Fiber Type Composition in Semitendinous Muscle of Wistar Rats and Effects of Intermittent Training on its Hypertrophy

Composición de los Tipos de Fibras del Músculo Semitendinoso de Ratas Wistar y los Efectos del Entrenamiento Intermitente en su Hipertrofia

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SUMMARY: Skeletal muscles respond to several stimuli changing their phenotype. Muscular fibers adaptation capability is related to the presence of several myosin heavy chains (MHC). These express four types of pure fibers: I, IIA, IID and IIB containing MHCI, IIA, IID and IIB, respectively. Among pure fibers, there are hybrid fibers, which can express two or more types of myosins. In this study, types of fibers constituting male Wistar rats semitendinous and their myosin heavy chains, as well as influence of intermittent training on hypertrophy of these fibers have been checked through MATPase histochemical technique and electrophoretic proteins separation. All types of pure and hybrid muscular fiber have been found, however the fibers of the types IIA, IID and IIB were predominant, featuring muscle as a fast-contracting one. Training has promoted muscular fibers transition with a significant increase of fibers of IC, IIAD and IIDB type. A cross-section increase of fibers of IIDB and IIB type has also been noticed. In summary, semitendinous muscle is essentially constituted by fast-contracting fibers and training could promote transition and hypertrophy of these fast fibers.

KEY WORDS: Semitendinous muscle; Muscle fibers; Intermittent training; Hypertrophy.

INTRODUCTION

The presence of different myosin heavy chains (MHC) allows skeletal muscles to adapt to several stimuli. These adaptations occur through gene changes that will express a new muscular fiber pattern, contributing this way to a heterogeneous phenotype (Demirel *et al.*, 1999).

In adult rat skeletal muscles, there are four myosin heavy chains: MHCI, which will express slow-contracting of I type and, MHCIIa, MHCIIId and MHCIIb, present in fast-contracting fibers of IIA, IID and IIB types, respectively (Campos *et al.*, 2002). Hybrid fibers can be found among pure fibers, expressing two or more myosin types (Tajsharghi *et al.*, 2004). This way, specific stimuli applied on muscles can provoke transition both from I to IIB (I=>IIA=>IID=>IIB) and from IIB to I (IIB=>IID=>IIA=>I), always passing through hybrid ones, thus changing their phenotype.

Physical exercise is one of the specific factors affecting muscular fiber change (Andersen *et al.*, 2000; Pette & Staron, 2001). For example, low-intensity and long-

duration physical training may induce a conversion from fast to slow fibers. On the other hand, a high-intensity and short-duration training can lead to an opposite transition, that is, from slow to fast (Caiozzo *et al.*, 2003).

The purpose of this study is to analyze fiber types constituting male Wistar rat semitendinous muscle and its myosin heavy chains, as well as to show these muscular fiber behavior when submitted to intermittent training.

MATERIAL AND METHOD

Animals. Sixteen Wistar breed and 21-day old male rats were used in the study. Two groups have been constituted with eight animals each, a control group (CG) and a trained group (TG). During the experimentation, animals were kept in a controlled-temperature environment (18 to 22°C), with a photoperiod of 12 hours and provided with food and water *ad libitum*.

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Training protocol. Animals have been trained on a motorized treadmill, during 6 days/weeks, for 8 weeks through intermittent training protocol (Table I) (Smolka *et al.*, 2000). CG animals have not performed any kind of exercise.

Table I. Training protocol of the rats.

Weeks	V (m/min)	T (min)	Interval (min)	Repeats
1 st	20	5	2	4
2 nd	30	2.5	1	4
3 rd	32.5	2.5	1	6
4 th	35	2.5	1	6
5 th - 8 th	35	2.5	1	6

Sample preparation. After the training period, animals were sacrificed with an overdose of Ketamine and Kyalasine Chloridrate (1:1). After this procedure, semitendinous muscle was removed and frozen in cooled isopentane in liquid nitrogen at -176°C and stored in a freezer at -80°C. Then, 12 mm thick cross section were obtained from samples, in a cryostat, at 24°C.

Histochemical and morphometric analysis. Sections have been submitted to mATPase (myofibrillar adenosine triphosphatase) reaction (Pette & Staron) after pre-incubations in pH of 4.3, 4.5 (Brooke & Kaiser, 1970) and 10.3 (Guth &

Samaha, 1970), and fibers types were identified and counted. Cross-section area of about fifty fibers of each type (I-IIA-IID-IIB) per animal was measured through Image ProExpress program.

Biochemical analysis. Sections of each sample have been collected for biochemical analysis. These were placed in 0.5ml of following medium: 10% (w/v) glycerol, 5% (v/v) 2 mercaptoethanol and 2.3% (w/v) sodium dodecylsulfate (SDS), in 62.5 mM Tris/HCL buffer (pH 6,8). Cuts were agitated during one minute and heated for 19 minutes, at 60°C. Extract portions (7 ml) were submitted to electrophoresis in gel (gradient of 7-10%) of polyacrylamine (SDS), with 17-18 hours of running at 120V. After coloration with silver (Blum *et al.*, 1987), gel was used to determine presence of MHC identified according to respective molecular masses. A soleum muscle sample was also included as control, as it presents in rats around 95% of MHCI and 5% of MHCII (Staron *et al.*, 1999).

Statistical analysis. Results have been analyzed and expressed through their averages and standard deviation. Statistical significance of differences between averages of both groups was calculated through test t of Student and Mann Whitney for independent samples, through Instat program. Significance for $p < 0.05$ was considered significant.

Fig. 1: mATPase histochemical reaction at pH 4.5(a,d), 4.3 (b,e) and 10.3 (c,f) in serial cross sections of the semitendinous muscle of Wistar rats showed the fibers types. Red portion (a, b, c) and white portion (d, e, f). I-type I, IIC- type IIC, A- type IIA, AD- type IIAD, D- type IID, DB- type IIDB e B- type IIB. Authors' name: Ferreira, AD and Campos GER.

Fig. 2: Electrophoretic separation of myosin heavy chain (MHC) isoforms from Wistar rat semitendinosus muscles. This separation own MHC-IIa, MHC-IIb, MHC-IIc and MHC-I. TG : Trained Group , CG : Control Group, SEM: semitendinosus muscle and SOL: soleus muscle. Authors' name: Ferreira, AD and Campos GER.

RESULTS

Fibers types and Myosin heavy chains (MHC). All pure fiber types were present in this muscle (I, IIA, IID and IIB), as well as hybrid fibers IC, IIC, IIAD and IIDB (Fig. 1).

Through electrophoretic separation, presence of 4 myosin heavy chains (MHCI, IIA, IID and IIB) was evidenced in both groups. The IIB was predominant, the IIA and IID have showed a slight difference as to migration distance, and a similar proportion, while band I was slightly noticed (Fig. 2).

Training effects. A significant increase on hybrid fibers of IC, IIAD and IIDB types could be noticed (Fig. 3) when was analyzed the all muscle. Cross-section area of red portion fibers of semitendinosus muscle presented no change (Table II.B). On white portion, only IIB fibers showed a significant increase in their area (Table II.C) and, when both muscle portions were analyzed together, it was noticed that IIDB hybrid fibers suffered hypertrophy (Table II.A).

DISCUSSION

According to histochemical analysis of Wistar rat semitendinosus muscle, all pure fibers were present, in which fast-contracting ones (IIA, IID and IIB) were the most expressive in the bouth groups, thus, semitendinosus muscle can be rated as an essentially fast-contracting muscle. Some studies carried out with the same muscle, but from other animal species, showed results that corroborate this information (Delp & Duan, 1996; Jurie *et al.*, 1998). Electrophoretic separation of myosins in semitendinosus muscle samples evidenced four bands: IIB, found in larger quantity, followed by IID, IIA and I, respectively. This way, data confirm existence and proportion of IIB, IID, IIA and I fibers seen in optical microscopy (Figs.1 and 2).

Physical exercise is an important factor for muscular phenotype conversion. It can lead to a fiber bi-directional transition (Goldspink, 2003), depending on its periodicity, which can be set according to continuous charge principle (continuous training), mainly in long-duration activities, with higher volume and lower intensities. Or periodic charge one, whose directing principle is a lower duration and higher intensity, features that can be

Fig. 3: Quantity of type fibers expressed in the whole semitendinosus muscle (red portion plus white portion) of the control group and trained group. The fiber type IC, IIAD and IIDB showed increased statistical significant. * Statistical significance.

Table II. Comparison between the cross-sectional area of different fiber types of the semitendinous muscle of Wistar rats of both groups (TG and CG) with their means and standard deviation and also statistical significance. A. presents the cross-sectional area in the whole muscle carrying hypertrophy in the IIDB fibers. B. presents the cross-sectional area in the red portion, and don't have statistical significance in none fiber types. C. presents the cross-sectional area in the white portion carrying hypertrophy in the IIB fibers.

A	Types of Fibers							
	I	IC	IIC	IIA	IIAD	IID	IIDB	IIB
TG	1337±222	1397±160	893±349	1519±412	1391±346	1899±471	2176±486*	3249±655
CG	1443±248	1438±490	1269±285	1650±394	1666±309	1853±251	1737±341*	2769±254
B								
TG	1322±234	1379±173	893±349	1511±424	1393±364	1927±497	2059±479	2832±616
CG	1443±252	1438±490	1269±285	1660±408	1685±432	1929±380	1647±474	2626±441
C								
TG	-----	-----	-----	1585±414	1585±414	1830±450	2283±873	3729±742*
CG	-----	-----	-----	1582±369	1611±288	1736±290	1923±246	2876±222*

* Statistical significance values

worked out in intermittent training, in which physical exercise is performed in a discontinuous manner and is interrupted at regular intervals and then, applied again.

This way, protocol applied in this study is considered as intermittent (Smolka *et al.*) and induced muscular fibers changes. Therefore, observing the Fig. 3, an increase of IC, IIAD and IIDB hybrid fibers could be noticed, suggesting that training has induced MHC levels changes, through disturbances in genes expressing these proteins (Harrison *et al.*, 2002), but was not able to fully convert them, maybe due to protocol application time or to and intensity that was not sufficiently high to promote full conversion. Nevertheless, fast-contracting fibers group presented the higher interference. So, that applied protocol recruits more fast-contracting fibers than slow-contracting ones. Training applied could not induce significant changes on red region muscular fibers size (Table IIB). On white region, there was a significant increase of fibers IIB area, and can be noticed

on the whole muscle a hypertrophy of the IIDB (Table IIC and A), both representing fast-contracting group. According to previous studies, most recruited fibers in short-duration exercises are fast-contracting ones, indicating a high proteins production of some of these fibers, provoking their hypertrophy (Bottinelli & Reggiani, 2000; Talmadge, 2000; Lin *et al.*, 2002).

In summary, it was demonstrated that semitendinous muscle is mainly constituted by fast-contracting fibers and that intervalled training could promote muscular fibers transition, with a significant increase of hybrid fibers IIC-IIAD- IIDB and a hypertrophy of fast-contracting fibers IIDB and IIB.

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RESUMEN: Los músculos esqueléticos responden a diversos estímulos cambiando su fenotipo. La capacidad de adaptación de las fibras musculares está relacionada con la presencia de diversas miosinas de cadena pesada (MHC). Estas miosinas expresan cuatro tipos de fibras puras: I, IIA, IID, IIB, que contienen MHCI IIA IId IIB, respectivamente. Entre las fibras puras hay fibras híbridas, las cuales pueden expresar dos o más tipos de miosinas. En este trabajo, se observaron los tipos de fibras y las cadenas pesadas de miosinas del músculo semitendinoso en ratas Wistar macho, así como también, la influencia del entrenamiento intermitente en la hipertrofia de aquellas fibras, a través de la técnica histoquímica de mATPasa y separación eletroforética de proteínas. Todos los tipos de fibras musculares puras e híbridas fueron encontradas, siendo las fibras de tipo IIA IID y IIB predominantes, por ser un músculo de contracción rápida. El entrenamiento promovió la transición de las fibras musculares con un aumento significativo de las fibras del tipo IC, IIAD y IIDB. En una sección transversal, un incremento de fibras del tipo IIDB y IIB también fue reportada. En resumen, el músculo semitendinoso está compuesto esencialmente por fibras de contracción rápida y el entrenamiento puede promover la transición e hipertrofia de las fibras musculares rápidas.

PALABRAS CLAVE: Músculo semitendinoso; Fibras musculares; Entrenamiento intermitente; Hipertrofia.

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