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Correlation between the proportion of fast fibers in the biceps brachii muscle and the relative torque during elbow flexion in subjects with clinical hypothesis of myopathy

Correlação entre a proporção de fibras rápidas do músculo bíceps braquial e o torque relativo da flexão do cotovelo em sujeitos com hipótese clínica de miopatia

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Abstract

Muscular strength production may be impaired in myopathic patients. Myopathies represent a heterogeneous group of diseases with distinct clinical and morphological characteristics. It has been hypothesized that functional performance findings may be related to the predominant fiber type expressed in the muscle analyzed. **Objective:** To correlate the proportion of type 2 fibers in the biceps brachii muscles of subjects with a clinical hypothesis of myopathy with their peak isometric and isokinetic torque during elbow flexion. **Method:** Seven subjects with a clinical hypothesis of myopathy participated in this study: four females of mean age 37 years (sd = 9), weight 73kg (sd= 26) and height 155cm (sd= 6); and three males of mean age 39 years (sd= 1), weight 88kg (sd= 5) and height 172cm (sd= 4). The muscle fiber proportion was analyzed using the mATPase technique. One month after taking biopsies, the subjects performed concentric isometric and isokinetic strength tests for elbow flexion and extension using an isokinetic dynamometer. The isometric and isokinetic peak torques at 90°s-^{1} and 180°s-^{1} were evaluated and the relative 90° (RT90) and 180° (RT180) torques were calculated. Spearman's correlation (r) was used for statistical analyses. **Results:** The proportion of type 2 fibers correlated positively with RT180 (r= 0.89, p= 0.01), and there was a moderate correlation with RT90 (r= 0.75, p= 0.05). **Conclusions:** The results suggest that the contractile behavior of type 2 fibers was not modified in these subjects. The isokinetic dynamometer was shown to be an instrument capable of noninvasively evaluating muscle fiber type predominance.

Key words: myopathy; skeletal muscle; biopsy; myosin; isokinetic test.

Resumo

A produção de força muscular pode estar comprometida em pacientes portadores de miopatias. Estas representam um grupo heterogêneo de doenças com distintas características clínicas e morfológicas. Supõe-se que achados de desempenho funcional estejam relacionados com o tipo predominante de fibra expresso no músculo em análise. **Objetivo:** Correlacionar a proporção das fibras tipo 2 (FT2) do músculo bíceps braquial de sujeitos com hipótese clínica de miopatia (HCM) com picos de torque isométrico e isocinético de flexão do cotovelo. **Materiais e métodos:** Participaram deste estudo sete sujeitos com HCM: quatro do sexo feminino com média de idade de 37 anos (dp= 9), peso de 73kg (dp= 26) e altura de 155cm (dp= 6); e três do sexo masculino com média de idade de 39 anos (dp= 1), peso de 88kg (dp= 5) e altura de 172cm (dp= 4). Pela técnica histoquímica de mATPase, foi realizada análise de proporção das fibras musculares. Após um mês da realização das biópsias, os sujeitos realizaram teste de força isométrica e isocinética concêntrica de flexão e extensão do cotovelo em dinamômetro isocinético. Avaliou-se o pico de torque (PT) isométrico a 90°s-^{1} e 180°s-^{1} e calculou-se o torque relativo 90 (TR90) e 180 (TR180). Para análise estatística, utilizou-se correlação de *Spearman* (r). **Resultados:** A proporção de FT2 se correlacionou positivamente com TR180 (r= 0,89, p= 0,01). Uma moderada correlação foi encontrada entre FT2 e TR90 (r= 0,75, p= 0,05). **Conclusões:** Os resultados sugerem que o comportamento contrátil das FT2 não foi modificado nestes sujeitos. O dinamômetro isocinético mostrou ser um instrumento que pode avaliar, de forma não invasiva, a predominância do tipo de fibra muscular.

Palavras-chave: miopatia; músculo esquelético; biópsia; miosinas; isocinético.

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Introduction

Some factors may influence the production of muscle torque during the human movement. We can highlight[1] the activation of the skeletal muscle by the central nervous system by means of the recruitment and firing rate of the motor units[2], the organization of the osteomioarticular system, that is, the patterns of the muscular fibers and levers formed by the articular anatomy[3] and the intrinsic mechanical characteristics of the muscular tissue[1].

The muscular tissue presents fibers with different shortening speeds. The miofibrilar ATPase (mATPase) located in the myosin heavy chain (MHC) isoforms perform the ATP hydrolysis which partially determines this difference[2-7]. The predominance of the slow isoform promotes the ATP's low hydrolysis, slow shortening speed and classifies the muscle fibers as being of the slow-twitch type. The predominance of fast isoform promotes high ATP hydrolysis, rapid shortening speed which characterizes the so-called fast-twitch fibers. In general, stemming from a histochemical analysis, the predominance of slow isoform classifies the fibers as being type-1 (T1F) and the predominance of fast isoform classifies the fibers as being of type-2 (T2F)[8]. Several fiber types in variable distributions coexist in the skeletal muscle. So, the mean of the performances of all fibers determines the muscular mechanic behaviour[9]. On the other hand, the predominance of a particular type of fiber determines the muscle function as a whole, that is, whether the muscle will be a fast or slow-twitch one [10].

Due to the differences in the shortening speed of the muscular fibers, T2F fibers demonstrate a greater torque production and power at high twitch speeds than the T1F ones [11,12]. This piece of information was obtained from the analysis of isolated muscular fibers *in vitro*. Nevertheless, with the advent of the isokinetic dynamometer, it has become possible to measure the muscular torque *in vivo* at controlled movement speeds. Studies were then begun whose objective was to evaluate whether the fibers' twitching contractile differences obtained *in vitro* could be observed with the analysis of the muscular performance *in vivo*. In order to achieve this, isokinetics aims at correlating variables such as torque peak, strength and workload at different speeds of the isokinetic test with the various fiber distributions, especially those of the T2F type of the skeletal muscle, both in ordinary individuals as well as in athletes[13-18]. On the whole, in these studies a positive association was observed between the percentage of type-2 fiber and isokinetic variables such as the peak torque.

Nevertheless, few studies have correlated muscle morphology and function to the diseases that primarily affect this tissue, as in myopathic patients. Most studies concerned with myopathies analyze muscle performance in order to evaluate the effectiveness of applying various protocols of maximal and submaximal resistance training measuring the peak torque and fatigue resistance isokinetically and comparing these findings with a control group[19-21]. Myopathies represent a group of diseases with heterogeneous physical pathologies and diversified clinical manifestations. The clinical signal of muscular weakness is extensively reported, with variable degrees of problems affecting these individuals' quality of life and independence[22]. Many myopathies can be classified according to the morphological findings obtained from the biopsies. However, sometimes these findings are considered too unspecific to classify a determined disease, such as the oscillation in the T1F and T2F proportions.

Taking the aforementioned data into account, it was hypothesized that myopathic patients with clinical signs of weakness might indicate, in the isometric and concentric tests carried out at different speeds, associations supposedly different from those observed in normal subjects between T2F and the muscular torque. By using fiber proportion analysis from biopsies of the brachial biceps muscle of subjects with a clinical hypothesis for myopathy, as well as isokinetic and isometric muscle performance of the elbow flexors, it was possible to observe that, in spite of the fundamental disease, the correlation between the functional performance analyzed by the peak torque and the isokinetic / isometric relations with the various T2F proportions was positive, suggesting that the contractile behavior of those fibers do not seem to have been modified.

Methods

Sample

Seven subjects with a clinical hypothesis for myopathy underwent a biopsy of the brachial biceps muscle for diagnostic confirmation, being assisted at the Neuromuscular Diseases Out-Patient Clinic of the Faculdade de Medicina de Ribeirão Preto (Ribeirão Preto Medical School) (ANEM- FMRP). The individuals signed a term of free consent participation form. This project was approved by the Research Ethics Committee of the Hospital das Clínicas de Ribeirão Preto (Protocol n° 10934/2003).

From this group of seven subjects, four were women, 37 years old on average (SD 9), with an mean weight of 73kg (SD 26), and a mean height of 155 cm (SD 6); and three were men, being on average 39 years old (SD 1), weighing 88kg (SD 5), and a mean height of172 cm (SD 4).

Morphometric study

The processing of the muscles followed the regular procedures of the Laboratory of Neuropathology of the Department of Pathology at the Faculdade de Medicina de Ribeirão Preto (Ribeirão Preto Medical School). The slides underwent a histoenzymologic reaction to pre-incubated myofribrilar ATPase (mATPase, E.C.3.6.1.3.) in an acid and alkaline environment for the characterization of Type-1 (T1F) and Type-2 (T2F) muscular fibers. The morphometric analysis was carried out with the assistance of the QualiView (Atonus) software. The images were obtained by means of a Leica 300 FX digital video camera attached to a Leica DM 2500 binocular microscope and to an IBC-PC microcomputer.

The proportion analysis for T1F and T2F was made by using images collected from five random fields of each muscle sample in slides processed in pH 9,4. A trained observer counted the different kinds of fibers that each field contained. For each subject the mean value set for each kind of fiber was obtained from mean of the five analyzed fields.

Quantitative evaluation of muscular performance

For the muscular performance analysis, we used a BIODEX computerized isokinetic dynamometer (model Biodex Multi-Joint System 2), including accessories for the evaluation of the elbow joint, and the Biodex v.4.5 Software, as well as an HP Deskjet 660 printer from the Physical Therapy Out-Patient Clinic of the Universidade Federal de São Carlos (UFSCar). The patients were previously warned that they should not ingest any alcoholic drinks or practice strenuous physical activities the day before the isokinetic test. Prior to the test, the subjects underwent a clinical physical therapy test aimed at evaluating their general physical condition, any reports of pain or fatigue in the biopsied limb and functional segment impairment. After the clinical test, the muscles of the upper limbs were "warmed up" on a load-free cyclo-ergometer for three minutes. After the warm-up, the patients were positioned seated on the isokinetic dynamometer chair, the back of which was set at a 90° angle, and were stabilized on the chair with belts adjusted onto their thorax, pelvis and tested arms. The dynamometer's mechanical rotation axis was aligned with the lateral humerus. The shoulder was positioned at 30° in the plane of the scapula, at 30° of abduction in the frontal plane, 0° of flexion and having the forearm in supine position. The device used to hold the forearm was attached to its middle finger and the resistance application was positioned on the middle part of the metacarpals, so that the individual exerted a pressure on the distal part of the resistance device. To familiarize the subjects with the equipment, two submaximal contractions were performed at each test speed prior to data collection.

The individuals performed a maximal isometric contraction for five seconds having their elbows positioned at a 90° flexion. Three measurements were taken, at one minute intervals. Next, the isokinetic evaluation was performed at reciprocal, continuous, and concentric modes of elbow flexion and extension at two angle speeds: 90°s^{-1} (five repetitions) and 180°s^{-1} (15 repetitions). The amplitude of the movement was set at between 60° and 130° of the elbow flexion. A 10-minute rest interval was taken between the isometric and the isokinetic tests, and between both isokinetic speeds, aimed at avoiding any possible effects of muscular fatigue. For each individual, the following data were obtained: isometric and isokinetic peak torque (PT) at 90°s^{-1} and 180°s^{-1}. Stemming from these results normalized for body weight, the relative torques (RT) were calculated in which the peak torque values isokinetically obtained were divided by the isometrically obtained peak torque and multiplied by 100, following the methodology suggested by Gür et al.[1]. During the tests, patients were given standardized and constant verbal encouragement in order to exert maximal strength during the contractions.

Statistical analyses

The descriptive analysis of the data was carried out and presented as means and standard deviations of the means. In order to correlate the morphometric and the functional performance results, Spearman's correlation coefficient (*r*) was used, with tests of significance[23]. For these tests, a level of significance of 5% was applied.

Results

T2F proportions

The mean percentage of T2F distribution among the individuals was of 56% (SD 11). The individual proportion ranged from 44 to 69%. The individual results can be observed on Table 1.

Table 1. Distribution of type 2 fibers (T2F), the diagnostic hypotheses (DH), isometric peak torques (PTI), isokinetic peak torques at 90 (PT90°s^{-1}) and at 180 (PT180° s^{-1}) degrees per second normalized by body mass and relative torques (RT90 and RT180) of the subjects.

Subject	Gender	T2F (%)	Diagnosis Hypothesis (DH)	IPT (%)	PT90°s^{-1} (%)	PT180°s^{-1} (%)	RT90 (%)	RT180 (%)
S1	F	69	Distal myopathy	47	41	32	87	68
S2	F	61	Mitochondrial myopathy	49	38	33	78	68
S3	F	58	Mitochondrial myopathy	30	21	18	70	61
S4	F	45	Metabolic myopathy	67	46	33	69	49
S5	M	44	Cannulopathy	81	58	44	71	54
S6	M	69	Metabolic myopathy	76	72	59	95	78
S7	M	47	Girdle dystrophy	43	37	28	86	66

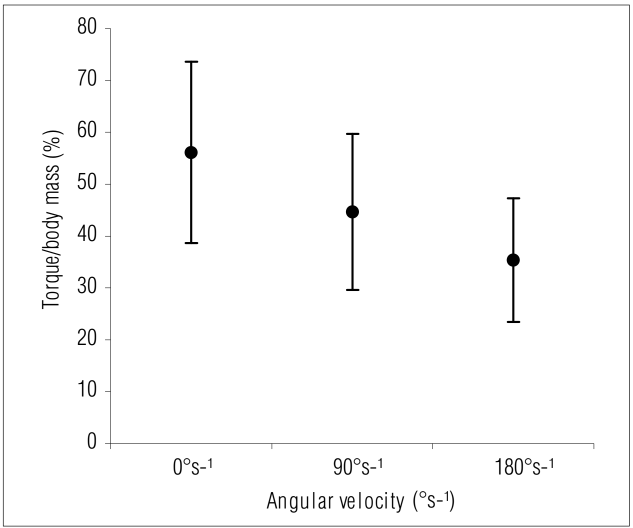

Figure 1. Mean values normalized by body mass to isometric peak torque (0°s-1), isokinetic peak torque at 90 (90°s-1) and at 180 (180° s-1). Vertical bars represent 95% confidence intervals.

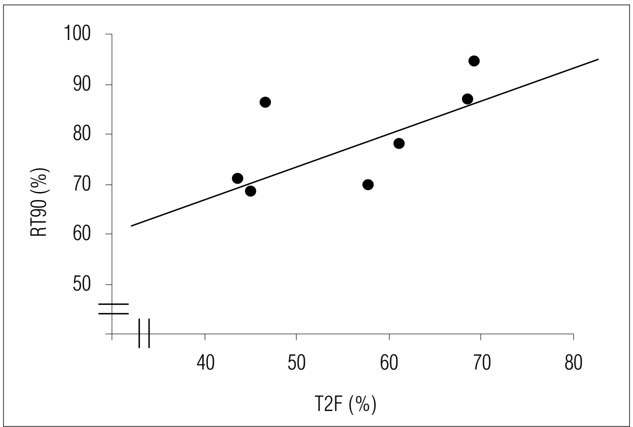

Figure 2. Values obtained at RT90 and type 2 fibers of subjects. Spearman correlation coefficient 0.75 (p= 0.05) n= 7.

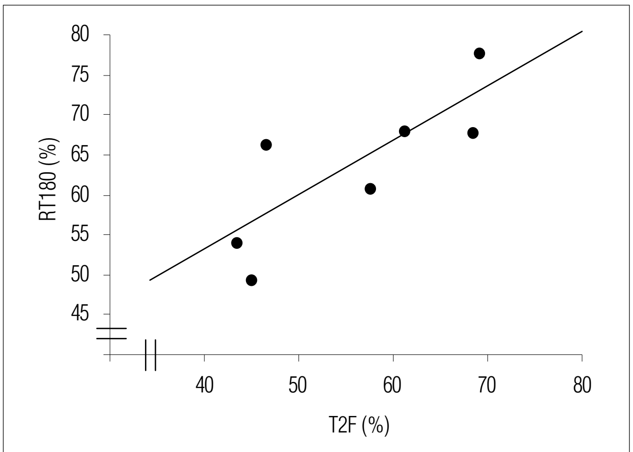

Figure 3. Values obtained at RT180 and type 2 fibers of subjects. Spearman correlation coefficient 0.89 (p= 0.01) n= 7.

Torque peaks and isokinetic / isometric torque relations

The mean values for isometric and isokinetic PT at 90°s-1 and 180°s-1, normalized for body weight, were 56% (SD 19), 45% (SD 16) and 35% (SD 13), respectively (Table 1 and Figure 1). The mean values for RT90 and RT180 were 79% (SD 10) and 63% (SD 10), respectively. The individual values for isometric PT ranged from 30 to 81%, for isokinetic PT at 90°s-1 ranged from 21 to 72% and at 180°s-1 ranged from 18 to 59%. For RT90 and RT180, the values ranged from 69 to 95% and from 50 to 78%, respectively (Table 1).

Correlations between T2F distribution and peak torque values and isokinetic / isometric relations

A moderate correlation was found between T2F proportion and RT90 for the Spearman's coefficient (r= 0.75, p= 0.05). For RT180 this positive correlation was higher (r= 0.89, p=0.01) (Figures 2 and 3).

Discussion

This study hypothesized that subjects with a clinical hypothesis for myopathy could present abnormalities in the association between functional performance and T2F percentage, different from the findings obtained with normal subjects, since they demonstrated clinical signs of muscular weakness. However, the results of the present study showed that, even in subjects with a clinical hypothesis for myopathy, the individual variations in the distribution of T2F correlated positively with muscular performance. It is relevant to point out that the specific diagnosis of a myopathy may not ultimately be characterized by the medical team, but only by the group of signs and symptoms demonstrated by the patient, including the analysis of the muscular biopsy. Nevertheless, the morphological changes may have directly interfered in the functional performance of the involved segment, as occurred in this study.

According to studies developed by Johnson et al.[24], the biceps brachii muscle of normal individuals demonstrate an T2F distribution interval ranging from 49.3 to 66.2%. Of the percentage values of the T1F and T2F proportions of the biceps muscles analyzed, we observed that only two subjects showed a pattern of muscle fiber distribution equivalent to the results suggested in the aforementioned literature; two subjects had a predominance of T2F; and three demonstrated T1F predominance.

It is known that an increase in the speed of muscular contraction leads to a diminishment of the production of muscular strength. This relation between strength and contraction speed was initially obtained in isolated muscular fibers[5]. However, stemming from isokinetic evaluations of several muscle groups, it was observed that the strength-speed relationship of the isolated muscular fiber obtained *in vitro* also holds true for the muscular torque-isokinetic speed relationships of the muscle groups[25]. As expected from the torque-speed relationship[25], all of the subjects in the present study showed a decrease in elbow flexion PT at higher isokinetic speeds. However, the PT variations in relation to angle speed demonstrated individual differences that were accompanied by different T2F percentages. This could be observed by the correlations of RT90 and RT180 to T2F percentage. For Gür et al.[1], the use of relative torque makes it possible to minimize the differences between individuals concerning muscle fiber patterns and positioning, as well as in relation to the mechanical levers formed by the articular anatomy, muscle and tendon elasticity, and the recruitment of motor units. Therefore, this kind of analysis is particularly useful for the study of heterogeneous samples, such as found in the present case.

The correlations obtained in this study between the percentage of T2F and the relative torque (isokinetic/isometric) of myopathic patients is in accordance with the contractile performance expected from the T2F fibers obtained in *in vitro* and *in vivo* studies in normal individuals and in athletes. According to Gülch[10], stemming from studies carried out *in vitro,* the T2F fibers, besides demonstrating higher shortening speeds, can also produce greater forces when speed and contractions increase, when compared to the T1F ones. The same seems to occur *in vivo* with speed increases in the isokinetic tests. Put another way, the individuals with higher T2F proportions have greater torques at higher isokinetic speeds than the individuals who have a low proportion of that fiber. Aagard and Andersen[26] found a positive correlation between T2F percentage, analyzed through *MHC*, of the *vastus lateralis* muscle and the peak torques obtained in tests developed at medium and high speeds, although the same results were not observed at low speeds. Gür et al.[1] observed a positive correlation between T2F percentage and isokinetic relative torque at 240°s-^{1} versus 30°s-^{1}.

The results of this study are in accordance those of other studies carried out with both normal individuals and athletes alike, in spite of the clinical hypothesis for myopathy and of the clinical signs of muscular fatigue reported by the subjects. It can be suggested that possible molecular defects of the myopathies that compose the group shown here, did not directly interfere with the strength production capacities at the analyzed muscular angle speeds. It is known that there is a direct relation between the development of muscular strength and myosin isoform[4,6,7]. Nevertheless, according to Oldfors et al.[27], only two types of myopathies were hitherto described as demonstrating myosin mutations located in the genes Glu706Lys and Arg1845Trp. These myopathies do not match the diagnostic hypotheses shown in this study.

On the other hand, in studies in which the skeletal-muscular tissue is directly affected, as in the inferior motor neuron diseases, the contractile characteristics of the muscle fibers seem to be altered by the disorder. Tollback et al.[28] evaluated subjects who made excessive use of the dorsiflexor muscles of the ankle due to poliomyelitis or to a previous injury of the L5 nerve root L5, by comparing them to a control group. The peak torque was measured at 30, 180 and 240°s^{-1}. In addition, angle speeds, and the proportion of fibers of the *tibialis anterior* with mATPase were evaluated. In the control group, there was an inversely proportional relationship between the relative torque and type-1 fiber proportion. In the other group, this relation was not found, although the proportion and the area of those fibers were significantly larger than in the control group. These authors concluded that, due to excessive use, the T1F had their contractile properties modified. Krivickas et al.[29] evaluated subjects with amiotrophycal lateral sclerosis (ALS) compared with a control group. The biopsied muscle was the *vastus lateralis*, with an analysis of the T2F percentage through *MHC*. The measurements of muscular performance were isokinetically carried out. The shortening speed of the individual muscular fibers was analyzed by the isolated fiber test. These authors found a higher T1F contraction speed among the ALS group rather than in the control group. The aforementioned result, in which the contractile behavior of the muscle fibers is modified, contrasts with those obtained by the present study.

Considering the data obtained in the present study, it can be summarized that: (1) There does not seem to be changes in the contractile behavior of the fibers in subjects with a clinical hypothesis for myopathy; (2) the isokinetic dynamometer can be used in a non-invasive manner to evaluate the predominance of a determined kind of muscle fiber; (3) and the most sensitive measurement to be taken in this evaluation is the relative torque, particularly at high speeds. And yet, data from other individuals must be added to the present ones in order to effectively confirm the findings in this paper.

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