b]	5.8±0.5[a,b,†]	5.6±0.4[a,b]	6.2±0.6[a]
Specific training programs								
v2.5								
5 min	4.3±0.7[a]	4.7±0.5[a]	4.7±0.6[a]	5.1±0.5[a]	4.8±0.7[a]	5.4±0.5[a,*]	5.2±0.5[a]	5.7±0.6[a]
15 min	4.9±0.7[b]	5.1±0.5[a]	5.1±0.5[a,b]	5.3±0.5[a]	5.4±0.5[b]	5.4±0.5[a]	5.8±0.7[b]	5.8±0.8[a]
25 min	4.6±0.5[a,b]	5.0±0.7[a]	5.0±0.5[a,b]	5.4±0.7[a,b]	5.1±0.3[a,b]	5.4±0.9[a]	5.4±0.6[a,b]	6.1±0.8[a,*]
v4								
5 min	4.9±0.6[b]	4.9±0.6[a]	5.2±0.6[b]	5.6±0.5[a,b]	5.3±0.6[b]	5.8±0.6[a,b,*]	5.7±0.6[a,b]	6.0±0.5[a]
15 min	4.6±0.7[a,b]	4.9±0.6[a]	5.2±0.7[b]	5.4±0.7[a,b]	5.3±0.7[a,b]	5.6±0.5[a,b]	5.7±0.8[a,b]	6.1±0.5[a]
25 min	4.6±0.6[a,b]	5.8±0.9[b,‡]	4.9±0.5[a,b]	5.9±0.7[b,‡]	5.2±0.6[a,b]	6.1±0.8[b,†]	5.5±0.8[a,b]	7.0±1.1[b,‡]
All specific training groups (n = 72 samples)	4.6±0.6	5.1±0.7‡	5.0±0.6	5.5±0.6‡	5.2±0.6	5.6±0.7‡	5.6±0.7	6.1±0.8‡

Values are means ± SD; n = 12. Significantly different from corresponding pretraining value: *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$. Within a column, means with different lowercase letters are statistically significant ($P < 0.05$).

induced MHC-isoforms and/or fiber-type conversions within the fast population in the direction of IIX to IIA. Advantages of the present study compared with previous work in this area include *1*) that these adaptations occurred after only 3 wk of specific training in already basically trained racehorses, and *2*) that we have compared in the same set of subjects the effects of different types of well-defined training programs. Of the six different protocols employed, the use of v4 as the exercise intensity for 25 min elicited the most consistent conversion of MHC-isoform and/or fiber-type content. The minimum training stimulus eliciting significant adaptations in any of these variables was obtained with the use of v2.5 as the exercise intensity for 15 min (Table 2, Fig. 6*A*). Within this range, the extent of training-induced changes in MHC and/or fiber-type composition was proportional to the volume of total work accomplished in each specific training program (Figs. 5 and 6*B*).

The short-term training-induced upregulation of MHC-IIa and downregulation of MHC-IIx found in the present study seems to be more dependent on the intensity than the duration of exercise during training. This finding agrees with the results of a previous study reporting significant muscular adaptations to training in thoroughbreds exercised at ~80% of $\dot{V}O_{2\,max}$ but not in horses worked at ~40% of $\dot{V}O_{2\,max}$ (29). As the degree of exercise load was constant in both groups of horses, the authors of this latter study (29) concluded that to achieve a significant training effect on muscle fiber composition, the total work accomplished is not the critical factor; rather, increasing exercise intensity may be necessary. However, the present results also imply that this prevalent effect of exercise intensity on MHC/fiber-type adaptation to training is modulated by the concurrent effect of the duration of exercise sessions. For example, for a given exercise intensity, exercises for up to 25 min/day are more effective on this adaptation than shorter (5 or 15 min) exercise sessions (see Fig. 6*A*). Together, not only exercise intensity but also exercise duration can interact as two important parameters in the extent of the short-term training-induced regulation of MHC/fiber-type expression. In practice, this means that the total exercise load accomplished during training may therefore be the critical factor in determining the degree of this local adaptation to training in thoroughbreds.

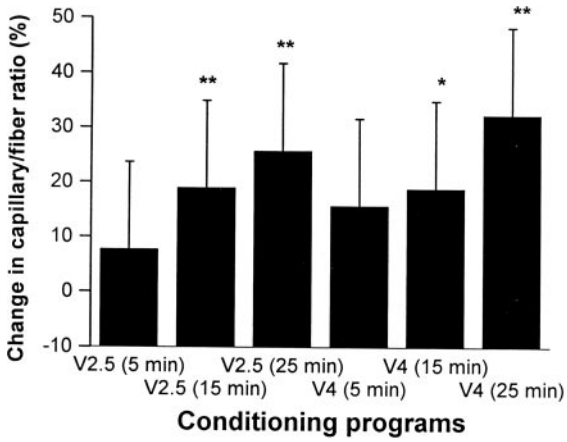

Fig. 8. Percent change in capillary-to-fiber ratio after 6 different conditioning programs of different intensity and duration of exercise training. Values are means ± SE; n = 12. Significant effect of this particular conditioning program: *$P < 0.05$, **$P < 0.01$.

The considerable myofiber hypertrophy observed in the present study after the 2-mo progressive training program has probably been caused by training itself and not by growth, even though significant changes in fiber areas have been found in nontrained thoroughbreds in the same age range (16, 30). The present study is in agreement with numerous previous studies that have reported a hypertrophic response for either all or specific muscle fiber types following short-term training in young racehorses (see 21 for a recent review). Hypertrophy appears to be the result of an increased rate of protein synthesis (19), which contributes to an absolute increase in the amount of contractile elements (3), and muscle strength and power (12). To our knowledge, however, no previous studies have investigated the effects of different types of training programs on equine muscle fiber size. Although the hypertrophic response took place in the present study following all training protocols as a whole, and it appeared to be independent of the exercise intensity and duration, significant differences between the training groups were noted both in absolute (Table 3) and relative (Fig. 7) terms regarding the specificity and extent of

Table 5. *Combined effect of intensity and duration of exercise training on fiber type histochemical SDH activity*

	SDH Activity, OD/min							
	Type I		Type IIA		Type IIAX		Type IIX	
Training Condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Basic training	0.46±0.04	0.51±0.05[b,†]	0.44±0.05	0.48±0.06[b]	0.39±0.08	0.40±0.08[a,b]	0.25±0.08	0.25±0.11
Specific training programs								
v2.5								
5 min	0.47±0.05	0.49±0.06[a,b]	0.44±0.07	0.45±0.07[a,b]	0.39±0.08	0.37±0.05[a,b]	0.22±0.11	0.24±0.06
15 min	0.46±0.04	0.48±0.05[a,b]	0.41±0.05	0.44±0.07[a,b]	0.34±0.07	0.39±0.09[a,b]	0.23±0.07	0.24±0.11
25 min	0.48±0.05	0.51±0.03[b]	0.42±0.07	0.48±0.04[a,b,*]	0.36±0.06	0.39±0.06[a,b]	0.25±0.07	0.26±0.09
v4								
5 min	0.46±0.03	0.48±0.03[a,b]	0.43±0.05	0.44±0.06[a,b]	0.35±0.04	0.36±0.07[a]	0.25±0.05	0.24±0.09
15 min	0.46±0.06	0.46±0.07[a]	0.41±0.07	0.43±0.07[a]	0.35±0.05	0.37±0.08[a,b]	0.25±0.06	0.21±0.08
25 min	0.46±0.03	0.51±0.05[b,†]	0.42±0.07	0.47±0.05[a,b,*]	0.35±0.06	0.42±0.07[b,*]	0.26±0.05	0.26±0.08
All specific training groups (n = 72 samples)	0.47±0.04	0.49±0.05†	0.42±0.06	0.45±0.06†	0.36±0.06	0.38±0.07*	0.24±0.07	0.24±0.09

Values are means ± SD; n = 12. SDH, succinic dehydrogenase; OD, optical density. Significantly different from corresponding pretraining value: *$P < 0.05$, †$P < 0.01$. Within a column, means with different lowercase letters are statistically significant ($P < 0.05$).

this adaptation. Thus myofiber hypertrophy only affected the fastest IIAX and IIX glycolytic fiber types following all three conditioning programs with the higher intensity, being maximized with the use of v4 as the exercise intensity for 15 min (Fig. 7*A*). Interestingly, a further significant increase in CSAs of these two muscle fiber types was not obtained when exercise durations were increased from 15 to 25 min at the same (constant) high intensity (Fig. 7*B*). It can be suggested from the present investigation that a significant improvement of strength and power can be elicited in gently pretrained young racehorses after short-term training (3 wk) from exercises of high intensity and low to moderate duration; and further that, at least at constant speed, additional enhancements in this muscular feature cannot be evoked by increasing the volume of the total work load. The fact that there seem to be significant hypertrophic responses of work bouts of only 5 min at v4 but not of longer exercises (25 min) at lower intensities (v2.5) (see Fig. 7*A*) is an essential part of the present results, providing the idea that this different hypertrophic outcome is affected by small differences in metabolic stress (v2.5 vs. v4) whereas duration of exercises is a secondary player in this matter.

In the present study, there was a consistent tendency for the number of capillaries per fiber to increase with training for all fiber types, indicating the formation of new capillaries within the muscle. Such findings have been reported previously following both aerobic and high-intensity training in racehorses (26, 31). A unique contribution of the present study is, however, that it provides potential variations in this response between different types of training. Accordingly, the magnitude of this adaptation depends on the exercise intensity and duration of training. Once again, the training stimulus that produced the biggest response was identified with the use of v4 as the exercise intensity applied for 25 min (Table 4). A significant response in capillary variables was, however, also obtained after exercise-training stimulus of either the same intensity and shorter duration (i.e., v4/15 min), lower intensity and the same duration (i.e., v2.5/25 min), or lower intensity and shorter duration (i.e., v2.5/15 min). As already proven in

Table 6. *Combined effect of intensity and duration of exercise training on fiber-type histochemical GPDH activity*

	GPDH Activity, OD/min							
	Type I		Type IIA		Type IIAX		Type IIX	
Training Condition	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Basic training	0.30±0.04[a]	0.32±0.05	0.37±0.07	0.34±0.06	0.43±0.07	0.45±0.08	0.50±0.07	0.50±0.09
Specific training programs								
v2.5								
5 min	0.31±0.06[a,b]	0.30±0.06	0.35±0.06	0.35±0.07	0.43±0.10	0.43±0.09	0.52±0.12	0.50±0.07
15 min	0.32±0.04[a,b]	0.32±0.06	0.37±0.07	0.36±0.05	0.46±0.08	0.44±0.08	0.50±0.06	0.53±0.11
25 min	0.30±0.06[a]	0.32±0.04	0.36±0.05	0.36±0.07	0.42±0.07	0.45±0.06	0.50±0.10	0.52±0.09
v4								
5 min	0.34±0.07[b]	0.31±0.03	0.37±0.06	0.37±0.04	0.44±0.05	0.46±0.06	0.49±0.07	0.55±0.08
15 min	0.32±0.06[a,b]	0.32±0.05	0.38±0.06	0.39±0.05	0.46±0.07	0.46±0.06	0.51±0.10	0.54±0.06
25 min	0.30±0.03[a,b]	0.33±0.07	0.37±0.06	0.35±0.06	0.45±0.08	0.46±0.10	0.49±0.08	0.56±0.09*
All specific training groups (n = 72 samples)	0.32±0.06	0.32±0.05	0.36±0.06	0.37±0.06	0.44±0.08	0.45±0.08	0.50±0.09	0.53±0.09*

Values are means ± SD; n = 12. GPDH, glycerol-3-phosphate dehydrogenase. Significantly different from corresponding pretraining value: *$P < 0.05$. Within a column, means with different letters are statistically significant ($P < 0.05$).

humans (3), these data suggest that volume training may have resulted in significant differences between conditioning programs, lending again support to the idea that capillary adaptations occur on a continuum that is based on the duration and intensity of training.

Muscle adaptations to training that have been described in the present investigation were accomplished with discrete but significant shifts in metabolic profiles of certain muscle fiber types (Tables 5 and 6). The quantitative SDH histochemical activity increased significantly for all three most-oxidative fiber types (I, IIA, and IIAX), whereas a significant improvement in glycolytic potential was obtained for type IIX fibers only. Training-linked metabolic adaptations in equine skeletal muscle are not, however, new findings (28, 31, 33). In agreement with two previous studies in thoroughbreds (5, 29), the metabolic response to training in skeletal muscle was independent of the exercise intensity during training. On the contrary, it appeared to be influenced by exercise duration, at least for the oxidative capacity of types I and IIA fibers (Table 5). For constant exercise intensity, training-induced improvements in SDH activities of these two fiber types were only found with exercises continued for 25 min. This observation agrees well with results reported by Gansen and coworkers (9), who concluded that exercise of long duration with the use of v2.5 as the exercise intensity are more effective to enhance muscle aerobic capacity in horses than shorter and faster exercises. Moreover, this finding has practical implications in thoroughbreds, since a reduction of training intensity is beneficial to minimize the risk of injuries in the musculoskeletal system (7). The development of the mitochondria (by increasing in number) is a key component of training, with this adaptation resulting predominantly from endurance-based training (2). Coyle and coworkers (4) proposed that the magnitude of the increase in mitochondria is influenced by the duration of training sessions.

In conclusion, the results of the present study demonstrate that both intensity and duration of exercise bouts are critical factors in determining the nature and magnitude of short-term (3 wk) training adaptations in skeletal muscle of adolescent thoroughbreds pretrained for 2 mo. Training-induced MHC and fiber-type conversions, as well as the myofiber hypertrophic response, seem to be more dependent on the intensity than on the duration of the exercise. On the contrary, duration was more prevalent than exercise intensity on the degree of training adaptation in the muscle oxidative capacity and capillarization. Overall, however, significant trends observed in most of muscular adaptations to training suggest that volume of the total work accomplished during training is the main determinant of the type and extent of this peripheral response.

With the exception of work bouts of only 5 min at v2.5, all remaining conditioning programs elicited significant muscular responses to training. The more prolonged (25 min) exercise sessions at v4 resulted in the largest muscular adaptation to training, except for the fiber size, which changed most with exercises of shorter duration (15 min) at the same intensity. Conditioning programs accomplished with exercises at v4 and either 15- or 25-min duration also induced significant shifts in the relative frequency, number of capillaries, and metabolic profiles of specific muscle fiber types. In practice, this means that local muscular endurance can be improved in young thoroughbred racehorses with exercise of moderate intensity (equivalent to v2.5) for ~15 min. In contrast, higher exercise intensities (equivalent to v4) applied for the same duration may be necessary to enhance muscular strength and power.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of Alexandra Köster and H. H. L. Sasse during the project. The treadmill was kindly provided by the Wissenschaftliche Gesellschaft der Schwarzwald-Tierklinik (Dr. H. Lauk), and laboratory space by the Institut für Klinische Biochemie der Universität Bonn (Dr. F. Bidlingmaier).

GRANTS

This project was possible through the financial support of Verein zur Förderung der Forschung im Pferdesport, Höveler Kraftfutterwerke, Boehringer Mannheim, and Horst Dieter Beyer, who provided the horses.

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