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Short Communication

Skeletal Muscle Fiber Composition of Untrained Mangalarga Marchador Fillies

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ABSTRACT

Mangalarga Marchador (MM) is the most important and numerous equine breed in Brazil. So far, no studies have been carried out on the breed's skeletal muscle composition, which is important to develop specific physical conditioning programs. To characterize the skeletal muscle fiber composition of young MM females, *gluteus medius* muscle biopsies were obtained from 13 fillies between 2.5- and 3-year-old using a biopsy needle at 60-mm depth. Types I, IIA, and IIX fibers were determined by the metachromatic staining method of ATPase activity in myofibers using preincubation followed by incubation in alkaline medium. Relative frequency (%F), average cross-sectional area (CSA), and relative cross-sectional area (%CSA) of each muscle fiber type were determined. Considering %F, 29.5 ± 5.4% were type I, 40.3 ± 5.5% were type IIA, and 30.2 ± 5.9% were type IIX fibers. CSA of type I fibers was 2,633 ± 798 μm^2, of type IIA was 3,407 ± 492 μm^2, and of type IIX was 5,856 ± 1,237 μm^2. %CSA was composed of 19.7 ± 4.9% of type I fibers, 35.4 ± 4.7% of type IIA, and 44.9 ± 7.4% of type IIX. The *gluteus medius* muscle of untrained MM fillies was predominantly composed of type IIA fibers, but the highest total relative area was occupied by type IIX fibers, suggesting moderate aptitude to the oxidative and glycolytic metabolisms.

1. Introduction

Mangalarga Marchador (MM) is the most important and numerous equine breed in Brazil. This breed's typical gait is the four-beat marcha, with alternate lateral and diagonal support interspersed by moments with triple support [1]. This movement dynamics allows the animal to always remain in contact with the ground during locomotion, which favors the stability of the animal's torso and provides more comfort to the rider. The smooth gait of MM horses is favorable to activities such as cattle work, cavalcade, working equitation, and therapeutic riding. In April 2015, the Brazilian Mangalarga Marchador Breeder's Association (ABCCMM) counted 9,895 associated members, 598,457 registered horses, 66 regional breeders organizations in Brazil, and four regional organizations abroad (Germany, Italy, Argentina, and USA).

Horse breeds have key intrinsic characteristics regarding morphology, temper, physical aptitude, and physiology. They also have particular muscle types related to the aptitude to the types of exercises to which each breed is subjected [2]. The muscle fibers of some horse breeds raised in Brazil have been characterized: Quarter Horse and

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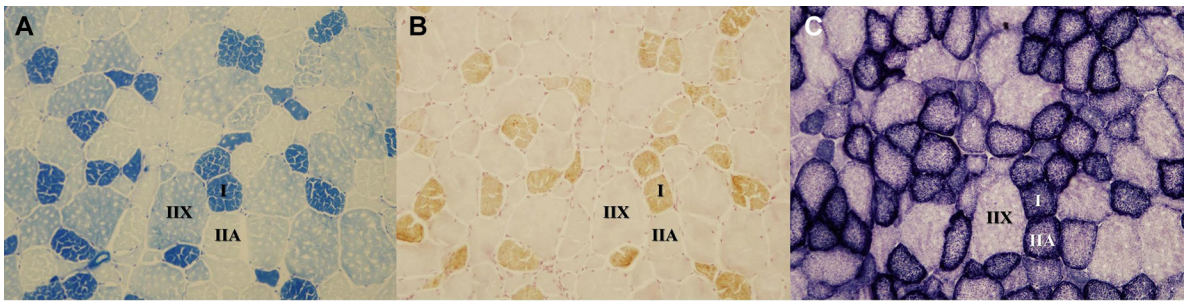

Fig. 1. Serial sections of M. *gluteus medius* of Mangalarga Marchador fillies. (A) mATPase histochemical staining: type I (oxidative, dark blue), type IIA (oxidative-glycolytic, light blue or no coloration), and type IIX (glycolytic, intermediate blue); (B) indirect immunohistochemical method: slow-twitch fibers or type I (golden color) and fast-twitch fibers or type II (lack of color); (C) NADH-TR: type I and type IIA (purple), and type IIX (light purple or no coloration; ×200). NADH-TR, nicotinamide adenine dinucleotide tetrazolium reductase.

Thoroughbred [3], Andalusian and Arabian horses [4,5], Crioulo [6], Mangalarga (of São Paulo state) [7], and Brasileiro de hipismo (Brazilian Sport Horse) [8]. Although the MM breed is responsible for the largest and most representative horse herd in Brazil, its muscle fibers have not been characterized yet. The present study aimed to characterize the composition of skeletal muscle fibers of MM fillies.

2. Materials and Methods

Thirteen MM fillies between 2.5- and 3-year-old with average body weight of 330 ± 30 kg that had never undertaken a physical conditioning program were used. Biopsies of the *gluteus medius* muscle were obtained at 60-mm depth using a Bergström needle with 6-mm external diameter as methodology adapted from Lindholm and Piehl [9]. Samples were frozen in hexane, precooled in liquid nitrogen, and stored at −80°C until analyzed. The experimental procedures were approved by the Ethics Commission on Animal Use (CEUA – UFMG) - protocol 237/2012.

Muscle samples were sectioned serially (12-μm thickness) in a cryostat (Mícron gmbH, H1599 OM, 69,190, walldorf, Germany) at −20°C. Histochemical analysis was used to identify or differentiate the types I, IIA, and IIX fibers and consisted of adapting the metachromatic staining method of ATPase activity in myofibers described by D'Angelis et al [10] for the preincubation in acid medium [11,12] at pH 4.45 to 4.55 for 5 to 6 minutes at 18°C to 20°C, followed by incubation in alkaline medium [13] at pH 10.50 to 10.55 for 25 minutes at 37°C. To verify the mATPase histochemical data, the indirect immunohistochemical method (peroxidase–antiperoxidase) [10] was used after incubation with monoclonal anti-slow myosin primary antibody (Clone NOQ7.5.4D; Sigma–Aldrich, Química do Brasil Ltda, São Paulo, SP, Brazil). The slow-twitch fibers (type I) and fast-twitch fibers (type II) were identified by the golden color of the diaminobenzidine precipitate formed in the antigen-antibody complex in the former and lack of color in the latter. The oxidative potential of the skeletal muscle fibers was assessed through nicotinamide adenine dinucleotide tetrazolium reductase [10,14]. Type I and type IIA fibers were stained purple, and type IIX were stained light purple or had no coloration. The muscle fibers were identified only through the mATPase histochemical staining as follows: type I (oxidative, dark blue), type IIA (oxidative-glycolytic, light blue or lack of color), and type IIX (glycolytic, intermediate blue; Fig. 1).

Three images were captured from each slide using a photomicroscope (Camedia Olympus 95-98 ME). These images were transferred to the image analysis software Scion Image, and the artifact-free regions that contained between 30 and 80 muscle fibers were selected. The relative frequency of muscle fiber types for each sample (%F) and the average cross-sectional area (CSA) were determined. The relative CSA (%CSA) that a fiber type occupied in a muscle sample was calculated by dividing the product of the percentage and the mean CSA of the fiber type by the sum of these products for all muscle fiber types [15]. Data were submitted to descriptive analysis, and results are expressed as means ± standard deviation.

3. Results

Results are presented in Table 1.

4. Discussion

According to the ABCCMM, MM horses are direct descendants of Alter breed horses from the "Coudelaria de Alter do Chão" situated in the Alentejo region in Portugal [16]. Horses of this stud farm are originated from Andaluz breed, and the common ancestry may explain the similarity between the distribution of skeletal muscle fibers in MM fillies and Andaluz specimens. As in the present study, Andaluz stallions [4] showed a prevalence of type IIA fibers (42.8%) followed by type IIX (29.5%) and type I (27.7%).

Table 1
Relative frequency (%F), average cross-sectional area (CSA), and relative cross-sectional area (%CSA) of types I, IIA, and IIX muscle fibers of Mangalarga Marchador fillies.

Variables	Type I	Type IIA	Type IIX
F (%)	29.5 ± 5.4	40.3 ± 5.5	30.2 ± 5.9
CSA (μm^2)	2,633 ± 798	3,407 ± 492	5,856 ± 1,237
%CSA (%)	19.7 ± 4.9	35.4 ± 4.7	44.9 ± 7.4

Abbreviation: SD, standard deviation.
Data are expressed as means ± SD.