Changes in the Mechanical Properties of Human Quadriceps Muscle after Eccentric Exercise

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Abstract. *Muscular adaptation which occurs following eccentric exercise-induced muscle damage has been associated with changes in the mechanical properties of muscle manifested as a shift in the length-tension relationship towards longer muscle lengths. However, it is not clear whether this shift is a long term adaptation to eccentric exercise. The purpose of this study was to investigate functional adaptations to skeletal muscle damage in humans, tracking such responses several days into muscle recovery. Ten healthy young men performed an eccentric exercise protocol involving the quadriceps muscle and functional measurements were performed before and on days 1, 2, 5, 8, 12 and 16 post-exercise. Blood samples were also withdrawn before and at 6 h, and 2 days, 5 days and 16 days post-exercise. The exercise protocol resulted in muscle damage, indicated by changes in clinical markers including increased serum creatine kinase activity and muscle soreness compared to pre-exercise levels (p<0.05-0.001). An acute, but not sustained shift in the quadriceps isokinetic and isometric angle-torque curves towards longer muscle lengths was observed post-exercise (p<0.05). It was speculated that the functional adaptations following eccentric exercise might be affected by the short resting and functional length of the quadriceps muscle, relative to its optimum. More studies are needed to confirm the hypothesis that a sustained shift in the muscle's length-tension relationship, as an adaptation after lengthening contraction-induced damage, is muscle specific.*

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Activity models such as muscle overload and muscle stretch or their combination, as occur in eccentric exercise where the muscle is being forcibly lengthened while generating active tension, have been shown to result in muscle damage (1-4). Exercise-induced muscle damage has been associated with disruption of the normal myofilament structures in the sarcomeres (5), damage to the sarcolemma, loss of fibre integrity and leakage of muscle-specific enzymes and proteins into the blood (6), delayed onset muscle soreness and loss of muscle force (7).

One functional response to eccentric exercise-induced muscle damage is a shift of the muscle's length-tension relationship towards longer muscle lengths (8-12), attributed to the over-extension of some weak sarcomeres. According to the "popping" sarcomere hypothesis, lengthening of active muscle does not occur by uniform lengthening of all sarcomeres, but by a non-uniform distribution of sarcomere length change, causing some weak sarcomeres to over-extend ("pop") beyond myofilament overlap (13). This would increase the in series compliance of the muscle, leading to an acute shift of the length-tension relationship to the right, *i.e.* towards longer muscle lengths, where the intact sarcomeres would reach their optimum lengths (1, 10, 14). Subsequently, the overstretching-induced acute shift is expected to partially reverse during the recovery period, because some damaged fibres die and therefore no longer contribute to the angle-torque curve, or, in other less damaged fibres, the "overstretched" sarcomeres recover their normal arrangement (8, 9). However, the possible addition of sarcomeres in series (sarcomerogenesis) in the regenerating myofibres (15) would result in the maintenance of the shift over longer periods of time, as a protective adaptation against damage (1, 3, 10). Such addition of sarcomeres in series has been shown in animal models (15-18) and has been proposed as a functional adaptation of skeletal muscle to lengthening contraction-induced damage.

Changes in the mechanical properties of the damaged muscles have been alternatively attributed to a failure in the

excitation-contraction coupling system (19) and/or decreased activation *via* reduced Ca^{2+} release and sensitivity (20, 21), however, these explanations remain a point of controversy (1, 8).

The phenomenon of the acute shift in the length-tension relationship following muscle-damaging exercise is well established and described in human muscles using the angle-torque curve and the optimum angle for peak torque generation (8-12, 22-25). However, there is currently little information regarding a sustained shift in muscle length-tension relationship following eccentric exercise in humans. Only two studies have reported a long-lasting shift, *i.e.* beyond 8 days after muscle damage (10, 22). In these studies, a sustained shift was observed after two separated (by 8 days) eccentric exercise bouts, and it is not clear whether the shift is sustained over a longer period of time after a single eccentric exercise bout. Therefore, the aim of this study was to determine whether the shift in the length-tension relationship is a persistent adaptation to muscle damage, using the human quadriceps muscle.

Patients and Methods

Ethical approval. Written informed consent was obtained from all the volunteers to participate in this study which was approved by the Ethics Committee of the National and Kapodistrian University of Athens and all the experimental procedures conformed to the Declaration of Helsinki.

Subjects. Ten healthy men (age 25±1.5 years, height 180.3±1.5 cm, body mass 76.8±2.4 kg, body mass index 23.6±0.5) participated in the study. The volunteers were physically active but were unaccustomed to high-intensity eccentric exercise and had not participated in any type of resistance training or regular exercise regime for at least 6 months before the study. These individuals were free of any musculoskeletal disorders or other lower extremity pathologies and they refrained from taking any medications or nutritional supplementations throughout the experimental period. The volunteers were specifically not allowed to perform any vigorous physical activities or quadriceps muscle stretching during the entire experimental period.

Experimental design. The volunteers performed a maximal eccentric exercise protocol of the knee extensor muscles with each leg. Post-eccentric exercise, one leg served for the assessment of muscle function (the functional assessment leg, FL) while the contralateral leg was used as the control (the control leg, CL) to assess any potential training effect of the repeated maximal torque testing on the functional measurements. The leg selected for functional assessment or as the control was alternated between subjects to account for possible variation between dominant and non-dominant legs. Blood serum analysis was also carried out. Furthermore, before the exercise protocol the volunteers completed two familiarization sessions in which they were acquainted with the measurements and the procedures of both the testing and the exercise protocols (see 'Testing of muscle function' and 'Maximal eccentric exercise protocol' below). The functional testing protocol consisted of a number of measurements performed before (PRE)

and on days 1, 2, 5, 8, 12 and 16 post-eccentric exercise. The main assessments included the maximal voluntary contractile (MVC) isometric as well as isokinetic torque of the knee extensors and the optimum angle for peak isometric and isokinetic torque generation of knee extensors. The time-points of blood sampling and functional measurements were chosen to cover a wide period in the regeneration and adaptation processes following damage. In addition, delayed-onset muscle soreness (DOMS) and changes in the maximal shortening ability of the knee extensors were also measured. All the functional testing measures were performed on both legs at each time-point of the testing protocol except for the maximal isometric and isokinetic torque and optimum angle for peak torque generation, which were performed on the FL at each time-point and on the CL before the eccentric exercise and on day 16 post-exercise.

Testing of muscle function. The volunteers were familiarized with the torque testing procedures on an isokinetic dynamometer (Cybex Norm Lumex Inc., Ronkonkoma, NY, USA) during two visits to the laboratory. During each familiarization session, the subjects warmed up for 10 min on a cycle ergometer at 50 W and thereafter they performed a number of comfortable submaximal trials of knee extension to become familiar with the procedures of the isometric and isokinetic torque measurements and to learn the proper execution of the exercise. On the day of the second familiarization, baseline measurements of the testing protocol (PRE) were performed with the knee extensors of each leg and the baseline measurements were reassessed post-exercise as mentioned above. To evaluate muscle function, the main assessments of the testing protocol as well as the muscle shortening ability were evaluated. The subjects were seated upright on the isokinetic dynamometer, also used for the exercise, with the knee joint aligned with the axis of rotation of the dynamometer's lever arm.

Maximal voluntary contractile torque and optimum angle for peak torque generation. Both the isometric and isokinetic MVC torque of the knee extensors were measured. The isometric torque was measured at eight different knee angles, *i.e.* 15, 30, 45, 60, 75, 90, 105 and 120 deg, in random order across subjects and across days for each subject. The knee angles used for the MVC torque testing were set using the dynamometer's visual display unit after entering a reference datum knee angle of 0 deg defined as full knee extension. Each subject performed two maximal voluntary isometric contractions of 3 sec duration at each angle and the best trial was taken as the MVC isometric torque of the angle.

The isokinetic MVC torque was measured at an angular velocity of 60 deg sec^{-1}, using also the isokinetic dynamometer switched to the isokinetic mode. Each subject performed two maximal voluntary concentric isokinetic contractions of ~2 sec duration and the best trial was recorded. The range of motion was from 130 to 0 deg (0 deg=full extension). A resting period between 60 sec and 90 sec was allowed between the repetitions. The order of torque testing (*i.e.* which type of muscle function, isometric or isokinetic, was assessed first) was also randomized across days for each subject.

The peak torque and optimum angle for peak torque generation were assessed using both the isokinetic and the isometric angle-torque curves which were determined for each subject by fitting a quadratic polynomial curve to the torque versus knee angle, as previously described (11).

Muscle shortening ability. The muscle shortening ability of the knee extensors was evaluated at the start of each functional measurement session by measuring the extended knee joint angle (EXANG), which was defined as the angle when the subject tried to fully extend the knee joint, *i.e.* an increase in knee angle corresponds to a decrease in shortening ability of the knee extensors. Full knee extension was set as 0 deg using the visual display unit of the isokinetic dynamometer which provided numeric feedback of the joint range of motion. The smallest knee angle (*i.e.* the angle that was closest to full knee extension) obtained across three trials was recorded.

Maximal eccentric exercise protocol. The subjects performed an eccentric exercise bout with the knee extensors of each leg on the isokinetic dynamometer switched to the isokinetic mode. The exercise protocol consisted of 2 sets of 25 maximal voluntary eccentric (lengthening) muscle actions with a 5-min break between the sets. The subjects were required to maximally resist the forced lengthening of their quadriceps through a range of motion of 130 deg, from almost full extension (5 deg) to almost full flexion. Each lengthening muscle action was performed at an angular velocity of 30 deg. sec^{-1}, lasted ~4 sec and was followed by 15 sec rest phase during which the leg was returned passively to the starting position (5 deg) by the motor of the dynamometer. The eccentric exercise protocol lasted for a total of ~22 min for each leg, and the order of limbs (*i.e.* which limb, FL or CL, was exercised first) was randomized across subjects. The subjects were instructed to contract as hard as possible and strong verbal encouragement was given to them during all the trials.

Muscle torque, power and work during eccentric exercise protocol. In order to evaluate the total mechanical activity of the exercised muscles during the eccentric exercise protocol, the peak torque, average power and total work were recorded throughout the exercise bout of each leg.

Muscle soreness. Muscle soreness was evaluated on both legs before any contractions were performed in each measurement session. The subjects visually recorded the perceived pain on a visual analogue scale that had a continuous line of 100 mm with the left end labelled "no pain" and the right end labelled "extremely sore". Instructions had been given to the subjects to rate soreness levels in two ways: (i) during one repetition of flexing and extending the knee joint throughout the entire range of motion and (ii) upon light palpation of the entire knee extensors area (*i.e.* the muscle belly and distal regions of the quadriceps muscle) always by the same investigator, with the thigh at rest (26). Subjects marked the scale and the distance from the left end of the scale to the mark was taken as the level of soreness. The average of the two values for each subject was used as the criterion score of the day. DOMS was assessed on each day of the functional testing protocol until the total disappearance of pain.

Blood sampling and serum measurements. At least ten days before the study initiation, the volunteers were subjected to pre-exercise (PRE) blood sampling. Blood samples were then withdrawn after the eccentric exercise *i.e.* at 6 h, 2 days, 5 days and 16 days post-exercise. The subjects were seated quietly for at least 30 min and 10 ml of blood were drawn from an antecubital vein. The blood sample was allowed to clot at room temperature for 30 min and

the serum was collected promptly after centrifugation at 4,000 RPM at 4°C for 10 min and stored frozen at –80°C until analysis. The serum was assayed for creatine kinase (CK) activity, as an indirect marker of muscle damage (26), with automated enzyme reactions (Roche/Hitachi ACN 057, Mannheim, Germany) at 37°C using a commercially available kit (Roche Diagnostics, Mannheim, Germany).

Statistical analysis. Changes in all the functional testing measurements were assessed using two-way analyses of variance (ANOVA) with repeated measures (SPPS v. 11 statistical package, SPSS Inc. Headquarters, Chicago, USA). A one-way ANOVA with repeated measures over time was employed to evaluate changes in serum measurements. Where significant F ratios were found for the main effects or interaction ($p<0.05$), the means were compared using Tukey's *post–hoc* tests. All the data are presented as mean±standard error of the mean (S.E.M). The level of significance was set at $p<0.05$.

Results

Assessment of eccentric exercise-induced muscle damage. All the volunteers reported a significant amount of perceived muscle soreness in both legs, which peaked on day 2 post-exercise and had almost disappeared by day 8 post-exercise. No significant differences were found between the CL and FL in the levels of muscle soreness post-exercise ($p>0.05$, Figure 1a). Serum levels of CK, the most common biochemical marker of muscle damage, were increased and remained elevated up to day 5 after the eccentric exercise protocol ($p<0.05$; Figure 1b).

Assessment of muscle function following eccentric exercise-induced damage. As expected, a significant gradual decrease in peak torque, mean power and total work was shown over the course of the eccentric exercise protocol. Significant decreases from the values recorded at the beginning of the eccentric exercise, (*i.e.* the means of the 5 first lengthening muscle actions), were observed in all the above measures of muscle function and in both legs over the exercise bout ($p<0.01$-0.001). There were no differences between the two legs in any of the measurements of muscle function (Figure 2a-c). The similar values of between the two legs indicated that both the functional assessment (FL) and the control (CL) leg underwent the same changes in muscle strength by the exercise protocol.

The EXANG was significantly increased on days 1 and 2 post-exercise in both legs, reflecting an impaired muscle shortening ability following exercise-induced damage, and returned towards the pre-exercise levels thereafter. No significant differences in EXANG were found between the two legs over time (Figure 3, a).

The peak torque (Figure 3, b) and the optimum angle for peak torque generation (Figure 3, c) changed significantly over time compared to the pre-exercise levels. These changes were assessed using two types of muscle function (*i.e.*
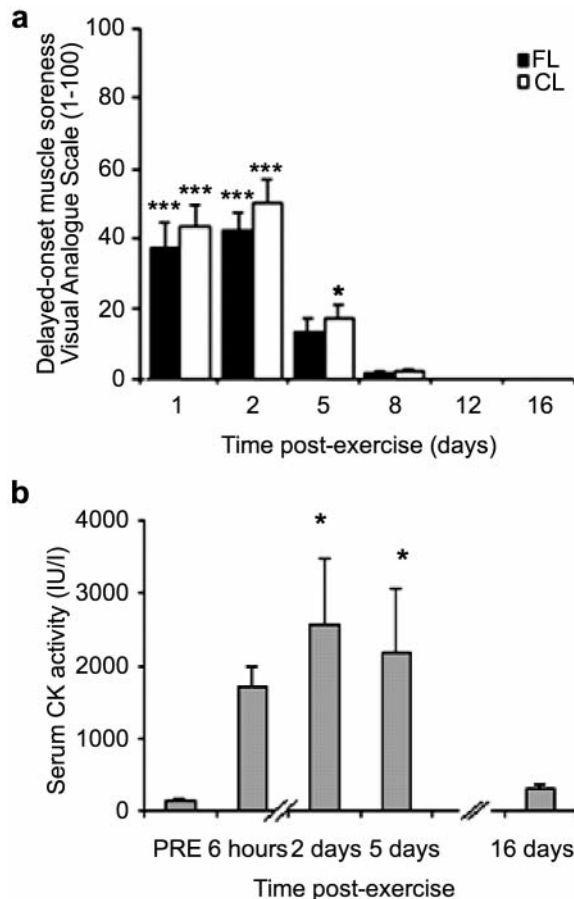
Figure 1. *Muscle soreness (a) and serum creatine kinase (CK) activity (b) before (PRE) and after maximal eccentric exercise of knee extensors of both legs (mean±S.E.M; n=10). FL: Functional assessment leg; CL: Control leg. Significantly different from pre-exercise *p<0.05, ***p<0.001.*

Figure 2. *Changes in peak torque (a), mean power (b) and total work (c) during the lengthening muscle actions of the knee extensors of both legs (mean±S.E.M.; n=10). FL: Functional assessment leg; CL: Control leg. **p<0.01, ***p<0.001.*

isometric and isokinetic) and no significant differences were found between the two types ($p>0.05$). Furthermore, there was no training effect of the repeated maximal torque testing on the functional responses of the FL and CL on day 16 post-exercise (*i.e.* no differences in percent changes of peak torque or in shift of the optimum angle were found between legs on day 16 post-exercise; Figure 3, b and c). Figure 4 shows changes in almost the entire isokinetic angle-torque curve and the shift in optimum angle as calculated by a quadratic polynomial curve fitting procedure over almost the entire range of the isokinetic muscle actions.

Discussion

The eccentric exercise protocol used in this study did result in skeletal muscle damage, as indicated by the sustained changes in the clinical markers of damage, *i.e.* post-exercise
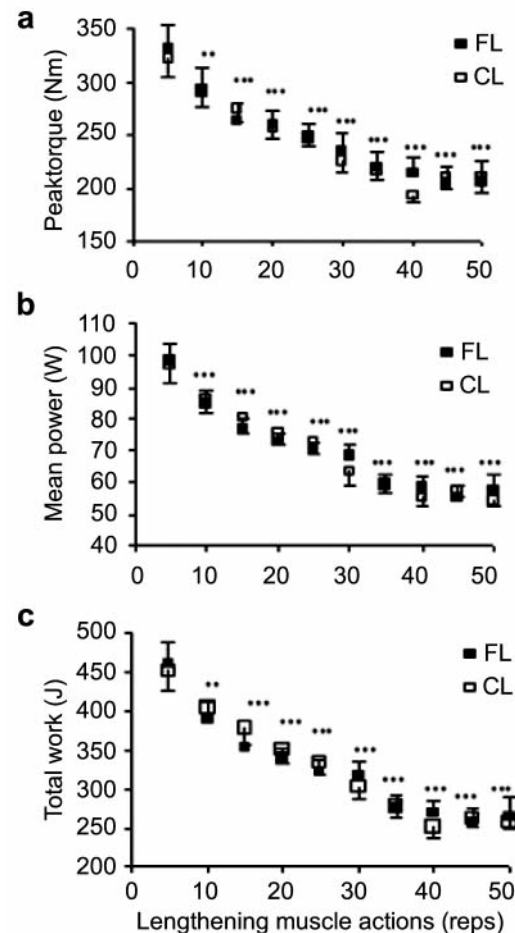
muscle soreness and increased serum CK activity levels. Moreover, the maximal eccentric exercise resulted in a shift in both the isometric and isokinetic angle-torque relationships, accompanied by other changes in the mechanical properties of the damaged muscles, such as impairment in their shortening ability and a deficit in maximal torque generation.

In contrast to a long-lasting shift previously found in human quadriceps (22) or hamstring muscles (10), only an acute shift in the human quadriceps length-tension relationship that reversed towards pre-exercise levels on day 8 post-exercise was observed in this study, which was not compatible with a longitudinal addition of sarcomeres in the damaged fibres during their regeneration.

Serial sarcomere number adaptations are expected under changes in fiber strain following eccentric exercise (15-17),
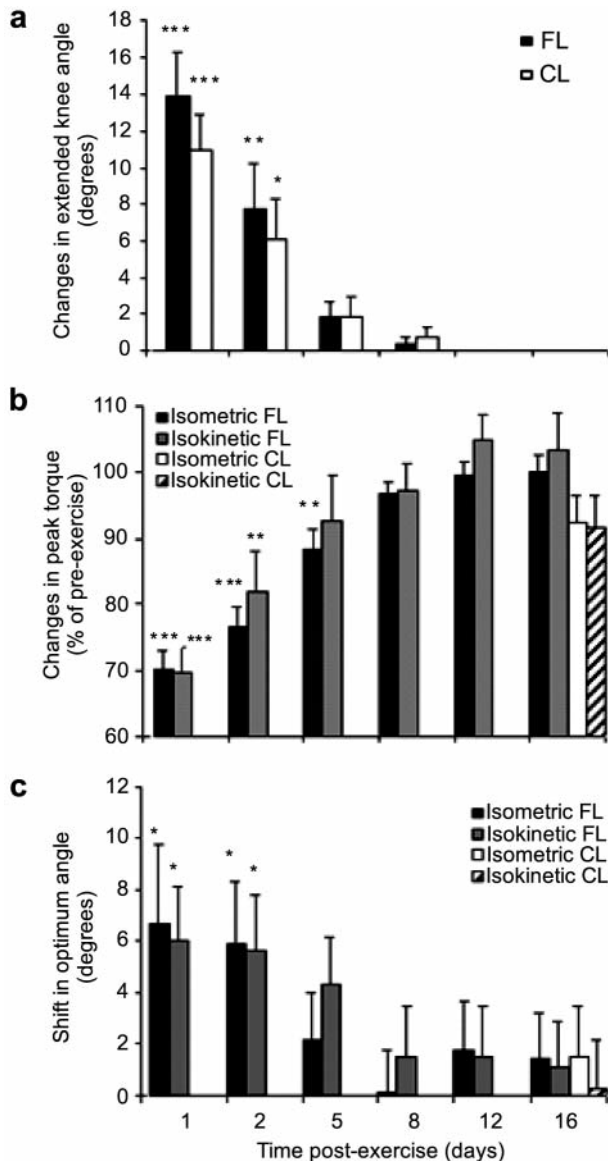
Figure 4. *Mean isokinetic angle-torque curves using data points from 115 to 30 deg (0 deg=full extension) of knee extension for each day (coefficients of determination for the fitted second order polynomial equation for each curve: r^2=0.97-0.99). Optimum angle for peak torque generation is indicated by crosses, while standard error bars have been omitted for clarity. Note the shift in optimum knee angle to the right, i.e. towards longer quadriceps lengths, after the damaging exercise (for statistically significance differences see Figure 3, c).*

Figure 3. *Changes in knee extensors shortening ability (i.e. maximally extended knee angle) (a), percent changes in peak isometric and isokinetic torque of knee extensors, calculated using the curve fitting procedure (b), and shift in optimum knee angle for peak torque generation (c), compared to pre-exercise (mean±S.E.M.; n=10). FL: Functional assessment leg; CL: Control leg. Significantly different from pre exercise *p<0.05, **p<0.01, ***p<0.001.*

functional length of the muscle appear to regulate the adjustment of sarcomere length to an optimum myofilament overlap for optimum shortening and, hence, optimum force generation (27, 30). In this way, the length-tension properties of a specific muscle are readjusted for optimum muscle function (9, 27).

Taking all these into account, the lack of a long-term shift in the quadriceps angle-torque curve, found in the present study, could potentially be attributed to the fact that the resting and functional length of the quadriceps muscle are short, *i.e.* below its optimum length. Such a short resting length would not favor the longitudinal addition of sarcomeres in the regenerating fibres (29, 30), while a shift towards longer lengths would impair quadriceps function by reducing the force at shorter lengths (1, 23), *i.e.* within its routine working range (see Figure 4). The impaired shortening ability of the exercised muscles also observed in this study was compatible with the overstretched sarcomeres hypothesis (1) and it could have reflected the detrimental effect of the acute shift on the quadriceps function at shorter muscle lengths. It could be argued that the existence of overstretched sarcomeres would impair the muscle fiber's ability to fully shorten, especially at shorter quadriceps lengths, *i.e.* at knee angles close to full extension, where the normal sarcomeres would be expected to be dramatically shortened due to the increased in series compliance of the muscle. The time courses of the recovery of shift in the optimum angle for peak torque generation and

while a change in the number of sarcomeres in series seems also to be the way in which the sarcomere length is adjusted to a change in functional muscle length (27). It was demonstrated that skeletal muscle has a preferred resting length, as well as a functional length, which can be acutely re-established by serial sarcomere number subtraction or addition (28-31). Thus, both the resting and
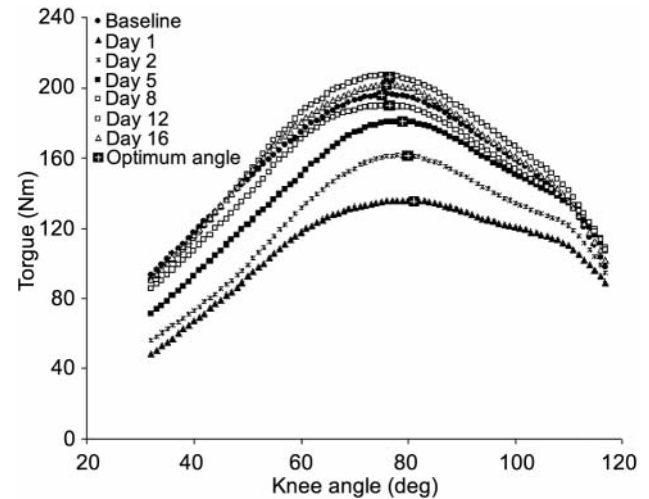
of the muscle's shortening ability were found to be similar (see Figure 3 a, c), however, a functional relationship between the shift and the impaired shortening ability remains to be determined. Thus, we postulate that the sustained shift previously found in quadriceps muscle (22) could be a training effect of the repeated eccentric exercise bouts separated by a relatively short time interval (*i.e.* 8 days), in contrast to the single exercise bout used in this study.

Our hypothesis regarding the role of the muscle's resting and functional length on the shift of its optimum length appears to be supported by the findings of another study on human hamstring muscles, which showed a sustained shift after eccentric exercise (10). The resting hamstring length is longer and human hamstrings also operate mainly at more lengthened positions, *i.e.* close to their optimum (32). In this case, the shift in the muscle's optimum length would have a beneficial and protective effect on the hamstrings (10, 33), since they operate routinely as well as more effectively at longer lengths (32). Indeed, there is evidence for such a sustained shift in optimum muscle length after four weeks of eccentric training of the hamstrings (34).

In conclusion, only an acute, but not a sustained shift in the human quadriceps angle-torque curve, provides evidence for transient changes in the mechanical properties of this muscle after a single bout of eccentric exercise. It is speculated that the functional adaptations following eccentric exercise might be affected by the muscle's specific resting and functional length relative to its optimum. Clearly, more studies are needed to confirm the hypothesis that a sustained shift in the muscle's length-tension relationship, as an adaptation following lengthening contraction-induced damage, is muscle specific.

Acknowledgements

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