

学 位 論 文 要 旨  
SUMMARY OF DOCTORAL THESIS

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題目 Title: Developmental change and recruitment pattern of muscle cells  
in the gluteus medius muscle of Thoroughbred horses

The Thoroughbred horse is a remarkable animal, with both speed and endurance abilities. It is considered that scientific information about these excellent abilities can provide significant insights into exercise physiology and health science not only for Thoroughbred horses, but also for human beings. Muscle adaptation associated with training and growth has been examined using various histochemical and biochemical measurements. However, little information is available with which to consider the training-induced improvements in horse skeletal muscle before age 2 year, because most racehorses are trained regularly from about 18 months of age. In addition, to consider optimal training programs for Thoroughbred horses, it is very important to know which intensity and duration is the most effective to stimulate the fastest muscle fiber in the main active muscle.

To characterize developmental changes in equine muscle fiber type, including hybrid fibers and their metabolic properties, we used a combination of immunohistochemical, histochemical and electrophoretic analyses. Twenty-four female Thoroughbreds, 2 months ( $n = 6$ ), 6 months (6), 12 months (6), and 24 months (6) old, were used in this study. Muscle samples were obtained from the gluteus medius muscle of each horse. Expression of myosin heavy chain (MHC) isoforms MHC-I, -IIa, -IIb, and -IIx in each muscle fiber was detected using 4 primary monoclonal antibodies, BA-D5, SC-71, BF-F3, and BF-35, respectively. Five muscle fiber types (types I, I/IIA, IIA, IIA/IIX, and IIX) were immunohistochemically identified. The area and succinate dehydrogenase (SDH) activity in each fiber type were determined by quantitative histochemical staining and image analysis. Although the proportion of type I and IIX fibers did not change with age, the proportion of type IIA and IIA/IIX fibers significantly increased and decreased, respectively, from 2 months to 24 months of age. The increased proportion of type IIA fibers with growth may be attributable to muscle fiber type transition from type IIA/IIX fibers, but not from type IIX fibers. Values for SDH activity and fiber area in hybrid fiber types were intermediate to those for their respective pure phenotypes. Hybrid fibers play an important role in determining the proportion of muscle fiber type in horses < 24 months old, and the metabolic and morphological properties of these hybrid fibers are well coordinated, as in mature horses.

Furthermore, we examined the recruitment pattern of muscle fibers including hybrid fibers

in well-trained Thoroughbred horses to consider the optimal training program. The horses performed exercise at three different intensities and durations; i.e. 100%  $\dot{V}O_{2max}$  for 4 min, 80% and 60%  $\dot{V}O_{2max}$  for 8 min on a treadmill with 10% incline. Muscle samples were obtained from the gluteus medius muscle before, during (4 min at 80% and 60%  $\dot{V}O_{2max}$ ), and after exercise. Four muscle fiber types (types I, IIA, IIA/IIX, and IIX) were immunohistochemically identified, then the optical density of Periodic Acid Schiff staining (OD-PAS) in each fiber type and the glycogen content of the muscle sample were determined by quantitative histochemical and biochemical procedures. The changes in OD-PAS showed that the recruitment of all fiber types were identical at the final stage of each exercise bout, i.e., 4 min running at 100%  $\dot{V}O_{2max}$ , and 8min running at 80% and 60%  $\dot{V}O_{2max}$ . Changes in OD-PAS of type IIA/IIX fiber were very similar to those of type IIX fiber. Recruitment of these fibers was obviously more facilitated by 4min running at 100%  $\dot{V}O_{2max}$  than by 4min running at 80 or 60%  $\dot{V}O_{2max}$ . Short duration with high intensity exercise, such as 4min running at 100%  $\dot{V}O_{2max}$  or 8 min running at 80% or 60%  $\dot{V}O_{2max}$ , is effective to stimulate type IIX fiber and IIA/IIX fibers, which have the fastest speed of contraction.

In a previous study, we emphasized the importance of type IIX fiber recruitment in Thoroughbred training. Because type IIX fibers provide the greatest contribution to total muscle volume, the increase in SDH activity in type IIX fibers would markedly affect the increase in whole-muscle SDH activity. We consider that the repetitive fastest muscle contraction within a short duration is accomplished by type IIX fiber with increased oxidative ability. Training of young Thoroughbred horses seems to facilitate muscle fiber hypertrophy and increase the oxidative capacity of type IIX fiber which could potentially enhance stamina at high speeds. Furthermore, in mature Thoroughbred horses, we established that short durations of high intensity exercise, such as 4min running at 100%  $\dot{V}O_{2max}$  or 8 min running at 80% or 60%  $\dot{V}O_{2max}$ , are effective to stimulate type IIX fiber and IIA/IIX fibers. To apply our results to developing practical training fields, differences in the recruitment pattern between continuous running (4 min) and intermittent running (1 min x 4 times or 2 min x 2 times) should be investigated in the near future.