Time Course of Neuromuscular Adaptations to Knee Extensor Eccentric Training

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Abstract
This study investigated the chronology of neural and morphological adaptations to knee extensor eccentric training and their contribution to strength gains in isometric, concentric and eccentric muscle actions. 20 male healthy subjects performed a 12-week eccentric training program on an isokinetic dynamometer, and neuromuscular evaluations of knee extensors were performed every 4 weeks. After 12 training weeks, significant increases were observed for: isometric (24%), concentric (15%) and eccentric (29%) torques; isometric (29%) and eccentric (33%) electromyographic activity; muscle thickness (10%) and anatomical cross-sectional area (19%). Eccentric and isometric torques increased progressively until the end of the program. Concentric torque and muscle mass parameters increased until the eighth training week, but did not change from this point to the twelfth training week. Eccentric and isometric activation increased at 4 and 8 training weeks, respectively, while no change was found in concentric activation. These results suggest that: 1) the relative increment in concentric strength was minor and does not relate to neural effects; 2) eccentric and isometric strength gains up to 8 training weeks are explained by the increased neural activation and muscle mass, whereas the increments in the last 4 training weeks seem to be associated with other mechanisms.

Introduction
Strength or resistance training is characterized by the execution of voluntary exercise against an external load or resistance [1,23]. In conventional resistance training (i.e., exercise with concentric and eccentric muscle actions), the strength increase in the first weeks of training is mainly attributed to an enhanced capacity of motor units activation (neural adaptation), whereas the increased strength after 6–8 weeks of training is commonly associated with the increase in muscle mass (morphological adaptation) [14,15,50]. Several clinical trials, however, have focused on the chronic effects of resistance training with eccentric actions in healthy subjects [3,6,12,19–21,27,33,38,40,43–45], regular resistance training practitioners [16,48] and athletes of different sports and competition levels [30,31]. Additionally, eccentric training has been proposed for the elderly [28,36,37,46] in order to counteract the deleterious effects of aging on the neuromuscular system [11,47], and has been widely used in musculoskeletal injury rehabilitation programs [22,26,49]. Knee extensor is the most frequently assessed muscle group and eccentric training programs have been usually performed using isokinetic dynamometers [3,6,19–21,27,28,33,38,40,46] or weight machines [36,37,44,45]. Although most studies have reported significant gains in strength when tests are performed eccentrically [3,19–21,28,33,37,38,40,45,46], increases in concentric strength have been observed in some studies [3,19,33,46] but not in others [20,21,36–38,40], and there are also inconsistent results regarding improvements on isometric strength [6,20,27,37,38,40,44–46]. Added to these conflicting results, there is a lack of studies focusing on strength temporal responses and the relative contribution of neural and morphological factors throughout an eccentric training program. Most studies assessing neural adaptations to knee extensor eccentric training suggest that motor unit activation increases in eccentric tests but not in concentric tests [19–21]. However, neural changes in isometric tests have shown inconsistent results with eccentric training pro-
strength gains in isometric, concentric and eccentric muscle actions require better addressed, as already done in conventional resistance training [32], isometric training [24] and chronic disease [10]. Therefore, the aim of this study was to verify the chronology of neural and morphological adaptations to knee extensor eccentric training and their contribution to strength gains in isometric, concentric and eccentric muscle actions throughout a 12-week eccentric training program.

Methods

Experimental design
This longitudinal study was conducted according to the ethical standards of the International Journal of Sports Medicine [18], and was approved by the local ethics committee. In order to verify the time course of neuromuscular adaptations to eccentric training, volunteers were evaluated 5 times during the 4 months of the study. Quadriceps femoris muscle mass, knee extensor torque and electrical activity during maximal isometric, concentric and eccentric tests were assessed in each evaluation session. A 4-week control period was respected between the first (Baseline) and the second (Pre-training) evaluations, during which subjects were instructed not to perform any systematic physical activity. Following the second evaluation, subjects initiated the 12-week knee extensor eccentric training program. Additional evaluations were performed after 4 (Post-4), 8 (Post-8) and 12 weeks (Post-12) of the eccentric training program (Fig. 1).

Subjects
Male healthy subjects between 20 and 35 years of age were invited to participate in this study. Since the training status represents an intervening factor on the adaptive responses to training [23], all volunteers were physically active university students who had not been enrolled in any kind of lower limb systematic resistance training program 6 months prior to the study. Exclusion criteria included: 1) history of lower limb musculoskeletal disorders that could be a contra-indication for maximal tests or that could impair performance during training and tests (e.g. patellar tendinitis, patellofemoral pain syndrome, knee surgeries, ruptured but not operated knee ligaments, recent muscle strains or joint sprains); 2) respiratory or cardiovascular diseases considered a risk or a limiting factor for maximal exercise; and 3) users of nutritional supplements, anabolic steroids or medications with analgesic and/or anti-inflammatory substances. Subjects were informed about the study design and the possible risks and discomfort related to the procedures, and all agreed to participate voluntarily through a written informed consent.

G*Power 3 software (Kiel University, Germany) estimated a sample size of 15 subjects (effect size = 0.30; significance level = 0.05; required power = 0.80). 22 subjects started the training program and 2 abandoned the intervention due to personal reasons. Therefore 20 volunteers (24.05 ± 3.73 years; 1.75 ± 0.06 m; 73.95 ± 6.99 kg) completed the full study schedule.

Strength assessment
Isometric, concentric and eccentric knee extensor peak torques were measured with a Biodex System 3 isokinetic dynamometer (Biodex Medical Systems, USA) in order to assess knee extensor strength adaptations to eccentric training. After a 5-min warm-up exercise performed on a cycle-ergometer, volunteers were positioned on the dynamometer according to the manufacturer’s recommendations for knee evaluations with the hip angle fixed at 85° and their trunk, hips and thighs firmly strapped to the apparatus. Subjects performed an additional warm-up protocol consisting of 10 knee extension/flexion repetitions at an angular velocity of 90°·s⁻¹ with a submaximal effort level. Subjects were previously instructed to execute all tests with the highest possible effort to develop maximal knee extension “as fast as possible”, and verbal encouragement was provided throughout the tests.

During isometric torque assessment, 3 maximal 5-s knee extensor isometric contractions were executed at 60° of knee flexion (0° = full extension), and a 2-min interval was observed between contractions. Peak torque values from each contraction were checked during data collection and an additional maximal knee extensor contraction was performed when torque variation was higher than 10% between the first 3 tests. For concentric torque evaluation, 3 consecutive maximal knee extensor concentric contractions were executed at an angular velocity of 60°·s⁻¹ and a range of motion between 90° and 10° of knee flexion. Subjects were instructed to perform maximal effort during knee extension and to passively return to initial position (90° of knee flexion). The test was repeated twice with a 2-min resting period between tests.

Eccentric torque was measured through 3 consecutive maximal knee extensor eccentric contractions at an angular velocity of 60°·s⁻¹ and range of motion between 30° and 90° of knee flexion. The concentric phase of the movement was executed with the investigator’s help and subjects initiated the active muscle contraction when the limb reached the start position (30° of knee flexion). 2 min were respected between tests. The highest peak torque values obtained during the isometric contractions (PTiso), concentric contractions (PTcon) and eccentric contractions (PTecc) were used for statistical analysis.
Neural activation assessment

An 8 channel EMG system AMT-8 (Bortec Biomedical Ltd, Canada) connected to a Windaq data acquisition system (Dataq Instruments Inc., USA) was synchronized with the dynamometer and used to evaluate the electrical activity of rectus femoris (RF), vastus lateralis (VL) and vastus medialis (VM) muscles during isometric, concentric and eccentric knee extensor maximal tests (Fig. 2).

Skin preparation and electrode positioning for EMG evaluation followed standard procedures [41]. Passive electrodes (Meditrace 100, Kendall, USA) were positioned in bipolar configuration (inter-electrode distance: 2.2 cm) on the RF (50% on the line from the anterior superior iliac spine to the superior part of the patella), VL (2/3 on the line from the anterior superior iliac spine to the lateral side of the patella) and VM (80% on the line from the anterior superior iliac spine and the joint space in front of the anterior border of the medial ligament) muscles. Maps on overhead transparency films were developed using anatomical reference points (i.e., border of patella) and skin marks (i.e., freckles and scars) to ensure the same position of the electrodes in all evaluations [9]. A reference electrode was fixed on the medial surface of the tibia.

Raw EMG signals were digitized with a sampling frequency of 2 000 Hz per channel with a DI-720 16 bits analogue-to-digital board (Dataq Instruments Inc., USA) and stored for subsequent analysis. Data were exported to SAD32 software, where they were filtered using a Butterworth band-pass filter, with cut-off frequencies of 20 and 500 Hz. Root mean square (RMS) values were calculated from 1-s segments of the EMG signals synchronized with knee extensor peak torque. The sum of RF, VL and VM RMS values in each test [isometric (ΣEMGiso), concentric (ΣEMGcon) and eccentric (ΣEMGecc) contractions] was used for statistical analysis in order to represent a large portion of the quadriceps femoris muscle activation [19].

Muscle mass assessment

Muscle imaging was performed with a B-mode Aloka SSD-4000 ultrasonography system (Aloka Inc., Japan), along with a linear array probe (60 mm, 7.5 MHz) to determine the morphological responses to knee extensor eccentric training. Muscle thickness from the same muscles chosen for EMG analysis (i.e., RF, VL and VM) was summed, and this sum (ΣMT) was considered as representative of quadriceps femoris muscle mass [9]. RF anatomical cross-sectional area (ACSA_{rf}) was also obtained with an ultrasound technique highly correlated with magnetic resonance imaging measurements [29]. These 2 parameters (ΣMT and ACSA_{rf}) were used to assess the morphological adaptations to eccentric training.

48 h without any vigorous physical activity were respected prior to the tests and subjects were evaluated in a supine position after 10 min of rest in this position [3]. Special attention was given to determine the specific sites where the images were collected from. Maps on overhead transparency films were made using anatomical reference points and skin marks to ensure that measurements were obtained from identical sites on each occasion [9]. All measurements were made by a highly experienced investigator using this technique.

3 ultrasound images were obtained with the ultrasonography transducer positioned longitudinally to the muscle fibers in each of the knee extensor muscles (Fig. 3). The midway point between the greater trochanter and the lateral condyle of femur was used as a reference point for RF and VL assessment, whereas VM measurements were made at 25–30% of this distance according to the subject’s characteristics. These ultrasound images were used for muscle thickness analysis. In addition, 3 transversal images at 50% of the distance between the greater trochanter and the lateral condyle of femur were taken for ACSA_{rf} analysis (Fig. 3). The ultrasonography probe was covered with a water-soluble transmission gel and placed on the scan site while not depressing the skin.

All ultrasound images were analyzed by the same experienced investigator with the Image-J software (National Institute of Health, USA). The distance between the deep and the superficial aponeuroses was measured at 5 different points in each longitudinal ultrasound image, and a mean value was used as the mean thickness of that ultrasound image. Mean values were obtained from 3 ultrasound images and taken as the muscle thickness for each muscle. A similar procedure was used for estimation of ACSA_{rf}. Five measures of RF area were made in each transversal ultrasound image and a mean value between the 3 ultrasound images was taken as the ACSA_{rf}. 
Eccentric training program

Volunteers were engaged in a 12-week knee extensor eccentric training program. Training sessions were performed twice a week, with a minimum interval of 72 h between sessions. Each training session comprised of a 5-min-warm up exercise on a cycle-ergometer followed by the knee extensor eccentric exercise on the same isokinetic dynamometer used during tests. The training program comprised of 3 sets of 10 repetitions during weeks 1–4, 4 sets in weeks 5–8 and 5 sets in weeks 9–12. A 1-min rest period was respected between sets. Subjects per-voluntarily initiated the study by training both lower limbs in order to avoid muscular unbalances due to training adaptations. However, some subjects experienced joint pain in one leg during the training sessions while others experienced musculoskeletal injury in one leg during their daily activities (unrelated to the training program). In these cases, only the uninjured segment from these subjects was considered for analysis. As a result, this study was conducted with 20 volunteers, 15 left and 5 right legs (or 16 non-dominant and 4 dominant legs). 18 volunteers completed the 21 training sessions and 2 subjects missed one session each due to personal reasons (adherence: 99.5%).

Statistical analysis

An intraclass correlation coefficient (ICC) was applied to verify the test-retest reliability between Baseline and Pre-training evaluations for all measurements. A repeated measures ANOVA was used to verify the effect of time between the 5 time points of data collection (i.e., Baseline, Pre-training, Post-4, Post-8 and Post-12), followed by Bonferroni post-hoc analysis when significant differences were detected. All statistical analyses used a significance level of p < 0.05. Results are presented in the figures as mean ± standard error of the mean (SEM).

Results

High scores of test-retest reliability between Baseline and Pre-training evaluations were observed for all tests: PT iso; r = 0.983; PT con; r = 0.958; PT ecc; r = 0.958; ΣEMG iso; r = 0.891; ΣEMG con; r = 0.848; ΣEMG ecc; r = 0.844; ΣMT; r = 0.956; ACSA rf; r = 0.971. Additionally, there were no significant differences in any variable between Baseline and Pre-training evaluations (p = 1.000 for all tests).

Figures 4–6 illustrate the adaptations observed throughout the training program for knee extensor strength (peak torque), neural activation (EMG activity) and muscle mass (thickness and ACSA), respectively. These figures show the significant differences between the 5 time points evaluated (Baseline, Pre-training, Post-4, Post-8 and Post-12) and the mean percent change observed at 4, 8 and 12 weeks of eccentric training. PT iso, PT con and PT ecc increased significantly at 4 weeks of eccentric training (p < 0.001, p = 0.001 and p < 0.001, respectively) and from Post-4 to Post-8 evaluation (p < 0.001, p = 0.001, p = 0.016). PT iso and PT ecc increased significantly between Post-8 and Post-12 evaluations (p = 0.007 and p = 0.013, respectively), whereas PT con remained at the same level in this period (p = 0.177) (Fig 4).

ΣEMG iso did not change significantly between Pre-training and Post-4 evaluations (p = 0.114), reached a significant increase at 8 training weeks (Pre-training vs. Post-8; p = 0.025) and stabilized between Post-8 and Post-12 evaluations (p = 1.000). Although the shape of ΣEMG con curve suggested a tendency for increment throughout the training period, this parameter did not change significantly throughout the study (Pre-training vs. Post-4:...
Training & Testing

Although several studies have evaluated the effects of eccentric training on the knee extensor muscles, to the best of our knowledge the present study is the first to simultaneously follow-up the neural and morphological changes during a 12-week eccentric training program, and to associate these adaptations with strength gains in isometric, concentric and eccentric tests. Our main findings were that a knee extensor eccentric training program 1) increased the isometric, concentric and eccentric peak torques, albeit by different magnitudes, 2) increased neural activation at 4 and 8 weeks of training in eccentric and isometric tests, respectively, but did not increase in concentric tests, and 3) promoted a significant hypertrophic response at 4 and 8 weeks of training, without further changes between 8 and 12 training weeks.

The experimental design of this study discarded the need of a non-trained control group, since all volunteers underwent a 4-week control period immediately before the eccentric training program. The high ICC scores and the absence of significant differences between Baseline and Pre-training evaluations supported the reliability of our measurement procedures. However, confounding factors directly affect the knee extensor adaptations to eccentric training, such as subjects characteristics [1,23], training periodization [1,23] and the use of isokinetic dynamometers or weight machines for training [17]. Thus, caution is required when comparing results from studies with different training programs or samples with distinct characteristics. Our data allowed us to estimate the percentage contribution from neural and morphological factors to the strength gains at the different time points during the eccentric training program, as previously performed in conventional resistance training [32]. However, it is important to note that our study, as well as

Discussion

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Fig. 5 Knee extensor activation in isometric (ΣEMGcon), concentric (ΣEMGcon) and eccentric (ΣEMGecc) tests throughout the study. α ≠ different from Baseline (p < 0.05); β ≠ different from Pretraining (p < 0.05); δ ≠ different from Post-4 (p < 0.05); λ = different from Post-8 (p < 0.05); θ = different from Post-12 (p < 0.05); Δ = percent change.

Fig. 6 Knee extensor muscle thickness (EMT) and rectus femoris anatomical cross-sectional area (ACSArf) throughout the study. α ≠ different from Baseline (p < 0.05); β ≠ different from Pretraining (p < 0.05); δ ≠ different from Post-4 (p < 0.05); λ = different from Post-8 (p < 0.05); θ = different from Post-12 (p < 0.05); Δ = percent change.
most resistance training studies, analyzed the electrical activity and muscle morphology at specific sites of the quadriceps femoris muscle. Since some eccentric training studies have demonstrated distinct adaptive responses between different regions of the same muscle [3, 19, 40, 44], extrapolation of the measurements for the full knee extensor muscle should be done with caution. Therefore, we did not determine values of percentage contribution from neural and morphological adaptations to the strength gains observed in our subjects, but we discussed the neural and morphological contributions to strength gains in isometric, concentric and eccentric tests throughout the eccentric training program through the time-course responses of torque, EMG and muscle mass data.

Higher increments in knee extensor eccentric strength compared to isometric and concentric strength were expected [3, 19–21, 33, 37, 38, 40, 45, 46], based on the specificity of strength gains due to eccentric training [17, 39]. However, contrary to studies that failed to show strength gains in isometric [37, 40, 45] and concentric tests [20, 21, 37, 38, 40], we found significant increases in torque with only 4 weeks of eccentric training. Isometric peak torque continued to increase through subsequent evaluations, but in magnitudes that were smaller than those observed in eccentric strength. The concentric peak torque also increased from the fourth to the eighth training week, but did not change from Post-8 to Post-12 evaluation. This behavior of knee extensor strength in isometric, concentric and eccentric tests is well associated with the EMG and ultrasound results, as discussed below.

EMG activity in eccentric tests increased at 4 training weeks and remained unchanged until the end of the study (Fig. 5), a result that can likely be explained by the motor learning related to the eccentric exercise. Higher levels of neural inhibition have been described in eccentric actions, and the most accepted hypothesis is related to differential programming of eccentric and concentric actions at the central nervous system, associated with the influence of proprioceptive feedback fromafferent neurons during the eccentric phase [13, 17]. Therefore, our results corroborate previous findings showing an increase in eccentric EMG activity with knee extensor eccentric training [19–21], but add an interesting point: this neural adaptation predominantly occurs in the first 4 weeks of eccentric training. Increments in EMG activity were observed from the Post-8 evaluation for isometric tests, while EMG activity did not change significantly throughout the study in concentric tests (Fig. 5). Hortobagyi et al. [21] also found improvements in isometric activation with isokinetic eccentric training, and most eccentric training studies agree with an absence of changes for concentric and isometric tests. It is tempting to speculate that the force produced per unit of muscle area was improved due to intrinsic adaptations of muscle cells not related to the hypertrophy response. However, there is the possibility that eccentric training decreased the intramuscular fat content [16] accompanied by a simultaneous and proportional increase in contractile content [21], without affecting muscle thickness or ACSA. This adaptation could explain why resistance training-induced changes in muscle fibers cross-sectional area are greater than changes in the whole muscle ACSA [14], as shown in a study involving elbow flexors eccentric training [43]. Furthermore, eccentric training can lead to an increase in the connective tissue stiffness [12], an adaptation likely linked to increased stimulus for collagen synthesis [25]. Therefore, the peak torque would be increased through the engagement of passive elements for force generation, since performance in high-intensity muscle actions relies in part on effective force transmission from the contractile elements to the skeleton [7].

In conclusion, this study provides information on the temporal responses of strength, neural activation and muscle mass of knee extensor muscles throughout a 12-week isokinetic eccentric training program. Our results suggest that eccentric training has a high specificity effect on strength gains in favor to eccentric and isometric contractions. Eccentric and isometric strength increments at 4 and 8 training weeks may be explained by the sum of neural and morphological adaptations, whereas the strength improvements from the eighth to the twelfth training week should be attributed to other mechanisms than neural activation increments or detectable changes in muscle mass.
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References


