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Research article

Assessment of the quadriceps femoris muscle in women after injury induced by maximal eccentric isokinetic exercise with low angular speed

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Abstract
The objectives of this study were to propose a model for exercise-induced muscle injury by way of a maximal eccentric isokinetic exercise at low angular speed, and assess the time course of functional recovery of the injured quadriceps femoris muscle from the maximal voluntary contraction (MVC) torque and electrical activity (root mean square – RMS and median frequency – MDF). The effectiveness of the proposed eccentric exercise in inducing injury was assessed from the activity of creatine kinase (CK). In addition, the presence of edema of the quadriceps femoris muscle was assessed by a visual inspection of the intensity of the magnetic resonance imaging (MRI) signal. These measurements were carried out before and after the exercise. Ten healthy women (21.9 ± 1.5) took part in this study. The injury was induced by 4 series of 15 maximal eccentric isokinetic contractions at 5º/s. The MVC torque reduced up to the 4th day after the exercise ($p < 0.05$). The RMS of the vastus medialis oblique (VMO) and the rectus femoris (RF) muscles decreased on the 2nd (VMO and RF; $p < 0.05$) and 3rd (RF; $p < 0.05$) days after. The MDF of the VMO increased immediately after ($p < 0.05$), whilst the MDF of the RF and VL decreased immediately after (RF; $p < 0.05$), on the 1st (RF and VL; $p < 0.05$) and on the 2nd (VL; $p < 0.05$) days after. The CK activity increased on the 2nd day after ($p < 0.05$). An increase in the intensity of the MRI signal was observed on the 2nd and 7th days after. In conclusion: 1- the eccentric exercise with low angular speed was effective in inducing injury, 2- the quadriceps femoris already started its functional recovery, as shown by the MVC torque and electrical activity, in the first week after the exercise, despite the presence of an increase in the intensity of the MRI signal.

Key words: Muscle damage, torque, electromyography, magnetic resonance imaging, creatine kinase.

Introduction

Exercise-induced muscle injury is a common phenomenon that is associated with strenuous or unaccustomed exercise, particularly physical activities involving eccentric contractions (Sotiriadou et al., 2006). The mechanisms responsible for skeletal muscle injury are physical strain induced by eccentric contractions and post-exercise inflammation-related events (Tiidus, 2000). During overexertion exercises, the strain placed on the muscle fibres results in damage to protein filaments, which, in turn, precipitates an inflammatory response (MacIntyre et al., 1995).

Damage from exercise is repaired within 2-10 days following exercise, depending on the nature and intensity of the activity (Clarkson and Hubal, 2001). Indirect indicators of damage include prolonged losses in muscle strength (Brown et al., 1997; Byrne et al., 2001; Child et al., 1998; Clarkson et al., 1992; Eston et al., 1996), alterations in range of motion (ROM) (Clarkson et al., 1992; Howell et al., 1985; Nosaka and Clarkson, 1995), increased levels of muscle proteins in the blood (e.g. creatine kinase, lactate dehydrogenase, aspartate aminotransferase, and myoglobin) (Clarkson et al., 1992; Hyatt and Clarkson, 1998; Lee et al., 2002; Lee and Clarkson, 2003; Sayers and Clarkson, 2003), and delayed onset muscle soreness (Bajaj et al., 2001; Clarkson et al., 1992; Cleary et al., 2002; Newham et al., 1987; Nosaka et al., 2002; Thompson et al., 1999; Weerakkody et al., 2001).

Maximal eccentric exercise has been used as the model for inducing muscle injury in humans when wishing to study the time course in recovery by way of indirect injury markers. However the studies vary with respect to the muscles tested and the injury inducing models. The majority of the studies asses the time course of recovery after inducing injury in upper limb muscles (Clarkson et al., 1992; Nosaka and Clarkson, 1996; Nosaka et al., 2002; Prasartwuth et al., 2005; Rinard et al., 2000). In addition, the studies that used maximal eccentric isokinetic exercises to induce injury, elaborated models with higher angular speeds (Chen, 2003; Evans et al., 2002; Linnamo et al., 2000; Lund et al., 1998; Prou et al., 1999).

Thus it is possible that the time course of recovery be different for lower limb muscles submitted to injury inducing models that use maximal eccentric isokinetic exercise with a low angular speed, firstly because of the function of these muscles in supporting body weight and secondly, because it appears that the pattern of recruiting motor units depends on the speed of movement (Ewing et al., 1990).

Thus the objectives of this study were: 1) propose a muscle injury inducing model using maximal eccentric isokinetic exercise with low angular speed; 2) assess the time course of recovery of the quadriceps femoris muscle injured by eccentric exercise with low angular speed, with special attention to functional recovery. For this, the maximal voluntary contraction (MVC) torque and electrical activity (root mean square – RMS and median frequency – MDF) of this muscle were assessed.

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Methods

Subjects

Ten healthy adult female subjects (mean ± SD: age 21.9 ± 1.5 years; height, 1.65 ± 0.04m; body mass, 58.4 ± 5.12 kg) volunteered for this study. All of them were sedentary undergraduate students and were not enrolled in any kind of weight training program in the three months that preceded the experiment. Exclusion criteria included the existence of current musculoskeletal pathology affecting the lower limb. Prior to beginning the experiment, all volunteers signed a consent form, and approval was obtained from the University Human Research Ethics Committee, which was conducted in accordance with the Helsinki Declaration.

Experimental design

The study was designed to assess the time course of functional recovery of the injured quadriceps femoris muscle in a group of healthy, sedentary subjects submitted to a protocol of muscle injury induction by maximal eccentric isokinetic exercise with low angular speed. For this: 1- 3 measurements were made on the 3 days preceding the eccentric exercise (3 visits before the eccentric exercise), of the MVC torque and electrical activity (root mean square – RMS and median frequency – MDF). The mean of the 3 measurements was used as the baseline; 2- After the eccentric exercise, MVC torque and electrical activity measurements of the quadriceps femoris muscle were carried out immediately after, during the first 7 days after and one more measurement made between the 21st and 30th days after the eccentric exercise (on one of the days in this period); 3- To determine the effectiveness of the protocol of eccentric exercise in inducing muscle injury, 4 measurements of creatine kinase (CK) activity and of magnetic resonance imaging (MRI) were carried out, the first measurements being made before the eccentric exercise (baseline), and the 2nd and 3rd measurements on the 2nd and 7th days after the eccentric exercise, respectively, and the fourth measurement between the 21st and 30th days after the eccentric exercise (on one of the days in this period); 4- The first measurement of MRI and the first blood sample for the first measurement of CK activity were taken before the baseline measurements for MVC torque and electrical activity.

Maximal voluntary contraction (MVC) torque

An isokinetic dynamometer (Biodex Medical Systems, Biodex Multi-Joint System 2, New York, NY, USA) was used to assess MVC torque.

To familiarize the subjects with this procedure, all subjects performed three submaximal voluntary isometric contractions, with hip and knee joints at 100° and 90° flexion, respectively.

The subjects were stabilized with two thoracic and one pelvic belts. The mechanical axis of rotation was aligned to the lateral femoral condyle. The resistance pad at the end of the lever arm was attached immediately above the medial malleolus of the subjects. MVC torque of the right quadriceps femoris muscle (dominant limb) was assessed through maximal voluntary isometric contraction, with hip and knee maintained as described above.

Six 4-second maximal voluntary isometric contractions were performed, each followed by a 2-minute pause. Therefore, six MVC torque values were obtained, one for each contraction.

Electromyography (EMG)

The electrical activity of the vastus medialis oblique (VMO), vastus lateralis (VL) and rectus femoris (RF) muscles was determined with simple active differential surface electrodes (Lynx Electronics Technologies) and a 16-channel signal-conditioning module (1000-V2, Lynx Electronics Technologies).

The simple active differential surface electrode consisted of two rectangular parallel bars of Ag/AgCl (1 cm in length, 0.2 cm in width, 1 cm apart from each other). These bars were coupled to a rectangular acrylic resin capsule 2.2 cm in length, 1.9 cm in width and 0.6 cm in height. In addition, the electrodes had a common mode rejection ratio with a minimum of 80 dB, internal gain of 20 times and input impedance higher than 10 GΩ.

The signal-conditioning module (1000-V2) had a digital analogue A/D converter (CAD 12/32-60K, Lynx Electronics Technologies) with a resolution of 12 bits, acquisition frequency of 1000 Hz per channel and the Aqdados data acquisition program version 4.6 (Lynx Electronics Technologies). This equipment also presented a Butterworth type filter with a 10.6 to 509 Hz bandpass and a gain of 50 times.

The electrical activity of the VMO, VL and RF muscles was measured simultaneously with the MVC torque of the quadriceps femoris muscle during maximal voluntary isometric contraction. The procedure used to assess the electrical activity was the same as that used for MVC torque.

Before recording the electrical activity of the muscles under study, the skin was shaved and cleaned with 70% alcohol and the electrodes fixed to the skin with micropore adhesive tape. A line from the anterior superior iliac spine (ASIS) to the centre of the patella was traced with a dermographic pen to guide electrode placement at different insertion angles of the portions of the quadriceps femoris muscle. For the recording of the electrical activity of the VMO muscle, the electrode was positioned 4 cm from the superior medial border of the patella (Hanten and Schulthies, 1990), at an inclination angle of 50–55° in relation to the reference line (Lieb and Perry, 1968). For the VL muscle the electrode was fixed 15 cm from the superior lateral border of the patella, with an inclination angle of approximately 13.6°, according to the anatomical study of Bevilaqua-Grosso et al. (1997). For such, a universal goniometer was used to measure the inclination angle of the VMO and VL muscles. The goniometer axis was aligned to the centre of the patella, the fixed arm was aligned to the reference line (line joining the anterior superior iliac spine to the centre of the patella) and the mobile arm was free to move to the desired inclination angle. For the RF muscle the electrode was positioned immediately distal to the bifurcation observed by the pennation of the muscle. This ensured that electrical activity was measured from the RF rather than the sartorius, since the sartorius bypasses this bifurcation (Signorile et al., 1995). In addition, palpation of the muscle belly with the subject in the testing position was also performed to confirm electrode placement. The electrodes were fixed to the midline of the muscle belly with the detection surface perpendicular to the muscle fibres, as suggested by DeLuca (1997). The reference electrode was fixed over the proximal anterior tibial shaft to eliminate possible external interferences. To ensure the reproducibility of the different measurements along the duration of this study, a plastic mould of the quadriceps femoris muscle was elaborated for each subject, in which the positions of all the electrodes were identified.

The electromyographic data was filtered with a bandpass of 10 to 450 Hz using post-processing procedures based on functions developed to calculate the root mean square (RMS, in µV) and the median frequency (MDF, in Hz), and then introduced into the Matlab 5.0 software. A Hanning window with a 90% overlap for the processing of Fast Fourier transformation was used to calculate the MDF, and data was normalized by the mean.

Assessment of serum creatine kinase (CK) activity

Approximately 5ml of blood were collected from the antecubital region by venipuncture using the Vacutainer and then centrifuged for 8 min at 3000 rpm(s) to obtain the plasma (approximately 400µl). The aspiration volume of the equipment was 6µl and the reagent volume 260µl. The test was carried out using the Express PLUS (Bayer) equipment at a temperature of 25ºC. The serum CK levels were determined using the Unitest kit CK-NAK UV (Laboratório Wiener).

Magnetic resonance imaging (MRI)

The Torm 0.5 Tesla MRI scanner, developed by the Group of Magnetic Resonance Imaging at the Institute of Physics of São Paulo University, was used to assess the exercised muscles.

The subjects lay supine with hip and knee joints in the neutral position. An initial sagittal localizing image of the quadriceps femoris muscle was obtained. Fifteen axial images were then acquired, starting at approximately 4 cm above the distal portion of the femoral condyle. However, only one of the images obtained from the middle third of the thigh was used to assess the muscle damage induced by eccentric exercise. The protocol adopted to assess muscle damage was the inversion recovery sequence, described as follows: repetition rate = 2000 ms; echo time = 70 ms; inversion rate = 140 ms; number of averages = 4; slice thickness = 15 mm; interslice distance = 17 mm; field of view = 256x256; resolution (X:Y) = 150x250; second-order flow compensation in the x, y, z; radio-frequency bandwidth = 6 KHz.

The images were visually inspected by a single examiner to assess the signal intensity. The signal intensity of the quadriceps femoris muscle after eccentric exercising was compared with that of the same muscle before exercising. The increase in signal intensity determined by visual inspection is considered as an indication of edema (Babul et al., 2003; Nurenberg et al., 1992; Shellock et al., 1991).

Muscle damage induction

Muscle damage was induced by eccentric exercise performed on the isokinetic dynamometer. The subjects were positioned according to the same procedures adopted for MVC torque.

Eccentric exercise of the quadriceps femoris muscle was performed between 40° and 110° of knee flexion (0° = full extension), at an angular velocity of 5 °/s. The subject's lower limb was passively returned to the initial position of 40°. The subjects were submitted to 4 sets of 15 maximal eccentric isokinetic contractions, each followed by a 5-minute rest period. Two subjects were unable to perform part of the last set due to muscle fatigue. Therefore these subjects only performed 6 contractions for the last sets, totalling 51 maximal eccentric isokinetic contractions.

Statistical analysis

The data distribution was verified prior to the statistical analysis, and the 6.5 version of the software *GB-Stat School Pak* was used for each statistical calculation. For the between-group comparison (before and after exercise), the one-way ANOVA with repeated measurements was applied. When a significant difference was observed between groups, the Dunnett's Test was applied. The significance level considered for all tests was $\alpha \leq 0.05$ (5%).

Table 1. Increase in signal intensity (edema) for the muscles Vastus Medialis (VM), Vastus Lateralis (VL), Rectus Femoris (RF) and Vastus intermedius (VI) of the 6 MRI-assessed subjects pre-exercise (Pre), on the 2nd day after (D2), 7th day after (D7), and between the 21st and 30th (D21-30) days after eccentric exercise.

Muscle	Days	Subjects					
		1	2	3	4	5	6
VM	Pre	-	-	-	-	-	-
	D2	+	+	+	+	+	+
	D7	+	+	+	+	+	+
	D21-30	-	-	+	-	+	-
VL	Pre	-	-	-	-	-	-
	D2	+	+	+	+	+	+
	D7	+	+	+	+	+	+
	D21-30	-	-	+	-	+	-
RF	Pre	-	-	-	-	-	-
	D2	+	+	+	+	+	+
	D7	+	+	+	-	+	+
	D21-30	-	-	+	-	-	-
VI	Pre	-	-	-	-	-	-
	D2	+	+	+	+	+	+
	D7	+	+	+	+	+	+
	D21-30	-	-	+	-	+	-

+ = presence of edema, - = absence of edema.

As described earlier, three measurements were recorded prior to the eccentric exercise to assess MVC torque and electrical activity (RMS and MDF). Therefore, to verify possible differences between pre-exercise measurements, the one-way ANOVA with repeated measurements was applied. Significant differences were not observed ($p > 0.05$) between the measurements made pre-exercise for both MVC torque and electrical activity (RMS and MDF). Consequently, the average of the scores

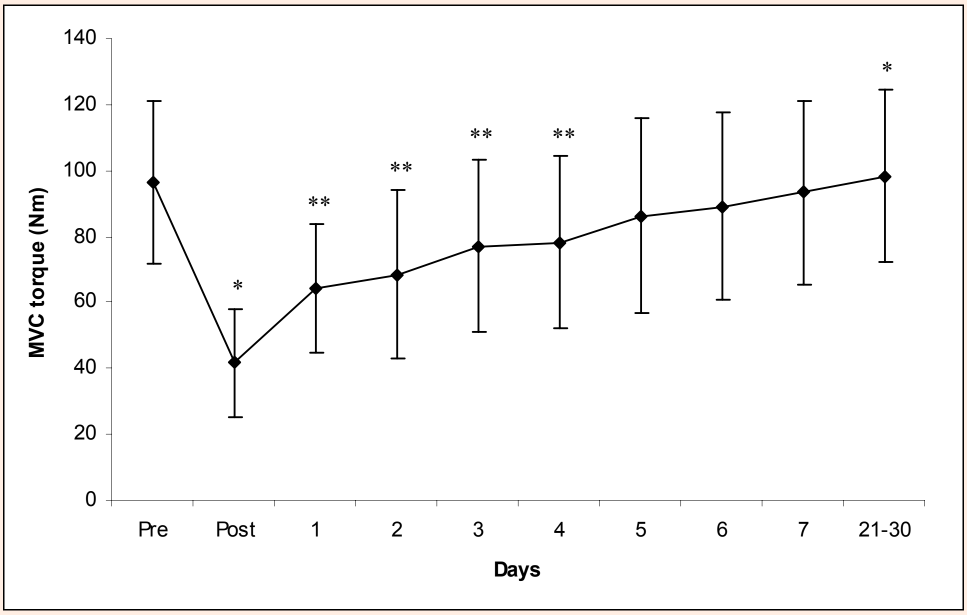

Figure 1. Average values and standard deviation for the MVC torque pre-exercise (Pre), immediately after (Post), during the 7 days after (1 to 7), and between the 21st and 30th (21-30) days after eccentric exercise.
* $p < 0.05$, ** $p < 0.01$ compared to the pre-exercise values.

obtained for the three measurements made before the eccentric exercise (baseline) was used to statistically analyze MVC torque and electrical activity (RMS and MDF).

Assessment of the MRI signal intensity was carried out in 6 subjects. An increase in signal intensity as determined by visual inspection was identified by a plus sign (+), and its absence by a minus sign (-) (Table 1).

Results

The results of this study demonstrated that immediately after eccentric exercise a significant decrease in MVC torque occurred in relation to the pre-exercise levels ($p < 0.05$). Although a progressive recovery was observed, MVC torque remained significantly lower during the 1st to 4th days after injury ($p < 0.01$) when compared to the pre-exercise value. However, the MVC torque obtained between the 21st and 30th day after eccentric exercise was significantly higher ($p < 0.05$) when compared to the pre-exercise value (Figure 1).

The RMS values of the VMO (Figure 2A) and RF (Figure 2B) muscles decreased significantly ($p < 0.05$) on the 2nd day after the eccentric exercise when compared to the pre-exercise RMS values, and on the 3rd day those of the RF muscle remained significantly lower ($p < 0.05$, Figure 2B). To the contrary, there was no alteration in the RMS of the VL muscle after the eccentric exercise ($p > 0.05$, Figure 2C).

The MDF of the VMO muscle increased significantly immediately after the eccentric exercise ($p < 0.01$, Figure 3A), but on the other hand, the MDF of the RF muscle decreased significantly immediately after and on the 1st day after the eccentric exercise ($p < 0.05$, Figure 3B). A significant decrease was also observed for the VL muscle on the 1st and 2nd days after the eccentric exercise ($p < 0.05$, Figure 3C).

With respect to serum CK activity, this was significantly greater ($p < 0.05$) on the 2nd day after eccentric exercise, returning to the pre-exercise levels on the 7th day (Figure 4).

The MRI assessment showed an increase in intensity of the signal of the vastus medialis (VM), VL and vastus intermedius (VI) muscles for all the subjects on the 2nd and 7th days after eccentric exercise. The RF muscle also showed an increase in intensity of the signal for the same period, with the exception of one subject. Between the 21st and 30th days after eccentric exercise, two subjects still showed an increase in the signal for all parts of the quadriceps femoris muscle (Figure 5 and Table 1).

Discussion

This study assessed the time course of functional recovery of the quadriceps femoris muscle after injury induced by eccentric exercise at low angular speed. According to Warren et al. (1999), the measurement of muscle function provides the best means of evaluating the magnitude and time-course of muscle injuries resulting from eccentric contractions. Muscle function is operationally defined as the ability to exert force under a given set of conditions, that is, over a given range of motion or at a fixed muscle length, at a given velocity or at a given external load, at a given level of activation and over a given number of contractions. A tool that assesses one or more of these components of muscle function is defined as a functional measurement tool. MVC torque and electromyography were the tools used in the present study to assess muscle function. In addition, CK activity was determined and MRI carried out, in order to verify the effectiveness of the eccentric exercise protocol in inducing muscle injury.

An increase in intensity of the MRI signal was shown for all parts of the quadriceps femoris muscle on the 2nd and 7th days after eccentric exercise, which remained high between the 21st and 30th days in two subjects. These results are in agreement with those of Shellock et al. (1991), who reported an increase in intensity of the signal for the elbow flexor muscles of 5 subjects after eccentric exercise. The authors found an increase in intensity of the signal during the days following eccentric exercise, which gradually decreased after the 10th day.

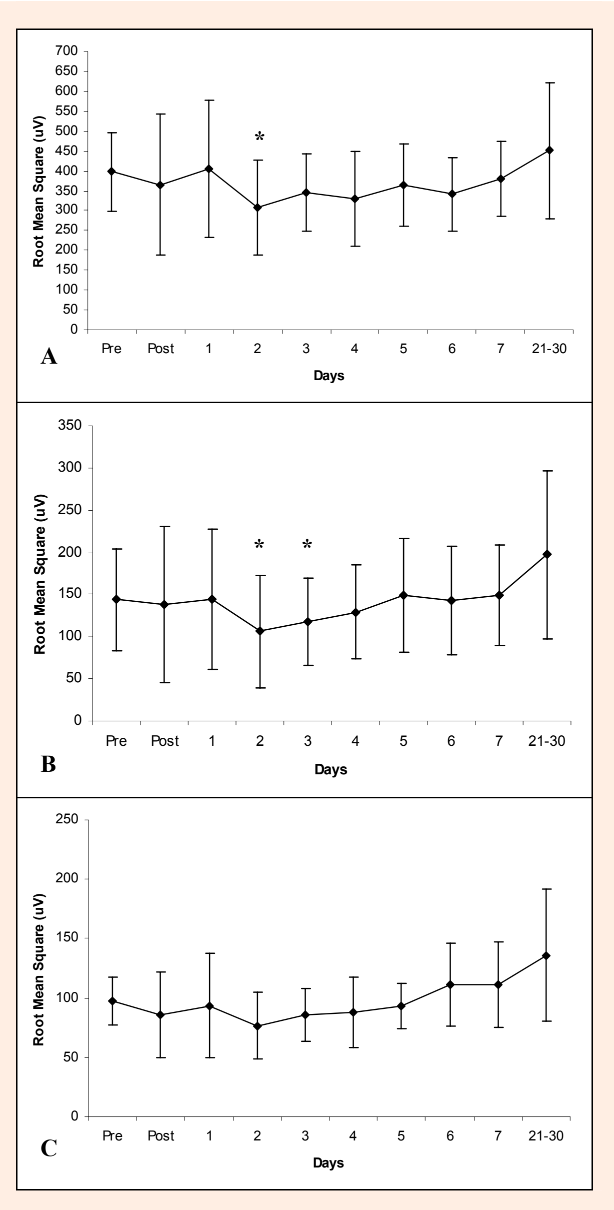

Figure 2. Average values and standard deviation for the Root Mean Square (RMS) of the VMO (A), RF (B) and VL (C) muscles pre-exercise (Pre), immediately after (Post), during the 7 days after (1 to 7), and between the 21st and 30th (21-30) days after eccentric exercise.
* $p < 0.05$ compared to the pre-exercise values.

Whilst in the present study alteration in intensity of the signal was used as the method, other studies have used the assessment of alterations in the T2 relaxation time as the method (Foley et al., 1999; Nosaka and Clarkson, 1996). It has been established that the T2 relaxation time increases during exercise, and then returns to the resting value within an hour post-exercise (Foley et al., 1999). However, Shellock et al. (1991) observed that a second increase in the T2 relaxation time developed gradually, giving a peak on the 3rd and 5th days after eccentric exercise, but not after concentric or isometric exercise. The time course and magnitude of this delayed increase in the T2 relaxation time, as also its relationship with other markers of muscle injury, have been described during the post-exercise period, with the general conclusion that the chronic T2 phenomenon reflects edema (Nosaka and Clarkson, 1996; Shellock et al., 1991).

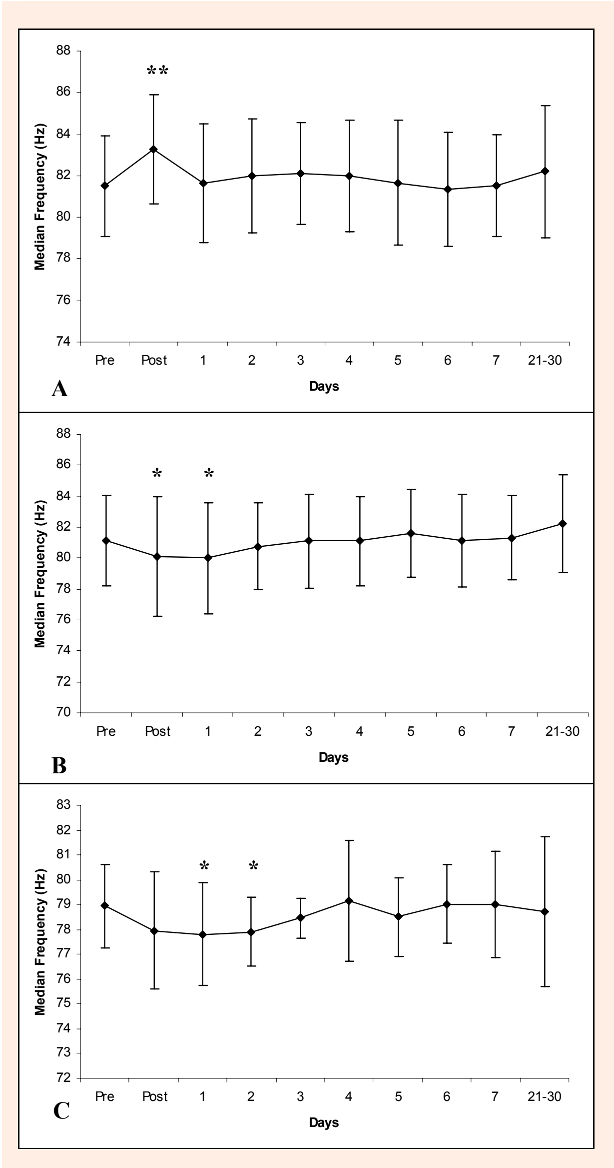

Figure 3. Average values and standard deviation for the Median Frequency (MDF) of the VMO (A), RF (B) and VL (C) muscles pre-exercise (Pre), immediately after (Post), during the 7 days after (1 to 7), and between the 21st and 30th (21-30) days after eccentric exercise.
* $p < 0.05$, ** $p < 0.01$compared to the pre-exercise values.

On the other hand, assessment of the increase in intensity of the signal after eccentric exercise, as identified by visual inspection, has been used in various previously published studies, being considered indicative of edema (Babul et al, 2003; Nurenberg et al., 1992; Shellock et al., 1991). According to Nurenberg et al. (1992), the immediate increase in intensity of the signal is a normal response occurring in parallel to the increase in intracellular and predominantly extracellular water that accompanies the exercise. To the contrary, the delayed rise in intensity of the signal seems to occur in parallel with delayed-onset muscle soreness and the ultra-structural injury, peaking from 48 to 96 hours after the exercise. These authors found high correlation between the increase in intensity of the signal assessed by MRI and the ultra-structural injury, as determined by an autopsy of some of the leg muscles (soleus, gastrocnemius lateralis, gastrocnemius medialis, anterior tibial and fibular longus) after carrying out the eccentric exercise.

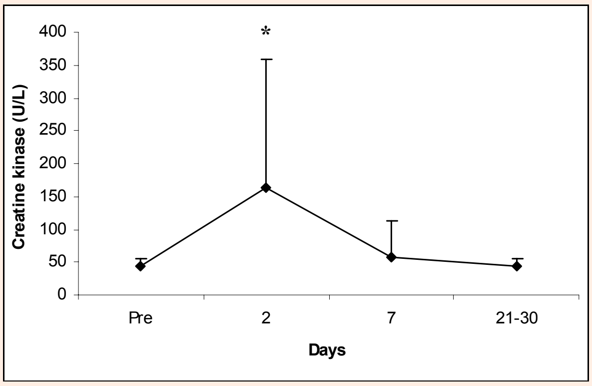

Figure 4. Average values and standard deviation for creatine kinase (CK) activity pre-exercise (Pre), on the 2nd day after (2), on the 7th day after (7), and between the 21st and 30th (21-30) days after eccentric exercise.
* $p < 0.05$ compared to the pre-exercise values.

Based on a visual inspection, Nurenberg et al. (1992) used a scale from 0 to 5 (0 = normal, 5 = very severe signal intensity increase) to grade the increase in intensity of the signal after eccentric exercise. Similarly, Babul et al. (2003) recorded the intensity of edema using a subjective score based on visual assessment, where: 0 = no visible edema, 1 = minimal muscle edema, 2 = moderate muscle edema and 3 = marked muscle edema. In the present study, only the presence or absence of an increase in signal intensity (edema) was registered and the affected muscle, since it was not the objective of the present study to assess the degree of increase in signal intensity during the injury recovery period.

Another important aspect refers to the MRI protocol used to assess the signal intensity. Part of the studies used T2-weighted images to assess the increase in signal intensity (Shellock et al., 1991). However, in the present study, after carrying out various pilot studies, assessment of the inversion-recovery sequence was chosen, described as preferable by some authors in identifying alterations in the amount of water observed in muscle injury (Fleckenstein et al., 1989).

The prolonged decrease in strength after eccentric exercise is considered to be one of the most valid and reliable indirect measurements of muscle damage in humans (Warren et al., 1999). In the present study MVC torque decreased 56% immediately after eccentric exercise and remained low up to the 4th day. This result is similar to that observed by Clarkson et al. (1992). Clarkson et al. (1992) detected a 50% decrease in maximal isometric strength of the elbow flexor muscles immediately after maximal eccentric exercise. Although a decrease in muscle strength after eccentric exercise is a frequent finding, differences exist between studies when comparing the recovery time. For example, in the present study, the MVC torque decreased significantly up to the 4th day after eccentric exercise. However, in other studies a longer time was required to return to the pre-exercise strength levels (Bottas et al., 2005; Clarkson et al., 1992; Chen and Nosaka, 2006). For example, Clarkson et al. (1992) reported that up to the 10th day after eccentric exercise, the strength had still not returned to the pre-exercise level. Possible reasons for these differences in muscle strength recovery time could be differences between the injury induction models (Chen and Nosaka, 2006) or the functional role of the injured muscle or muscle group.

Doubts still exist with respect to the mechanism involved in decreasing muscle strength after eccentric exercise (Clarkson and Hubal, 2002). According to McHugh (2003), the loss in strength following a bout of eccentric exercise could theoretically be due to a physical disruption of the force-generating structures (including a loss of myofibrillar contractile proteins) or a failure to activate intact force-generating structures within the muscle fibre (excitation-contraction coupling). Impaired excitation-contraction coupling has been estimated to account for 50-75% of strength loss in the first 5 days following a damaging eccentric exercise (Warren et al., 2001). However, this estimate is based on electrically stimulated maximal contractions in an animal model, and little is known about the effects in human skeletal muscle with voluntary contractions. Jones (1996) and Hill et al. (2001) reported that the manifestation of a decrease in muscle strength after eccentric exercise is the phenomenon known as low-frequency fatigue (LFF). There is a decreased capacity to produce strength with a low-frequency stimulus after exercise induced muscle damage, which can extend for one week after the exercise. Edwards et al. (1977) suggested that LFF occurred due to a failure in the excitation-contraction coupling process. Animal-conducted studies indicated a reduction in calcium released by the sarcoplasmic reticulum after exercise-induced muscle damage as the primary cause of LFF, offering evidence that muscle inability to produce maximal strength after eccentric exercise results from the impairment of excitation-contraction coupling processes (Byrd, 1992). Other studies also indicated failure in the excitation-contraction coupling process as a plausible cause of decrease in muscle strength after eccentric exercises in humans. Hill et al. (2001) reported that along with a 33% decrease in quadriceps femoris maximal voluntary torque, a significant decrease in torque production was observed with low-frequency stimulation, which presented significant correlation with the depression in calcium release.

With respect to electrical activity, it was shown that the RMS of the VMO and RF muscles decreased significantly on the 2nd day after eccentric exercise, remaining low up to the 3rd day for the RF muscle. On the

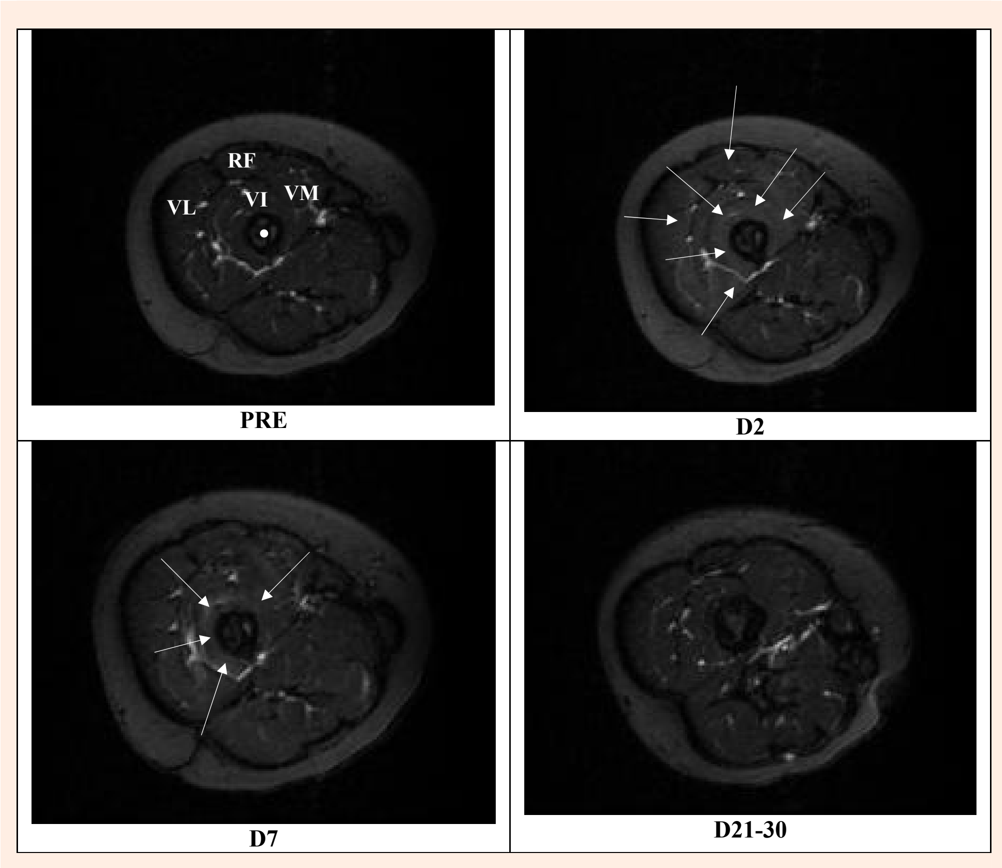

Figure 5. Magnetic Resonance Imaging of the middle third of the right thigh of one of the subjects, before (Pre), on the 2nd day (D2), on the 7th day (D7), and between the 21st and 30th days (D21-30) following eccentric exercise. Arrows indicate an increase in signal intensity (edema). Vastus Medialis (VM), Vastus Lateralis (VL), Rectus Femoris (RF), Vastus intermedius (VI) and Femur (•). The level of the section is the same for every image.

other hand there was no alteration in RMS of the VL muscle after eccentric exercise. The RMS results for the VMO and RF muscles immediately after and on the 1st day after the eccentric exercise were similar to those found by McHugh et al. (2000) and Bajaj et al. (2002), in which the authors reported no alterations in this period. However, a decrease in RMS was found in the present study for the VMO and RF muscles, on the 2nd day after eccentric exercise.

According to Basmajian and De Luca (1985), the RMS represents the number of activated motor units during contraction, and is generally used as a muscle activity measurement. At the same time, Fridén et al. (1983) studied myofibrillar injury and observed focal disorder in the striated band pattern in 32%, 52% and 12% of the muscle fibres, 1 hour, 3 days and 6 days, respectively, after intense eccentric exercise in humans. Thus these authors demonstrated that the proportion of severely injured muscle fibres was greater between the 1st and 3rd days after eccentric exercise. This period coincided with the decrease in RMS of the VMO and RF muscles, observed in the present study, and thus one can suggest that this decrease could have been due to a reduced number of activated muscle fibres during the isometric contraction.

The MDF of the VMO muscle increased significantly immediately after eccentric exercise. On the other hand, the MDF of the RF muscle decreased significantly immediately after and on the 1st day after eccentric exercise. A significant decrease was also observed for the VL muscle on the 1st and 2nd days after eccentric exercise.

MDF has been associated with the average speed of conduction of the muscle fibre action potential. Thus, a decrease in MDF during fatigue has been attributed to a decrease in conduction speed in activating muscle fibres (Arendt-Nielsen and Mills, 1985; Merletti et al., 2001).

Motor unity recruitment with the increase in strength production progress from slow-oxidative motor units to fast-oxidative motor units and then to fast-glycolytic motor units (Henneman et al., 1965). However, there is evidence that during eccentric contraction, preferential recruitment occurs with respect to fast-glycolytic motor units (Nardone et al., 1989). Therefore, this preferred recruitment of fast-glycolytic motor units explains the greater susceptibility to fast muscle fibre damage.

Linnamo et al. (2000) reported that a decrease in muscle fibre conduction speed might be related to selective damage of fast muscle fibres. Following selective damage of the fast fibres after eccentric exercise, slow fibres would be preferentially recruited. Since the conduction speed of the slow fibre action potential is lower, a decrease in MDF is to be expected. Thus, considering that the VL and RF muscles show greater amounts of fast fibres (Johnson et al., 1973; Staron et al., 2000), one could suggest that the selective injury of these fibres would explain the decrease in MDF after eccentric exercise. On the other hand, the different behaviour of the MDF of the VMO could be related to the higher proportion of slow fibres (Travnik et al., 1995) that this muscle possesses. As a function of the decreased proportion of fast fibres in comparison with the VL and RF muscles, it is possible that the eccentric exercise had a reduced influence on the VMO fibres, not causing a reduction in MDF. On the other hand, the increase in MDF of the VMO muscle could be related to the increase in firing rates of the activated motor units, with the objective of producing isometric force comparable with the pre-exercise values.

Differently from the majority of studies using eccentric isokinetic exercise to induce muscle injury (Chen, 2003; Linnamo et al., 2000), the eccentric exercise of the present study used low angular speed. It has been speculated that fast motor units are recruited during high speed movements, whilst slow motor units are recruited during low speed movements (Ewing et al., 1990). Based on this speculation, one can assume that the induction of injury at different angular speeds would result in different behaviours of the MDF, since different types of fibre would be recruited. The influence of angular speed on the pattern of motor unit recruitment during isokinetic exercise was studied by Hutchins et al. (1998). However, these authors found no difference in the MDF of the quadriceps femoris muscle (vastus medialis and vastus lateralis), suggesting that the speed does not alter the motor unit recruitment pattern (no selective recruitment) during isokinetic exercise. The decrease in MDF of the VL and RF muscles encountered in the present study, allows for the suggestion that low angular speed eccentric exercise (5°/s) promotes a recruitment pattern of these muscles similar to that of the eccentric isokinetic exercise at higher speeds used by Linnamo et al. (2000) and Chen (2003) (115°/s and 60°/s, respectively), since these authors also reported a decrease in MDF of the elbow flexor muscles after exercise.

Serum CK activity was higher on the 2nd day after eccentric exercise, returning to the pre-exercise level on the 7th day after the proposed exercise. The time period during which higher serum CK activity occurred seems to be dependent on the type of exercise used to provoke muscle damage. The majority of studies that evaluated the CK level used maximal eccentric contraction or even downhill running, where sub-maximal eccentric contraction of the quadriceps femoris muscle occurs. Some studies (Byrnes et al., 1985; Eston et al., 1996) demonstrated that peak serum CK activity after downhill running occurred between the 1st and 2nd days, while other studies (Byrne et al., 2001; Clarkson et al., 1992; Lee and Clarkson, 2003; Schwane et al., 2000) observed the peak serum CK activity for maximal eccentric exercises between the 4th and 6th days after exercise.

It was not the purpose of the present study verify during which day peak serum CK activity occurred. However, the increase observed on the 2nd day after the eccentric exercise was an indirect evidence of the occurrence of injury.

Inter-subject variability is highlighted as one of the major problems with respect to the use of the CK activity measurement as a muscle damage indicator. Nosaka and Clarkson (1996) noted a great variability in CK response amongst individuals submitted to the same eccentric exercise protocol. Accordingly, Fridén and Lieber (2001) did not consider CK activity as a reliable measurement of the extent of muscle damage. These authors reported poor correlation between serum CK activity and ankle dorsiflexor muscle function (torque evoked by nervous stimulation) in rabbits on the 1st, 2nd, 7th, 14th and 28th day after eccentric exercise damage. The authors also reported that this result was not unexpected, since muscle fibre permeability to intramuscular enzymes may or may not be correlated to the cell contractile function. Although Fridén and Lieber (2001) reported that the CK activity level was not correlated with the magnitude of muscle damage, they suggested that high levels of CK provided evidence of muscle damage in a binary manner – injured or not injured.

Therefore, the high inter-subject variability with respect to the CK response may be the explanation of the great standard deviation observed for the measurements performed on the 2nd day after eccentric exercise in the present study.

The level of circulating estrogen appears to be a factor influencing CK activity. Thus sex is described as a factor affecting rupture of the sarcolemma, and hence, the release of CK. Previous studies found lower levels for CK release in women than in men, both in the basal condition and during exercising to a degree comparable with working (Van der Meulen et al., 1991). This difference would be a result of the exposition of the musculoskeleton to estrogen, which appears to protect the muscle from CK release (Bär et al., 1990). Based on this idea, one could imagine that the CK activity would vary depending on the phase of the menstrual cycle during which injury was induced, since the level of estrogen circulating fluctuates. Recent studies, both in animals (Sotiriadou et al., 2003; 2006) and in humans (Thompson et al., 2006) offer support to the idea of estrogen influencing CK activity. Although there was no control in the present study of the phase of the menstrual cycle during which muscle injury was induced, the present authors believe this did not influence interpretation of the results, since correlating the intensity of the CK response with the magnitude of muscle injury was not an objective of the present study, aiming simply to use CK to confirm the presence of injury.

Finally, based on the increase in signal intensity (2nd and 7th days after eccentric exercise) and on the increase in CK activity, one can conclude that the protocol of low angular speed eccentric exercise resulted in muscle injury, and can thus be used as a model to induce injury in future studies aiming to assess recovery time.

Conclusion

Within the limits of the study, the results allowed for the conclusion that:

- The quadriceps femoris muscle injured by eccentric exercise, already started its functional recovery, as demonstrated by the behaviour of MVC torque and electrical activity, in the first week after eccentric exercise, despite the presence of an increase in the intensity of the MRI signal;

- The RMS of the VMO and RF muscles decreased on the 2nd day after eccentric exercise, remaining low until the 3rd day for the RF muscle. This result suggests a decrease in the number of activated muscle fibres during this period after eccentric exercise;
- A significant decrease in MDF of the RF muscle was observed immediately after and on the 1st day after eccentric exercise, whilst for the VL muscle there was a decrease on the 1st and 2nd days after injury. These results are consistent with the hypothesis of selective injury of fast fibres after eccentric exercise.
- The MDF of the VMO muscle increased significantly immediately after eccentric exercise. This result is consistent with a possible increase in the firing rates of the activated motor units with the objective of producing isometric force comparable with the pre-exercise values.
- The low angular speed eccentric exercise was effective in inducing injury of the quadriceps femoris muscle. Thus, in addition to the models already described in the literature using eccentric exercise with higher angular speeds, low angular speed eccentric exercise can also be used as a model to induce muscle injury in future studies.

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Key points

- The low angular speed eccentric exercise was effective in inducing injury of the quadriceps femoris muscle, and could be used as a muscle injury inducing model in future studies;
- The quadriceps femoris muscle injured by eccentric exercise started its functional recovery in the first week after low angular speed eccentric exercise.

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