

Differences in Muscle Fiber Recruitment Patterns between Continuous and Interval Exercises

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We evaluated differences in muscle fiber recruitment patterns between continuous and interval training to develop an optimal training program for Thoroughbred horses. Five well trained female thoroughbred horses (3–4 years old) were used. The horses performed two different exercises on a 10% inclined treadmill: 90% $\dot{V}O_2$ max for 4 min (continuous) and 90% $\dot{V}O_2$ max for 2 min \times 2 times with 10-min interval (interval). Muscle samples were obtained from the middle gluteal muscle before and immediately after the exercises. Four muscle fiber types (type I, IIA, IIA/X, and IIX) were immunohistochemically identified, and the optical density of periodic acid Schiff staining (OD-PAS) in each fiber type and glycogen content of the muscle sample were determined by quantitative histochemical and biochemical procedures, respectively. No significant differences were found in the OD-PASs and glycogen contents between the continuous and interval exercises, but the decreases in OD-PAS of fast-twitch muscle fibers were obvious after interval as compared to continuous exercise. Interval exercise may be a more effective training stimulus for the glycolytic capacity of fast-twitch muscle fiber. The data about muscle fiber recruitment can provide significant insights into the optimal training program not only for thoroughbred horses, but also for human athletes.

Key words: *glycogen, interval exercise, muscle fiber, Thoroughbred*

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Thoroughbred race horses often run at full speed for relatively short periods (less than 3 min). It is important to establish safe and effective training programs based on scientific evidence. The fast-twitch (type II) fiber recruitment within targeted muscles is considered to be one of the most important factors during exercise training. In our previous study [19], it was reported that exercise at 100% $\dot{V}O_2$ max for 4 min was sufficient stimulus to induce type II muscle fiber recruitment. However, in the practical training field, continuous training at high speeds (100% $\dot{V}O_2$ max) for a relatively long time (4 min) is not frequently performed because of the risk of extreme fatigue, poor performance and injury.

Therefore, it would be interesting to know whether training effects are similar when continuous high

intensity exercise for 4 min is divided into two 2-min high intensity exercises bouts. If the training effects are the same between continuous and interval training, our previous results may be important knowledge in the practical training field. The main purpose of this study was to investigate the recruitment pattern of each muscle fiber type in both continuous and interval exercises in Thoroughbred horses.

Materials and Methods

Animals

Five Thoroughbred horses (female; 3–4 years old) that weighed 449 ± 8 kg were used. Before the experiments, the horses had acclimatized to exercise on a treadmill (Mustag 2200, Kagra AG, Fahrwangen, Switzerland), and were well trained for 3 months in

conventional training. All procedures used in this study were approved by the Animal Experiment Committee of the Equine Research Institute, Tochigi Branch, Japan.

Measurement of $\dot{V}O_2\max$

A week before the experiment, we performed an incremental exercise test (IET) on a 10% inclined treadmill to measure maximum oxygen consumption ($\dot{V}O_2\max$) of each horse. The IET protocol was as follows: after 2 min walking (1.8 m/sec) and 5 min trotting (3.6 m/sec), the speed was increased to 6, 8 m/sec and then 1 m/sec increments every minute were continued until exhaustion. The measurement of $\dot{V}O_2\max$ was performed by an open flow system (Vice Medical, Chiba, Japan). We measured O_2 and CO_2 concentrations and temperature and relative humidity continuously [1]. All instrument signals were stored on a computer with an analog-to-digital converter, and then calculated using a software analysis package (DATAQ Instruments, Akron, OH, USA). The average $\dot{V}O_2$ of the last 15 sec of each cantering speed was determined as the $\dot{V}O_2$ for the speed, and $\dot{V}O_2\max$ was determined at a leveling off point by regression line analysis. The running speeds corresponding to 90% $\dot{V}O_2\max$ were determined based on the $\dot{V}O_2\max$ for each horse.

Exercise protocol and sample collection

All exercise tests were performed on a high speed treadmill. Horses ran in two different exercise protocols; i.e. 90% $\dot{V}O_2\max$ for 4 min on the 10% inclined treadmill (continuous) and 90% $\dot{V}O_2\max$ for 2 min \times 2 times with 10-min interval walking (1.8 m/sec) on the 10% inclined treadmill (interval). Each exercise test was separated by 4 days.

According to the sampling method of Lindholm and Piehl [9], muscle samples were obtained from nearly the same portion of the middle gluteal muscle and at the same depth (5 cm from the skin surface). Muscle biopsy was performed before exercise (Pre) and after each exercise (Post) under local anesthetic with 2% lidocaine (Fujisawa Pharmaceutical Co., Osaka, Japan). All muscle samples were frozen in melting isopentane cooled by liquid nitrogen, and then they were stored at -80°C until analyzed. The samples were analyzed at the same time by histochemical, immunohistochemical and biochemical procedures.

Histochemical and immunohistochemical analysis

Frozen pieces of muscle were cut with a freezing microtome (CM 510, Leica, Tokyo, Japan) into eight transverse sections (thickness of each section, 8 μm). Four of these sections were reacted for glycogen with the periodic acid Schiff (PAS) procedure [17]. The sections were incubated in 0.5% periodic acid for 5 min at room temperature (25°C), rinsed in distilled water and placed in Schiff's reagent for 15 min at room temperature. After the staining, microscopic images of muscle fibers were obtained by a personal computer and image-processing system (DS-U1, Nikon, Tokyo, Japan). During the analysis, optical intensity was kept constant. To measure the optical density of PAS staining (OD-PAS) in muscle fibers, luminosity was expressed at 256 Gy. Luminosity was calibrated by use of 4 filters with differing transmissivity (100, 25, 6, and 1.5%). The OD-PAS was represented by a relative value for pre OD-PAS in each exercise bout.

The other four transverse sections were used for immunohistochemical analysis with anti-mouse IgG or IgM. The sections were allowed to warm to room temperature and then pre-incubated in normal horse serum in phosphate buffer at 25°C for 10 min. A primary monoclonal antibody was then applied: 1) BA-D5, which specifically labels MHC-I; 2) SC-71, which specifically labels MHC-IIa; 3) BF-F3, which specifically labels MHC-IIb; and 4) BF-35 for the detection of MHC-IIx. The specificity of these monoclonal antibodies has been previously demonstrated in the horse [13]. The sections were incubated at 25°C for 180 min, then washed with phosphate buffer and reacted with a horseradish peroxidase (HRP)-labeled secondary antibody at 25°C for 180 min, and then washed with phosphate buffer again. Diaminobenzidine tetrahydrochloride was used as a chromogen to localize peroxidase in all primary antibodies. To avoid inter-batch variation, all samples from each horse were processed simultaneously. On the basis of examination of the immunohistochemical staining images, muscle fibers were classified as type I, I/IIA, IIA, IIA/IIX and IIX fibers. The OD-PAS and muscle fiber area were measured in at least 25 muscle fibers of type I and IIA/IIX fibers, and 50 muscle fibers of type IIA and IIX fibers. Type I/IIA fibers were excluded from the result, because little of this fiber type existed in all muscle samples (less than 0.1%).

Biochemical analysis

Muscle glycogen contents were determined by the

Table 1. Body weight (BW), $\dot{V}O_2$ max and running speed in each Thoroughbred horse.

Horse	BW (kg)	Age (year)	$\dot{V}O_2$ max (ml/min/kg)	Running speed (m/sec)	
				Continuous (4 min)	Interval (2 min \times 2 times)
1	457	4	181	9.1	9.1
2	436	3	184	8.7	8.7
3	452	3	185	9.4	9.4
4	452	3	191	9.1	9.1
5	449	3	186	8.4	8.4
Mean	449		185	8.9	8.9
SD	8		4	0.4	0.4

Values are presented as mean \pm standard deviation (SD).

Anthrone method after alkaline digestion [5]. Samples were boiled and melted with 30% KOH at 100°C for 30 min. After addition of 98% ethanol, samples were centrifuged at 3,000 rpm for 30 min and the supernatants were discarded. The deposits were diluted with 3 ml of distilled water, and then 1.5 ml of 0.09% anthrone (0.25 g of anthrone in 272 ml of 68% H₂SO₄) was added to 0.5 ml of the solution. The samples were placed in boiling water for 15 min. The optical density (OD) at 620 nm of the solution was determined by spectrophotometry in triplicate. OD was calibrated by glucose solution at different concentrations (0, 12.5, 25, 50 and 100 μ g/ml).

Statistical analysis

All values were reported as mean \pm standard deviation. To examine the significance of difference between exercise stages (pre and post) and exercise tests (continuous and interval), a non-paired *t*-test was used. Significance was established at $p < 0.05$.

Results

Animal conditions

The physical properties and running speeds corresponding to 90% $\dot{V}O_2$ max of each horse are summarized in Table 1. During the experimental period, the variations of body weights in each horse were 1 to 6 kg. The mean value of running speed at 90% $\dot{V}O_2$ max was 8.9 ± 0.4 m/sec.

Histochemical analysis

The immunohistochemical stain with each antibody could detect MHC I, IIa, and IIx isoforms, but not the MHC IIb isoform. Muscle fibers were classified as type

I, IIA, IIA/IIx and IIx fibers (Fig. 1). In the muscle sample obtained before the first exercise bout, the mean percentages of type I, IIA, IIA/IIx and IIx fiber were $11.7 \pm 4.1\%$, $38.3 \pm 5.9\%$, $4.4 \pm 0.7\%$ and $45.6 \pm 9.1\%$.

Staining images of PAS stain before and after each exercise are shown in Fig. 1. And the changes in OD-PAS of each fiber type are summarized in Fig. 2. As compared to each pre-exercise value, the relative values of OD-PAS in continuous and interval trainings decreased to 71.6% and 60.4% in type I fibers, 71.7% and 59.9% in type IIA fibers, 77.9% and 65.1% in type IIA/IIx fibers, and 77.4% and 66.1% in type IIx fibers, respectively. Each post-exercise value in all fiber types was significantly lower than pre-exercise values. Although there were no significant differences ($p = 0.08 \sim 0.15$), these relative values in all fiber types were lower in interval training than those in continuous training.

Biochemical analysis

Biochemical analysis showed significant glycogen decreases in the whole muscle after all exercises (Fig. 3). After the continuous and interval trainings, the glycogen contents of muscle samples decreased to 69.5% and 55.6% of pre-exercise values, respectively. There was a significant decrease in glycogen content after each exercise test. Although there was no significant difference ($p = 0.07$), the glycogen content was lower in interval exercise than in continuous exercise.

Discussion

The muscle fiber recruitment pattern during continuous exercise has been investigated in horses [6, 8, 15, 16, 19]. In this study, continuous and

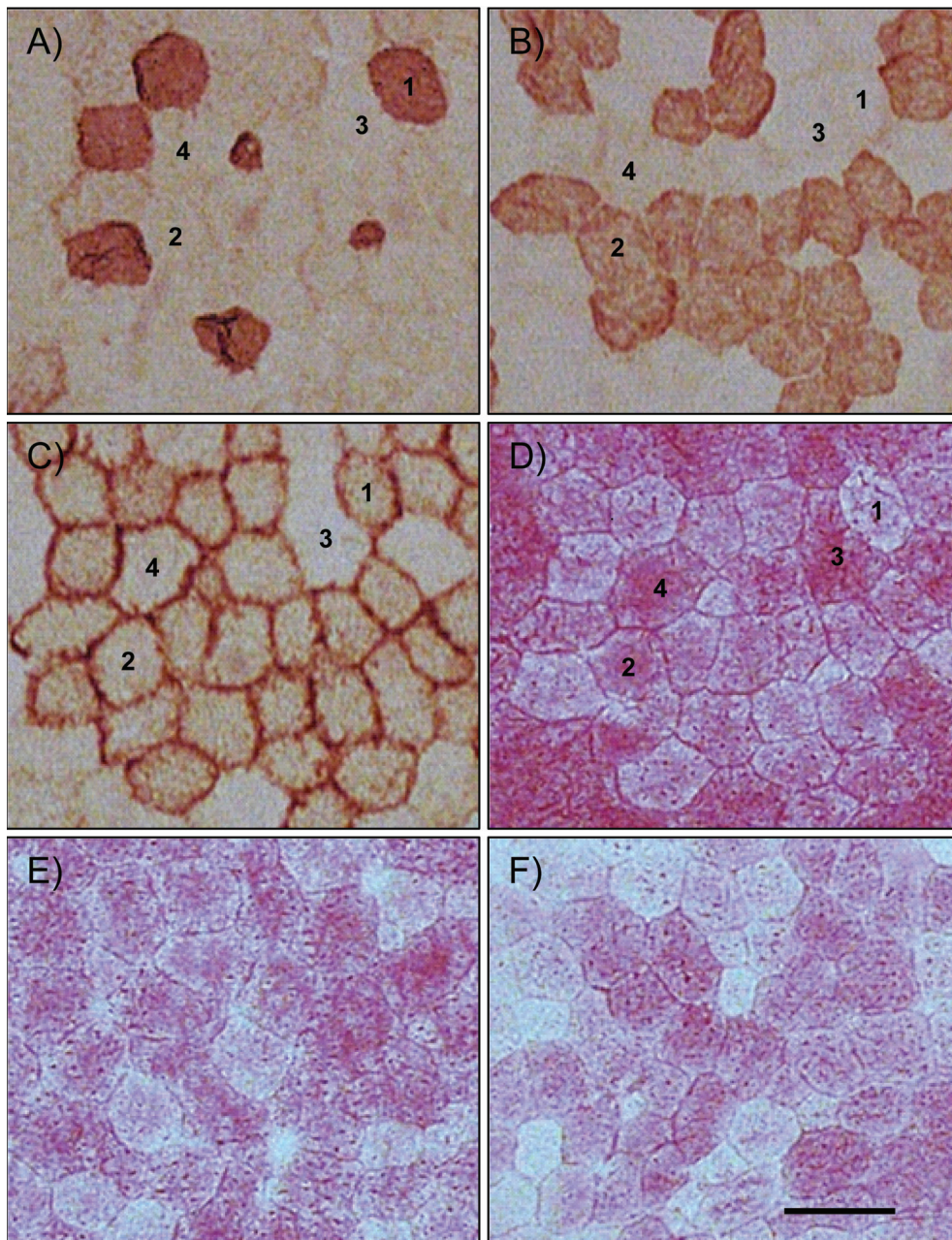


Fig. 1. Photomicrographs of muscle fibers obtained from the middle gluteal muscle of horse. Staining image of immunohistochemical with each antibody: A) BA-D5 (for MHC-I), B) SC-71 (for MHC-IIa), C) BF-35(MHC-IIx). And staining image of PAS stain: D) before exercise, E) after continuous exercise and F) after interval exercise. 1: type I fiber, 2: type IIA fiber, 3: type IIX fiber, 4: type IIA/IIX fiber. Bar=100 μ m.

interval exercises were performed at the same intensity (90 % $\dot{V}O_2$ max), and similar recruitment patterns were found in all fiber types. Therefore, the results suggest that both continuous and interval

training may demand basically similar recruitment patterns of muscle fiber types when the total running time is the same.

Although the study was non-quantitative

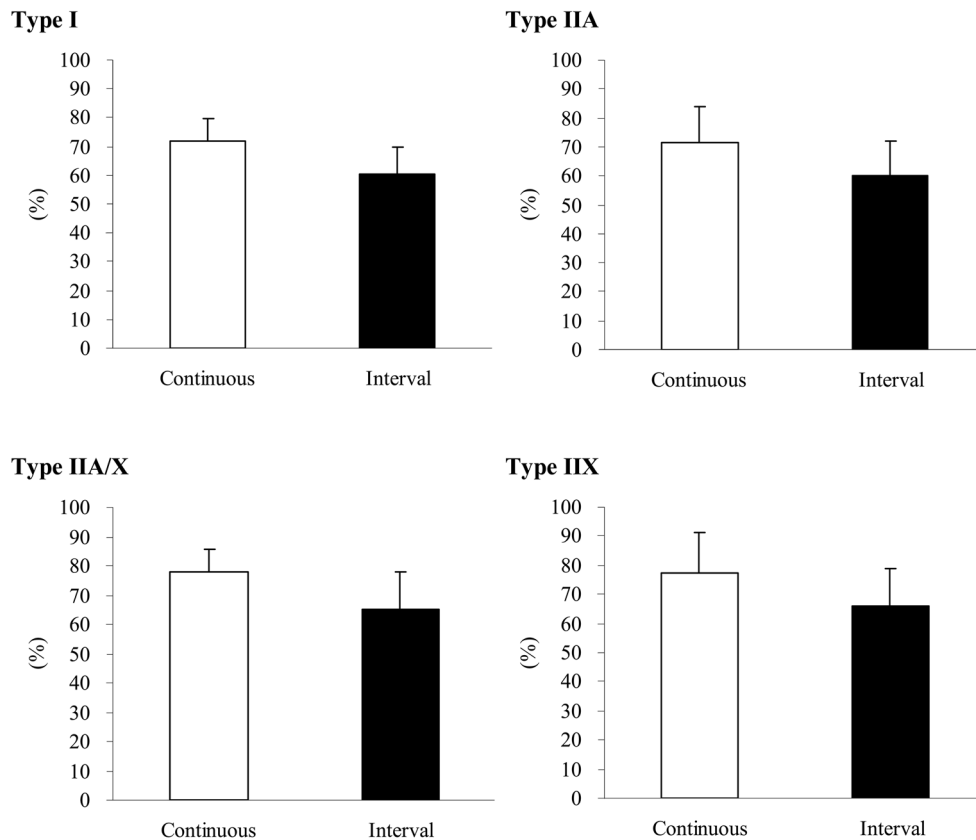


Fig. 2. Comparison of optical densities of PAS stain in each fiber type between continuous and interval exercises. Data are represented by relative values for pre OD-PAS in each exercise bout. Although there are not significant differences, these relative values in all fiber types are lower in interval exercise than those in continuous exercise.

examination, Gottlieb [6] demonstrated that walking (2 m/sec) with 34 kp and 80 kp load induced only type I fiber recruitment at least 30 min in hindlimb muscles of Standardbred horses. Furthermore, it is well described that only type I fiber was recruited for walking in the cat hindlimb muscles [18]. Based on the results in previous studies, we speculate that recruitment of type I fibers and II fibers in our interval exercise are mainly induced by walking during interval period and exercise running, respectively.

In this study, although there were no significant differences in recruitment patterns of muscle fiber type between continuous and interval exercises, we found that fast muscle fibers (i.e. type IIA, IIA/X, IIX) tended to be recruited more frequently in interval exercise. This tendency is considered to be attributed to oxygen deficit at the start of exercise. The cardio-respiratory system can not supply sufficient oxygen to working

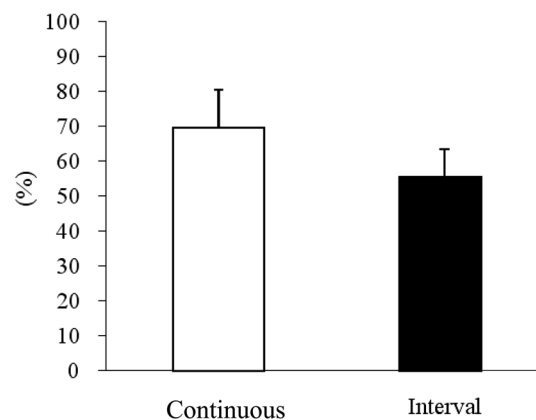


Fig. 3. Comparison in glycogen content between continuous and interval exercises in muscle samples. Although there is no significant difference, these relative values in all fiber types is lower in interval exercise than continuous exercise.

muscles at the start of exercise. Therefore, the working muscles need to rely on anaerobic (glycolytic) energy metabolism for contraction, and as a result, fast-twitch muscle fibers with rich glycolytic enzymes, more than oxidative slow-twitch muscle fibers, would be recruited. In another point of view, the difference of recruitment in this study might be attributed to the altered stride and pitch at the start of exercises. We consider that whatever the reason, the facilitation of type II fiber recruitment is preferred exercise effect in the interval training. In a previous study [2], we reported that high intensity training (80–100% $\dot{V}O_2\text{max}$, for 12 weeks) induced increased glycolytic enzyme activity and improved anaerobic capacity. It is possible that high intensity interval exercise stimulates the fast-twitch glycolytic muscle fibers more frequently in the working muscle and enhances the contraction ability of the fibers more efficiently, compared to continuous exercise.

In the practical training for horses, a general training protocol consists of aerobic training as the first stage, a combination of aerobic and anaerobic training as the second stage, and anaerobic training as the third stage [3]. In the second stage, the best training intensity is 70–85% of race speed (blood lactate concentration; 15–20 mmol/l). This training intensity can also induce an increase in the population of fast-twitch fibers, muscle buffering capacity, and aerobic and anaerobic enzymatic activities [10, 14]. However, because of the risk of overtraining and injury, continuous, low intensity training is generally preferred as second stage training. Based on our results, we speculate that interval training can improve the anaerobic energy-supplying system with less possibility of overtraining and injury. In fact, Lovell and Rose [10] reported that lactate dehydrogenase activity was increased by interval training on a 5%-inclined treadmill, but not in continuous training. Furthermore, Harkins and Kammerling [7] reported that interval training was performed for over 7 months without injury in Thoroughbred horses. The main purpose in the third stage of Thoroughbred training is to enhance the maximal running speed and the accelerating force at the start of exercise. If high intensity training in this stage is divided into a couple of same intensity and shorter periods (i.e. interval training), improvement of anaerobic capacity without overtraining and injury may be achieved. In fact, it is reported that accelerating force was improved by interval training in Standardbred horses [4].

In addition, it has been proposed that there are various potential advantages for interval training. It has been shown that, in Standardbred racehorses, interval training on a treadmill improved metacarpal bone quality [11]. Midgley and Mc Naughton [12] have reported that, in humans, intermittent running protocols have been found to be more effective than continuous protocols for increasing time at or near $\dot{V}O_2\text{max}$. In the present study, we provide a scientific base demonstrating the advantages of interval training by the examination of recruitment patterns of muscle fiber type in Thoroughbred horses.

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