INTRAMUSCULAR GLYCOGEN AND LIPID CONTENTS OF ACTIVE AND INACTIVE YOUNG HORSES OF THE BRASILEIRO DE HIPISMO BREED

CONTEÚDO DE GLICOGÊNIO E LIPÍDIOS INTRAMUSCULAR DE EQÜINOS JOVENS DA RAÇA BRASILEIRO DE HIPISMO INATIVOS E ATIVOS

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SUMMARY

We aimed at determining the lipid and glycogen contents in the *gluteus medius* muscle fibers of horses of the *Brasileiro de Hipismo* (BH) breed, analyzing the variables of age, gender, muscle biopsy depth and low-intensity physical activity. For so, we used 89 horses, out of which 78 were inactive and 11 underwent to moderate physical activity. The intramuscular glycogen analysis was performed through the PAS staining, and the lipid determination through the Oil red O staining. For the typification of the I, IIA and IIX fibers, we employed the NADH-TR technique. There was no difference in the glycogen content in the comparison among ages. We observed differences between sexes, once females presented a higher glycogen content compared to males, especially in the IIA- and IIX-type fibers. Regardless on the age, sex and biopsy depth, the lipid content in the I-type myofibers was higher. The same result was seen regarding physical activity, once there were no differences concerning glycogen and lipid contents when we compared the staining intensity in the inactive animals with those trained for 10 months. Henceforth, mild physical activity was not able to change the glycogen reserves while resting. On the other hand, females presented higher glycogen content, what can suggest a better performance of those animals in energy-demanding situations of low and average duration and intensity.

KEY-WORDS: Brasileiro-de-Hipismo. Horse. Exercise, Glycogen. Lipids

RESUMO

Objetivou-se determinar o conteúdo bioquímico de lipídeos e glicogênio nas fibras do músculo *gluteus medius* de cavalos da raça Brasileiro de Hipismo (BH), observando-se as variáveis idade, gênero, profundidade da biópsia e atividade física de baixa intensidade. Para tanto, utilizaram-se 89 eqüinos, sendo 78 inativos e 11 com atividade física moderada. A análise de glicogênio intramuscular foi realizada por meio da coloração de "PAS" e, a determinação de lipídios utilizando-se a coloração de "oil red O". Para a tipificação das fibras I, IIA e IIX empregaram-se a técnica de NADH-TR. Não houve diferença no conteúdo de glicogênio na comparação entre as idades. Observou-se diferenças entre os sexos, sendo que as fêmeas apresentaram maior conteúdo de glicogênio em relação aos machos, principalmente nas fibras do tipo IIA e IIX. Independente da idade, sexo e profundidade da biópsia muscular o conteúdo de lipídios nas miofibras tipo I foi maior. O mesmo resultado foi verificado em relação à atividade física, não havendo diferenças em relação ao conteúdo de glicogênio e lipídeos quando se comparou as intensidades de coloração nos animais inativos e treinados por 10 meses. Concluiu-se que a atividade física leve não foi capaz de alterar as reservas de glicogênio em repouso. Por outro lado, as fêmeas apresentaram maiores conteúdo de glicogênio podendo sugerir um melhor desempenho desses animais em situações de demandas energéticas de curta e média duração e intensidade.

PALAVRAS-CHAVE: Brasileiro de Hipismo. Cavalo. Exercício. Glicogênio. Lipídeos.

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INTRODUCTION

Among the main substrates for muscle contraction, carbohydrates, lipids and proteins are highlighted. Carbohydrates are stored mainly as glycogen in the muscles and liver, while blood glucose represents an immediate energy supply. A great amount of the energy used during the exercise of moderate to high intensity comes from the skeletal muscle glycogen stocks. The glycogen stocks in the muscles depend on the glucose availability and on the activity of the glycogen synthase enzyme. High blood concentrations of insulin and glucose, together with a high activity of the glycogen synthase, promote the storage of glycogen within the muscle (POWERS E HOWLEY, 1997).

Several studies determined the patterns of glycogen use in different types of muscle fibers. As the exercise intensity increases, there is a progressive recruitment of more powerful and fast-contracting muscle fibers (IIX). During the high-intensity exercise, there is a preferential use of glycogen in the fast-contracting glycolytic muscle fibers – IlB (WHITE AND SNOW, 1987), with little use occurring in the I-type slow-contracting oxidative fibers.

Lindholm et al. (1974) concluded that short-duration high-intensity exercises consume from 20 to 35% of the muscle glycogen reservations. Inversely, although the rate of glycogen use is low during a sub-maximal long-duration exercise, the total glycogen depletion can be very significant. It is not an uncommon fact that the muscle glycogen reservations of endurance animals are used in more than 50-75% after 160km of circuit (SNOW et al, 1981). Suitable muscle glycogen reservations seem to be important for the good development both in short periods of intense exercise and in long-duration exercise.

Those authors, studying the glycogen in a group of horses participating of an endurance competition, tried to relate the performance of the animals and the muscle fiber composition of the *gluteus medius* muscle. They observed that the animals with a high proportion of I-type fibers and a appropriate stock of glycogen presented a better development in the 80-km endurance competition, with a low and high temperature of 4ºC and 18ºC, respectively.

During exercises of moderate to low intensity, the release of fatty acids may represent a greater source of energy supplying the whole body energy demand (TARNOPOLSKY et al., 1990). The endurance training leads to several skeletal muscle adaptations including the increase of the oxidative metabolism capacity of lipids and carbohydrates. The increase of the fatty acid oxidation is eased by the greater capacity of income of the acid into the myocytes, its subsequent mitochondrial transportation and β-oxidation. Snow et al. (1981), using horses that participated of an 80-km endurance competition, found few alterations in the intramuscular lipid content compared to that found by Lindholm et al. (1979) who, using biochemical techniques for analysis, reported a 65%-decrease in the triglyceride content during the first hours of endurance exercise.

The aim of this study was to determine the variations in the glycogen and lipid composition of the *gluteus medius* muscle fibers of horses of the *Brasileiro de Hipismo* breed, according to age, sex, muscle biopsy depth and low-intensity activity.

MATERIAL AND METHODS

We used 78 inactive horses (9 geldings, 35 stallions and 34 females) of the *Brasileiro de Hipismo* (BH) breed coming from the Haras Pólo Regional de Desenvolvimento Tecnológico de Agronegócios da Alta Mogiana, located in the town of Colina, São Paulo State.

In order to analyze the differences regarding the age, the animals were selected according to the following groups: the first group, composed by 1-year-old animals (n = 21) with 390 ± 23kg of body weight; the second group, composed by 2-year-old animals (n = 33) with 420 ± 25kg of body weight; the third group, composed by 3-year-old animals (n = 13) with 460 ± 24 kg of body weight; and the last group, only 4-year-old males (n = 11) with 490 ± 20 kg of body weight. Concerning the analysis of the sex difference, over the skeletal striated muscle fiber, those animals were divided into groups of males and females.

All animals were kept inactive in pickets of *Panicum maximum* Jacq. var. maximum and *Panicum maximum* var. Tanzania, supplemented, in the dry period, with concentrated feed once a day and mineralized salt and water *ad libitum*.

To analyze the training effect, we used males of the BH breed (9 geldings and 2 stallions), aged 4 years old at the beginning of the experiment. This phase of the experiment was entirely developed at the "Nove de Julho" Military Police (PM) Battalion, São Paulo State (SP), and all animals were kept in individual bays during the training period. In that period, the horses were supplemented with 6 kg of daily feed[4], 4 kg of *Cynodon dactylon* hay, mineralized salt and water *ad libitum*. The animals were exercised for a 10-month period by the São Paulo State Military Police to be employed in police patrolling assignments. Each animal underwent mild physical activity conducted always by the same person, 10 daily hours at 90% at walk gait from 4 to 5 times a week, with an interval for feeding twice a day. Every 15 days, a specific training during 1 hour and 30 minutes was conducted with 70% of exercise at trotting gait, 20% at galloping gait and 10% at walk gait. No skeletal muscle lesion was seen during the entire period during which the animals took part in the police patrolling assignments.

The percutaneous muscle biopsy through 6.0-mm Bergström needle allowed the collection of a fragment of the left *gluteus medius* muscle in the 20-mm and 60-mm depths, which was processed according to D'Angelis et al. (2005).

The intramuscular glycogen analysis through the PAS staining and the observation of intramuscular lipids using the Oil red O staining were conducted according to the protocol described by Dubowitz

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(1985), using fragments collected for the hystochemical analyses. In order to determine the typification of the I, IIA and IIX fibers, the NADH-TR technique (NOVIKOFF et al., 1961) was used. For the determination of the intramuscular glycogen and lipid contents, the procedures described by Snow et al. (1981) was performed. The hystochemical analyses of the histological cuts were conducted from images captured by a photomicroscope and analyzed by an image analysis program coupled to a computer. The glycogen content of those fibers was subjectively characterized into 5 categories: high and with difficulty of cell visualization (++++), high (+++), intermediate (++), low (+) and neglectable (-). The lipid contents were divided into 3 categories: high (+++), intermediate (++) and neglectable (-).

RESULTS AD DISCUSSION

The glycogen results (Table 1; Figure 1A and C) did not show differences among the ages, but did so among the sexes. In the females, the IIX-type fibers presented an intense staining and difficulty of cell visualization (++++) due to a larger quantity of glycogen droplets in the cytoplasm, being an identical result for the IIA-type fibers. The glycogen content in the I-type fibers was shown with intermediate intensity (++), regardless on the age and muscle biopsy depth. In the males, the glycogen content was shown to be high (+++) for the IIA- and IIX-type fibers, and intermediate (++) for the I-type fibers. Nevertheless, for the intramuscular lipid staining (Table 2; Figure 1B and C) the I-type myofibers were stained with a strong intensity, showing a high lipid content (+++), followed by IIA-type fibers that presented an intermediate content (++) and, at last, the IIX-type fibers, showing a neglectable lipid concentration (-). That result was regardless on the age, sex and muscle biopsy depth.

In the BH horses undergoing mild activities for 10 months, the glycogen results (Table 1) showed that the IIA- and IIX-type fibers were intensively stained revealing a high (+++) glycogen concentration, and the I-type fibers were stained with intermediate intensity (++), both in inactive animals and in those having trained for 10 months. Regarding the intramuscular lipid content, the I-type myofibers revealed a high concentration (+++) followed by the IIA-type fibers, which presented intermediate concentrations and, at last, the IIX-type fibers, presenting a neglectable concentration(-). We did not observe differences regarding glycogen and lipids when we compared the staining intensities in inactive animals and in those having trained for 10 months.

The highest accumulation of glycogen presented by the females in all types of fibers compared to the males of the same breed corroborated the studies of BRAUN & Horton (2001) and Hamadeh et al. (2005) who, studying the endurance exercise in women, confirmed the important role of the strogenous hormones in the regulation of metabolic substrates. In animal models, strogene promotes lipolysis and an increase in the availability of fatty acids (HATTA et al., 1988, KENDRICK & ELLIS, 1991) with a reduction in the total oxidation of carbohydrates during the exercise due to the decrease in the capture of blood glucose and other carbohydrates, such as glycogen (D'EON et al., 2002). This way, females seem to have an increase in the sensitivity towards insulin in the skeletal muscles, what may cause, according to WISMANN & Willoughby (2006), an increase in the muscle glycogen stocks.

The fact that no changes in the glycogen and lipid contents in inactive animals when compared to the animals trained for 10 months were not observed, may be due to the fact that sample collections of the *gluteus medius* were performed with animals at rest. For a better analysis of the glycogen and lipid reservations during a training program, Serrano et al. (2000) suggest the collection of muscle biopsies shortly after a test exercise, what was not possible to be accomplished during this project.

One can conclude that the 10-month physical activity, in the way it was conducted at the "Nove de Julho" Military Police (PM) Battalion of the São Paulo State (SP), was not able to change the reservations of glycogen during rest. On the other hand, the fact that females presented larger reservations of glycogen might suggest a better performance of those animals in energy-demanding situations of short and average duration and intensity. Another noteworthy point, related to the biopsy depth, is that it can be made at a 20-mm depth, what, theoretically, would be less traumatic than deeper biopsies.

Table 1. Glycogen content in the different types of muscle fibers of the *Gluteus medius* muscle of inactive horses of different ages and both sexes and in active males of the BH breed in the 20-mm and 60-mm depths.

	Age	Sex	Glycogen content					
			20 mm			60 mm		
			I	IIA	IIX	I	IIA	II
Inactive	1 year old	F	+++	++++	++++	+++	++++	++++
		M	++	+++	+++	++	+++	+++
	2 years old	F	+++	++++	++++	+++	++++	++++
		M	++	+++	+++	++	+++	+++
	3 years old	F	+++	++++	++++	+++	++++	++++
		M	++	+++	+++	++	+++	+++
	4 years old	M	++	+++	+++	++	+++	+++
Active	4 years old	M	++	+++	+++	++	++	+++

F: female; M: male; N: number of animals per group; ++intermediate; +++ high; ++++ high and with difficulty of cell visualization.

Table 2. Lipid content in the different types of muscle fibers of the *Gluteus medius* muscle of inactive horses of different ages and both sexes and in active males of the BH breed in the 20-mm and 60-mm depths.

	Age	Sex	Lipid content					
			20 mm			60 mm		
			I	IIA	IIX	I	IIA	II
Inactive	1 year old	F	+++	++	-	+++	++	-
		M	+++	++	-	+++	++	-
	2 year old	F	+++	++	-	+++	++	-
		M	+++	++	-	+++	++	-
	3 years old	F	+++	++	-	+++	++	-
		M	+++	++	-	+++	++	-
	4 years old	M	+++	++	-	+++	++	-
Active	4 years old	M	+++	++	-	+++	++	-

F: female; M: male; N: number of animals per group; +++ high; ++intermediate ; - neglectable

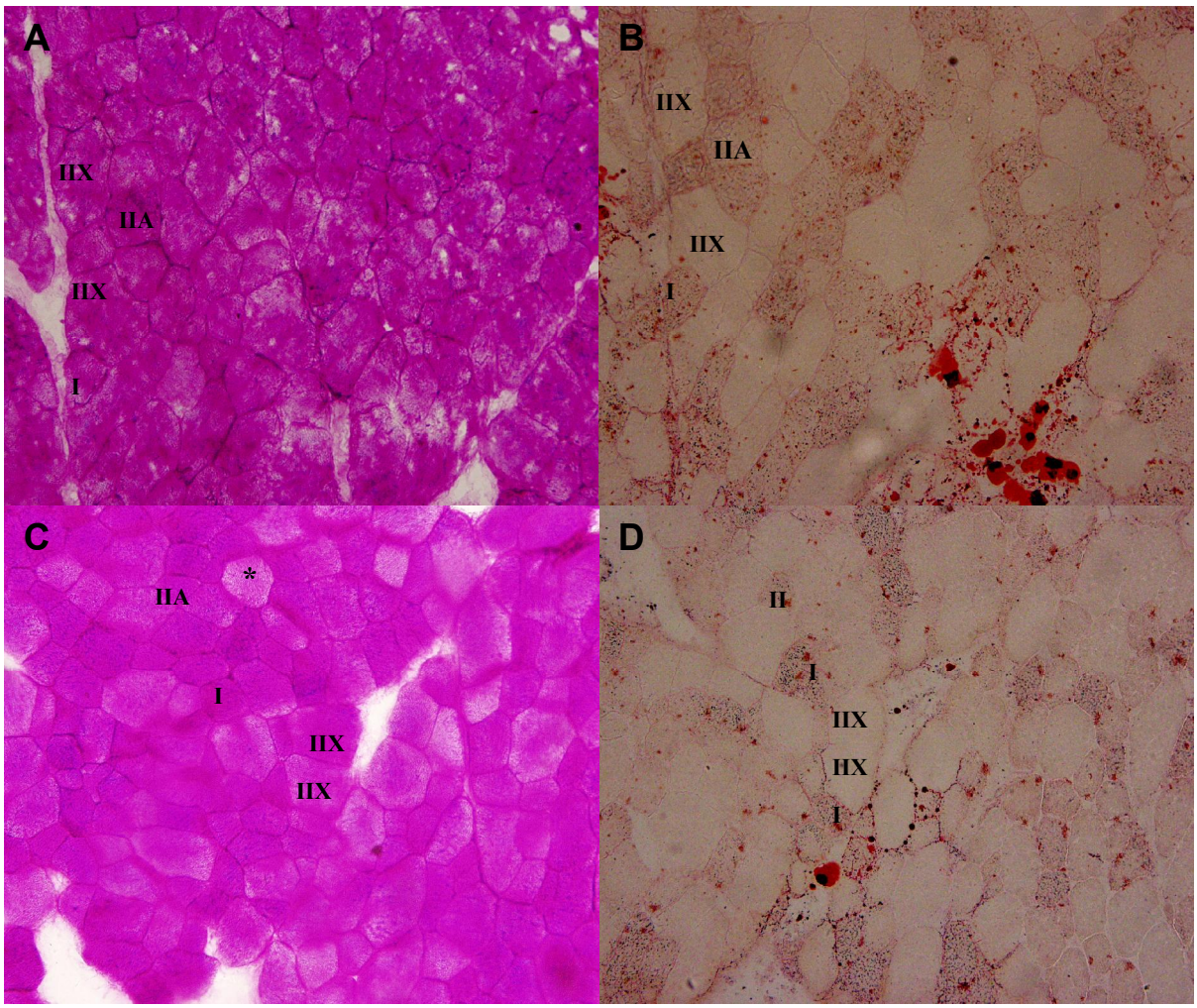

Figure 1. Photomicrographies of transverse serial histological cuts of the *gluteus medius* muscle of inactive stallions of the BH breed in the 60-mm biopsy depth. A) PAS staining for glycogen and B) Oil red O staining for lipids, in 4-year-old horses. C) PAS staining for glycogen and D) Oil red O staining for lipids, in 3-year-old horses. Glycogen content: I (++), IIA(+++), IIX(+++); Lipid content: I (+++), IIA (++), IIX (-); * Intermediate glycogen content (++) in an I-type myofiber (atypical result); 200X.

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