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ORIGINAL ARTICLE

The Effects of Short-Term Resistance Training and Subsequent Detraining on Neuromuscular Function, Muscle Cross-Sectional Area, and Lean Mass

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Abstract

Purpose To examine and compare the effects of three days of dynamic constant external resistance (DCER) and isokinetic (ISOK) training and subsequent detraining on thigh muscle cross-sectional area (TMCSA) and thigh lean mass (TLM), ISOK peak torque (PT), DCER strength, isometric force, muscle activation, and percent voluntary activation (%VA).

Methods Thirty-one apparently-healthy untrained men (mean ± SD age = 22.2 ± 4.2 years; body mass = 77.9 ± 12.9 kg; height = 173.9 ± 5.4 cm) were randomly assigned to a DCER training group ($n = 11$), ISOK training group ($n = 10$) or control (CONT) group ($n = 10$). Subjects visited the laboratory eight times. The first visit was a familiarization session, the second visit was a pre-training assessment, the subsequent three visits were for unilateral training of the quadriceps (if assigned to a training group), and the last three visits were the post-training assessments conducted at three days, one week, and two weeks after training ended.

Results DCER strength increased from pre- to post-training assessment 1 in both limbs for the DCER group only, and remained elevated during post-training assessments 2 and 3 ($P < 0.05$). In addition, surface EMG for the biceps femoris was higher at post-training assessment 3 than at the pre-training assessment, and post-training assessments 1 and 2 ($P < 0.05$). No other training-related changes were found.

Conclusion The primary finding of this study was that DCER strength of the trained and untrained limbs can be increased with three days of training. This has important implications for injury rehabilitation, where in the initial period post-injury strength gains on an injured limb can possibly be obtained with short-term contralateral resistance training.

Keywords Twitch interpolation · Electromyography · Muscle activation · Strength training · Injury rehabilitation

Abbreviations

BF%	Body fat %
BM	Body mass
CSA	Muscle cross-sectional area
DCER	Dynamic constant external resistance
DXA	Dual-energy X-ray absorptiometry
EMG	Electromyography
ISOK	Isokinetic
MMG	Mechanomyography
MVC	Maximum voluntary contraction
PT	Peak torque
TF%	Thigh fat %
TLM	Thigh lean mass
TMCSA	Thigh muscle cross-sectional area
RPE	Rating of perceived exertion
%VA	Percent voluntary activation

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Introduction

Resistance training has been the hallmark of exercise and rehabilitation programs. Recently, interest has been shifted to short-term resistance training. Allied health professionals, such as physical therapists and athletic trainers, may benefit from a rapid increase in strength of a patient or athlete

recovering from injury [3, 11, 15]. In theory, if an individual's strength can be increased within a short amount of time, they are more likely to comply with a rehabilitation program and perhaps decrease the risk of reinjury [3]. Accordingly, short-term resistance training has been shown to increase muscle strength and isokinetic performance after only days of training [5, 11, 15, 55]. Those who are injured could benefit greatly from this type of training because they often receive limited physical rehabilitation treatments due to minimal insurance coverage or lack of compliance [11, 15, 70], or simply because not enough time is available before an athlete needs to return to play. If patients do not improve quickly, the risk of injury reoccurrence may increase [15]. In addition, if short-term resistance training can improve performance quickly, patient compliance may increase, risk of reinjury might be reduced, and it may offer an alternative to more expensive and oftentimes invasive procures [11, 15]. Because of this potential for short-term resistance training to improve muscular performance in a relatively shorter period of time, it would have important implications for professionals working in rehabilitation settings [3, 11, 15].

Increases in muscular strength following a resistance training program can be attributed to neural and hypertrophic factors [23, 42, 46, 57, 58, 60, 64]. Thus, voluntary strength increases due to not only the quantity and quality of muscle mass, but also to the extent that the muscle mass is able to activate [6, 58]. In general, neural factors are believed to account for most of the increases in strength in the early stage of a resistance training program, whereas hypertrophic factors gradually become prevalent after several weeks of training [23, 36, 38, 40, 46, 47, 58]. Hence, initial improvements in strength and muscular performance reported following short-term resistance training are generally attributed to neural adaptation instead of muscle fiber hypertrophy [1, 46]. For example, Akima et al. [1] reported increases in peak torque (PT) after two weeks of resistance training but no changes in muscle cross-sectional area (CSA) or fiber area suggesting strength increases occurred without muscle hypertrophy [1]. Nevertheless, Akima et al. [1] suggested future studies should investigate the mechanisms underlying strength gains obtained with short-term resistance training [1].

When training a muscle on one limb, strength can be increased on the contralateral side in an outcome termed contralateral strength training effect [9] or cross-education [12, 25, 39, 43, 49]. A meta-analysis concluded that overall, unilateral resistance training led to -2.7% to 21.6% changes in the initial strength of the untrained limb, with a mean improvement of 7.8%, which was 35.1% of the effect on the trained limb [49]. A more recent meta-analysis reported a pooled mean increase in strength of 15.9% with isotonic training compared to 11.3% with concentric-only training [43]. Nevertheless, the mechanisms for contralateral strength gains are unclear and there is no consensus on proposed mechanisms [49]. In addition, most of the increases in strength found with resistance training are lost after several weeks of training [13, 28, 29, 35, 50, 66]. However, Colliander and Tesch [13] revealed that a resistance training program incorporating combined concentric and eccentric leg extension repetitions retained more of the recently-acquired strength gains than the concentric-only repetitions [13]. Because isokinetic muscle action training is primarily concentric in nature, it is unknown whether dynamic constant external resistance (DCER) training, which uses coupled concentric and eccentric muscle actions, or isokinetic training would affect detraining differently.

The neuromuscular system undergoes numerous adaptations following a resistance training program [1, 23, 42, 46, 47, 57, 64]. Research suggests early adaptations to resistance training programs are related to improvements in neuromuscular efficiency, which perhaps indicates an increased capacity to activate skeletal muscle voluntarily [1, 11, 15, 40, 55, 60]. However, the specific mechanisms of such adaptations in short-term training are not fully understood [11]. Traditionally, isokinetic muscle actions have been used in rehabilitation and testing scenarios. Several studies have examined the effects of isokinetic training on strength and/or muscle CSA, and isokinetic training allows development of maximum tension throughout the range of motion [1, 3, 5, 11, 15, 55]. However, DCER training would offer a more accessible, convenient, cost-effective, and practical method of training, in addition to perhaps providing a greater stimulus to elicit increases in strength [14, 39, 41]. Therefore, the purpose of this study was to examine and compare the effects of three days of DCER or isokinetic (ISOK) training and subsequent detraining on thigh muscle CSA and thigh lean mass, isokinetic PT, DCER strength, isometric force, surface electromyography (EMG) and mechanomyography (MMG) of the leg extensors during maximal leg extension muscle actions, and percent voluntary activation (%VA).

Methods

Subjects

Thirty-one healthy men between the ages of 18 and 35 who were naïve to the intent of the experiment volunteered to participate in this study (Table 1). Prior to any testing, all subjects read and signed an Informed Consent form and completed a health and exercise status questionnaire. Any individual with degenerative neuromuscular or joint disorders or injuries distal to the waist within six months prior to screening were also excluded from the study. Subjects were untrained in resistance training within six months prior to the start of the study. Thus, those who reported engaging

Table 1 Sample sizes and means (±SE) for age (yr), body mass (kg), height (cm), body fat percentage (%), thigh fat (%), thigh lean mass (g), and thigh muscle cross-sectional area (cm^2) for the DCER, ISOK, and CONT groups

Groups	Age (year)	BM (kg)	HT (cm)	BF%	TF%	TLM (g)	TMCSA (cm^2)
DCER ($n=11$)	20.3 ± 0.5	77.9 ± 3.6	173.2 ± 2.2	24.4 ± 2.5	30.3 ± 2.7	6570.3 ± 298.5	152.1 ± 9.5
ISOK ($n=10$)	23.7 ± 1.8	79.4 ± 3.4	176.3 ± 1.3	20.6 ± 2.0	25.4 ± 1.7	7064.5 ± 284.6	171.0 ± 9.6
CONT ($n=10$)	22.9 ± 1.3	77.3 ± 5.3	172.3 ± 1.2	24.8 ± 2.9	28.1 ± 2.9	6329.6 ± 278.0	151.4 ± 9.9

DCER dynamic constant external resistance, *ISOK* isokinetic, *CONT* control, *BM* body mass, *HT* height, *BF* body fat, *TF* thigh fat, *TLM* thigh lean mass, *TMCSA* thigh muscle cross-sectional area

in one or more lower-body resistance training exercises for six months prior to the start of the study were excluded. Subjects were asked to maintain their daily activities, but refrain from any exercise and/or nutritional supplements throughout the course of the study. In addition, individuals who had been taking nutritional supplements three months prior to screening were not allowed to volunteer. This study was approved by the university's Institutional Review Board for the protection of human subjects.

Research Design

A mixed factorial design was used to examine the effects of three days of DCER versus ISOK training with the leg extensors. Participants were randomly assigned to a DCER training group, ISOK training group, or CONT group. Participants visited the laboratory on 8 occasions separated by 2–7 days. The first visit was a familiarization session, the second visit was a pre-training assessment, the subsequent three visits were for training, and the last three visits were the post-training assessments (Fig. 1). Pre-training assessments were performed 48 h prior to the start of training. Dependent variables in this study were [a] body mass (BM); [b] body fat % (BF%); [c] thigh fat % (TF%); [d] thigh lean mass (TLM); [e] thigh muscle cross-sectional area (TMCSA); [f] isokinetic PT; [g] DCER strength; [h] isometric force; [i] EMG; [j] MMG; [k] percent of voluntary muscle activation (%VA) at 30%, 50%, 70% and 100% of

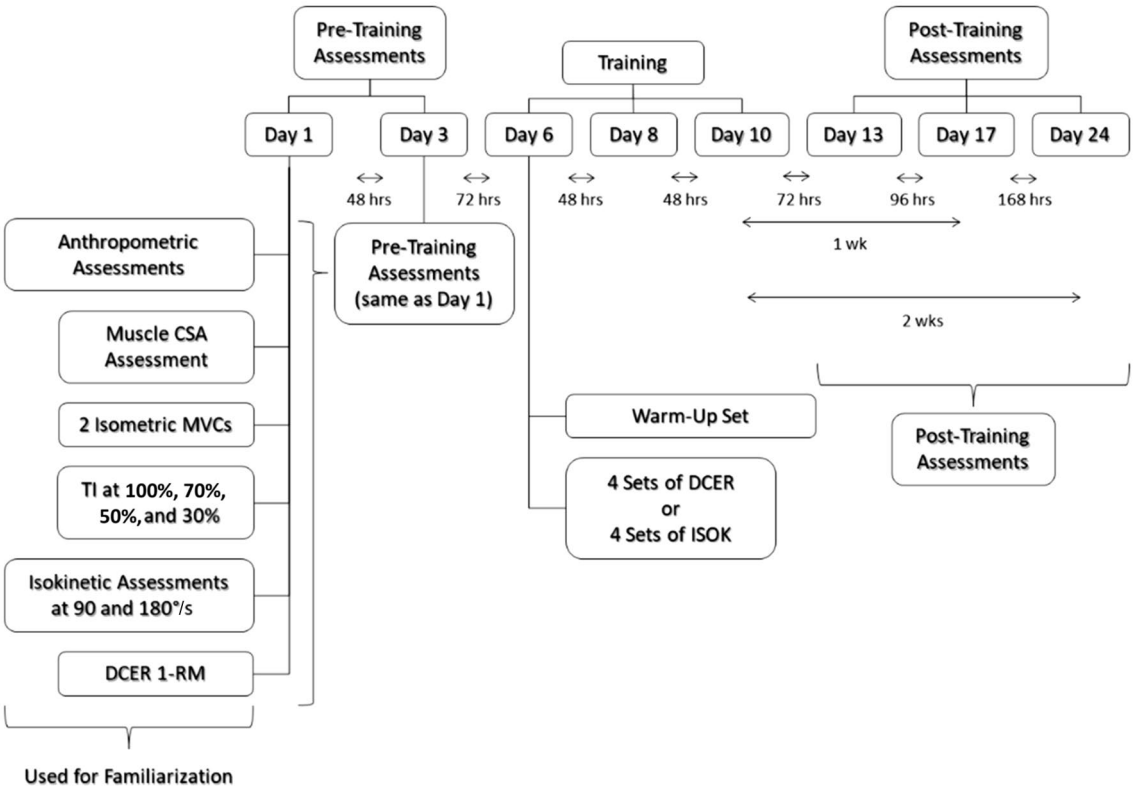

Fig. 1 Study design flowchart

maximum voluntary contraction (MVC); and (l) rating of perceived exertion (RPE). After the three training sessions, post-training assessments were performed in an identical manner to the pre-training assessments. In order to examine the time course of the effects of the different training modes, post-training assessments were performed 72 h, 1 week and 2 weeks after the final training session. All pre- and post-training assessments were conducted at approximately the same time of day.

Familiarization Trial

During the familiarization visit, subjects underwent screening and upon approval, were familiarized with the ISOK and DCER leg extension machines and exercises to allow the inexperienced participants to become better accustomed to the proper exercise technique execution. In order to reduce the potential of any learning effect, all strength assessments were practiced during the familiarization session. The familiarization visit included anthropometric assessments (BM, height and body composition) followed by the maximal isometric force, ISOK PT and DCER strength tests (in listed order). These assessments and tests were performed in an identical manner during the pre- and post-training assessments.

Dual-Energy X-ray Absorptiometry

Dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Advance 300532, General Electric Healthcare, Madison, WI) was used to assess BF%, TF% and TLM. Each day prior to testing, a quality assurance phantom was scanned to ensure calibration. Before each assessment, the subjects' height, BM, sex, and race were entered into the computer DXA software program (enCORE™ 2006, version 10.50.086). Subjects were positioned supine on the DXA table with hands pronated and flat on the table. Total body mode was selected for each scan, and scanning thickness was determined by the DXA software. Thigh lean mass was determined using regions of interest around the dominant thigh. Region of interest markers were placed by the primary investigator (P.B.C.) using the DXA software crossing diagonally the femoral neck and horizontally through the inferior border of the femur condyles (Fig. 2).

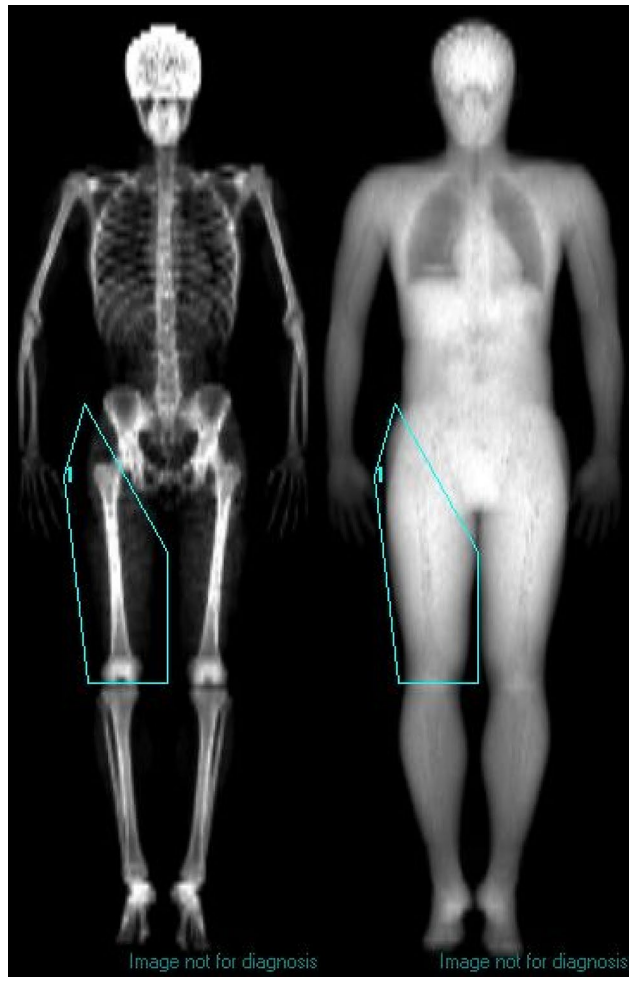

Fig. 2 Region of interest around the dominant thigh

Muscle Cross-Sectional Area

Thigh muscle CSA (TMCSA) was estimated by measuring circumference and correcting for subcutaneous fat [46, 47]. In short, the circumference of the thigh was measured, the radius calculated, and TMCSA was estimated by subtracting the mean of four skinfolds [46]. Thigh circumference was measured at the midpoint between the inguinal crease and the proximal border of the patella [69]. Circumference was measured to the nearest millimeter using a tension-gauged measuring tape (Gullick II; Country Technology, Inc., Gays Mills, WI). Skinfold thickness was measured using four skinfolds from the anterior, lateral, posterior, and medial region of the thigh, and measured to the nearest millimeter using a Lange caliper (Santa Cruz, CA), midway between the proximal border of the patella and the inguinal crease [69]. The skinfold values were averaged after three measurements were taken at each site. Muscle CSA was estimated using the equation by Moritani and deVries [46, 47]:

$$CSA = \pi \left[\frac{Circumference}{2\pi} - \frac{\sum_{i=1}^{4} skf_i}{4} \right]^2$$

where *Circumference* is defined as the circumference of the thigh, and *skf* is defined as the thigh skinfold thickness of each of the four sites.

Isokinetic Assessments

Maximal concentric ISOK PT of the left and right leg extensors was measured using a calibrated Biodex System 3 ISOK dynamometer (Biodex Medical Systems, Inc., Shirley, NY) at the randomly-ordered velocities of 90 and 180°/s. Subjects were in a seated position with restraining straps over the pelvis, trunk, thigh, and ankle in accordance with the Biodex User's Guide (Biodex Pro Manual, Applications/Operations. Biodex Medical Systems, Inc., Shirley, NY. 1998). The input axis of the dynamometer was aligned with the axis of rotation of the knee. Prior to the ISOK assessment, each participant's range of motion was individually determined and limb weight measured for subsequent gravity correction. Three submaximal warm-up repetitions of increasing intensities (i.e., approximately 25%, 50% and 75% of the subject's perceived maximum) preceded three maximal muscle actions at each velocity. During the tests, loud verbal encouragement was provided by the investigator such that each subject was instructed to "kick out" as hard as possible throughout the entire range of motion. A 1-min rest was allowed between velocities [52].

Dynamic Constant External Resistance Assessments

The maximal strength of both leg extensors was assessed using a DCER Nautilus leg extension machine (Nautilus, Inc. Vancouver, WA) (Fig. 3). The input axis of the machine was aligned with the axis of rotation of the knee. The distal anterior portion of the leg superior to the ankle was used as the load bearing point. Three submaximal warm-up sets of increasing tester-selected intensities (i.e., 6–8, 3–5 and 1–2 repetitions) preceded the maximal strength attempt. When one attempt was successful, the load was increased by 2–5 kg until a failed repetition occurred. A failed repetition was defined as the inability to complete the full range of motion with the assigned load. During the tests, loud verbal encouragement was provided by the investigator. Each subject was instructed to provide maximal effort throughout the entire range of motion. The greatest load moved through a complete leg extension range of motion was considered the one repetition maximum 1-RM. A 1-min rest was allowed between each successive attempt [44, 68].

Fig. 3 Dynamic constant external resistance assessment set-up with isokinetic dynamometer on the background

Electromyography

Pre-amplified, bipolar surface EMG electrodes (EL254S, Biopac Systems Inc.; Santa Barbara, CA, gain = 350) with a fixed center-to-center inter-electrode distance of 20 mm were taped over the rectus femoris (RF), vastus lateralis (VL), and biceps femoris (BF) muscles of the dominant leg. For the RF, the electrodes were placed at 50% of the distance between the inguinal crease and the superior border of the patella. For the VL, the electrodes were placed at the anterior border of the iliotibial band along the muscle's longitudinal axis at 50% of the distance from the greater trochanter to the lateral epicondyle of the femur. Finally, the electrodes for the BF were placed at the midpoint of the distance between the ischial tuberosity and the lateral epicondyle of the tibia. A single pre-gelled, disposable electrode (Ag–Ag Cl, Quinton Quick Prep, Quinton Instruments Co., Bothell, WA) was placed over the spinous process of the 7th cervical vertebrae to serve as a reference electrode. Prior to electrode placement, the skin was shaved to remove hair and the recording sites were carefully abraded with an abrasive pad and cleaned using isopropyl alcohol to reduce the inter-electrode impedance. All electrodes were placed in accordance with the recommendations of Hermens et al. [32]. The EMG signals (recorded in microvolts) were differentially amplified with a bandwidth of 1 to 500 Hz, input impedance of 2 M (differential), common mode rejection ratio of 110 dB, maximum input voltage of 10 V, sampling rate of 1,000 (ISOK PT) and 10,000 (%VA) Hz, and gain of 1,000 (EMG100C; Biopac Systems, Inc., Santa Barbara, CA). After the electrodes were removed, a permanent marker was used to indicate the positions of the electrodes for replacement during subsequent post-training assessments.

Mechanomyography

The MMG signals were detected with active miniature accelerometers (EGAS-FS-10-/VO5, Intran Inc., Fairfield, NJ) that were pre-amplified with a gain of 200, frequency response of 0–200 Hz, sensitivity of 70 mV/ms^2 and range of ± 98.1 mV/ms^2. The accelerometers were placed over the RF and VL muscles, respectively, and fixed to the skin with double-sided foam tape (3M, ST. Paul, MN). MMG

accelerometers were placed superior to the EMG electrodes. After the accelerometers were removed, a permanent marker was used to indicate the positions of the electrodes for replacement during subsequent post-training assessments.

Isometric Assessments

Two isometric MVCs were recorded using a Biodex System 3 ISOK dynamometer (Biodex Medical Systems, Inc., Shirley, NY) configured for isometric mode and modified with an in-line load cell (Omegadyne LC402, Stamford, CT). The participant was seated in a similar manner as the isokinetic assessments, with the input axis of the dynamometer aligned with the axis of rotation of the knee and the leg fixed at an angle of 90° below the horizontal plane. During the tests, loud verbal encouragement was provided by the investigator such that each subject was instructed to "kick out" as hard as possible for approximately 4–6 s. A 1-min rest was allowed between each assessment. The highest force output between the two trials was selected as the representative MVC value.

After the MVC trials, each subject performed submaximal isometric step muscle actions at 30%, 50% and 70% of the peak MVC. The order of the submaximal isometric step muscle actions was randomly ordered during each experimental trial. During the isometric step, participants were asked to track their force production on a computer monitor placed in front of them that displayed in real-time, digitized force signals overlaid onto a programmed template. Horizontal lines were programmed as templates on the computer monitor that served as the target force lines. The isometric step muscle action templates and real-time force overlay were programmed and displayed using LabView version 8.5 (National Instruments, Austin, TX). A 1-min rest was allowed between each isometric assessment.

Twitch Interpolation

The twitch interpolation technique was used to determine %VA at 30%, 50%, 70% and 100% of MVC. Transcutaneous electrical stimuli were delivered to the femoral nerve using a high-voltage (maximal voltage = 400 V), constant-current stimulator (Digitimer DS7AH, Herthfordshire, UK). A small bipolar electrode was pressed over the lateral portion of the femoral triangle. Single stimuli were administered to the femoral nerve at a low current (amperage = 20 mA) to determine the optimal probe location by the investigator based on visually inspecting a computer monitor displaying the compound muscle action potential (M-wave) and Hoffmann reflex (H-reflex) of the RF muscle. Once the best location was determined and marked, the maximal M-wave was achieved with incremental (5 mA) amperage increases until a plateau in the peak-to-peak (p-p) M-wave was observed after three successive amperage increases. To ensure a supramaximal stimulus, 120% of the stimulus intensity (mA) that elicited the maximal M-wave was used during the evoked twitches and TI procedures. A single stimulus was defined as a 200-µs duration square wave impulse, while a doublet consisted of two single stimuli delivered successively at 100 Hz. Doublets were administered with the supramaximal stimulus intensity during the MVC trials to increase the signal-to-noise ratio and minimize the series elastic effects on force production [20]. In accordance with the TI procedure, supramaximal stimuli were administered 3–5 s into the MVC plateau (superimposed twitch) and again 3–5 s after the MVC trial at rest (control twitch). The extent of voluntary activation was quantified by the following equation [2, 45]:

$$\%VA = \left[1 - \left(\frac{Superimposed\ Twitch}{Control\ Twitch}\right)\right] \times 100$$

where the *Superimposed Twitch* is the force increase during the MVC at the time of stimulation and the *Control Twitch* is the response in the relaxed muscle.

Signal Processing

ISOK torque, EMG and MMG signals were sampled at 1000 Hz during ISOK PT assessments and at 10,000 Hz during MVC force and %VA assessments. Signals were recorded simultaneously with a Biopac data acquisition system (MP150WSW, Biopac Systems, Inc.; Santa Barbara, CA) during each assessment from the dynamometer interfaced with a laptop computer (Inspiron 8200, Dell Inc., Round Rock, TX) using proprietary software (AcqKnowledge version 3.7, Biopac Systems, Inc., Santa Barbara, CA) and stored on a personal computer for off-line analysis. Torque, force, EMG and MMG signals were processed off-line with custom-written software (LabView 8.5, National Instruments, Austin, TX). ISOK PT was calculated as the highest 0.25-s average torque value for the repetition that yielded the highest PT value. Consequently, the same (concurrent) 0.25-s epochs were selected for all EMG and MMG signals. Isometric MVC force (N) was calculated as the highest average force value that occurred during a 0.05-s epoch. The force value for the TI was calculated during a 0.25-s epoch immediately prior to the stimulation for all submaximal and the two MVC (ITT MVC) trials. EMG and MMG signals were filtered with a bandpass (zero phase 4th order Butterworth filter) of 10–500 Hz and 5–100 Hz, respectively. The amplitudes of the EMG and MMG signals were quantified by calculating the root-mean-square (RMS) values for each signal epoch.

Isokinetic and Dynamic Constant External Resistance Training Protocol

After a rest period of 48 h following the pre-training assessment, the training groups performed three ISOK or DCER training sessions separated by 48 h. Participants in the training groups performed 4 sets of 10 repetitions of knee extension muscle actions. For the ISOK training group, a velocity of 90°/s was used, whereas moderate speed in a controlled manner was used for the DCER training group. Each training session began with ten warm-up repetitions at approximately 25% of the resistance used for the DCER training session and 25% of the individual's perceived maximal for the ISOK training session. Approximately 80% of the 1-RM obtained during the DCER maximal strength assessment was used as the starting load for the DCER group. A 2-min rest period was allowed between each training set. Training load for the DCER group was continually increased and adjusted as each participant was able to tolerate a given load with ease in order to assure that the subject reached failure at approximately the 10th repetition. Participants of the ISOK training program were seated and strapped to the machine similarly to the manner used during the maximal ISOK assessment. All participants taking part in either ISOK or DCER training interventions were supervised during all training sessions.

Rating of Perceived Exertion

Rating of perceived exertion (RPE) was used to compare effort between the DCER and ISOK training modes [17, 21, 22, 26, 65]. Prior to the start of the study, subjects received instructions on how to use the RPE scale to rate their perceived exertion. A Category-Ratio scale (CR-10) was used, where "0" was classified as rest (no effort) and "10" was classified as maximal effort (most stressful exercise ever performed). The CR-10 has been slightly modified to reflect American English (e.g., easy and hard instead of light and strong, respectively) [26]. Subjects were asked to provide a number on the scale to rate their overall effort immediately after each set was completed and after the entire training session. These RPE assessments were conducted during each session by showing the scale and asking subjects "How would you rate your effort?" and "How would you rate your entire workout?" immediately after each set of training and after each training session, respectively. Therefore, in this study, "set RPE" was defined as the RPE reported by the subject after each set, while "session RPE" was defined as the RPE reported each day after the training session was completed.

Statistical Analyses

Four one-way factorial ANOVAs were used to compare the means at baseline for age, BM, height, BF%, TF%, TLM, and TMCSA among the DCER, ISOK, and CONT groups. Five two-way mixed factorial ANOVAs (time [pre- vs. post-training assessment 1 vs. post-training assessment 2 vs. post-training assessment 3] × group [ISOK vs. DCER vs. CONT]) were used to analyze the anthropometric, body composition, and MVC data. A three-way mixed factorial ANOVA (time [training session 1 vs. training session 2 vs. training session 3] × group [ISOK vs. DCER] × set [1 vs. 2 vs. 3 vs. 4]) was used to analyze RPE assessed after each set during training. A two-way mixed factorial ANOVA (time [training session 1 vs. training session 2 vs. training session 3] × group [ISOK vs. DCER]) was used to analyze training session RPE. One three-way mixed factorial ANOVA (time [pre- vs. post-training assessment 1 vs. post-training assessment 2 vs. post-training assessment 3] × group [ISOK vs. DCER vs. CONT]) × limb [right vs. left]) was used to analyze 1-RM data. A four-way mixed factorial ANOVA (time [pre- vs. post-training assessment 1 vs. post-training assessment 2 vs. post-training assessment 3] × group [ISOK vs. DCER vs. CONT]) × velocity [90 vs. 180°/s] × limb [right vs. left]) was used to analyze ISOK PT. A three-way mixed-factorial ANOVA (time [pre- vs. post-training assessment 1 vs. post-training assessment 2 vs. post-training assessment 3] × group [ISOK vs. DCER vs. CONT]) × intensity [30% vs. 50% vs. 70% vs. 100%]) was used to analyze the TI data. A four-way mixed factorial ANOVA (time [pre- vs. post-training assessment 1 vs. post-training assessment 2 vs. post-training assessment 3] × group [ISOK vs. DCER vs. CONT]) × muscle [RF vs. VL] × velocity [90 vs. 180°/s]) was used to analyze quadriceps EMG and MMG. A three-way mixed factorial ANOVA (time [pre- vs. post-training assessment 1 vs. post-training assessment 2 vs. post-training assessment 3] × group [ISOK vs. DCER vs. CONT]) × velocity [90 vs. 180°/s]) was used to analyze the BF EMG. When appropriate, follow-up analyses were performed using lower-order two- and one-way repeated measured ANOVAs, and paired sample *t*-tests. An alpha level of $P < 0.05$ was considered statistically significant for all comparisons. Predictive Analytics SoftWare (PASW) version 18.0.0 (SPSS Inc., Chicago, IL) was used for all statistical analyses.

Results

Anthropometric and Body Composition Characteristics

There were no significant differences among groups for anthropometric measurements (age, BM, height, BF%, TF%,

TLM and TMCSA) at baseline ($P > 0.05$) (Table 1). Table 2 displays the means (± SE) for all anthropometric data. There was no two-way interaction for time × group for BM, BF%, TF%, TLM or TMCSA ($P > 0.05$). However, there was a significant main effect for time for body mass ($P < 0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed across groups) indicated a mean decrease of 0.6 (± 2.3) kg from pre- to post-training assessment one for all three groups ($P < 0.05$). There were no other significant anthropometric changes for BM, BF%, TF%, TLM or TMCSA ($P > 0.05$).

Isokinetic Assessments

Table 3 contains the means (± SE) for ISOK PT assessments for the dominant and non-dominant legs. There was no four-way interaction for time × group × velocity × limb, no three-way interactions for time × limb × velocity, time × group × velocity, time × group × limb, or group × velocity × limb, and no two-way interactions for time × limb, group × time, or group × limb ($P > 0.05$). However, there were significant two-way interactions for time × velocity, limb × velocity and group × velocity ($P < 0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed across time and group, group and limb, and time and limb) indicated that PT for the dominant leg was significantly higher than the non-dominant leg and PT was significantly higher at 90°/s than 180°/s (Table 3) ($P < 0.05$).

Dynamic Constant External Resistance Assessments

Table 4 contains the means (± SE) for 1-RM strength in the dominant and non-dominant legs. There was no three-way interaction for time × group × limb ($P > 0.05$), no two-way interaction for group × limb ($P > 0.05$), but there were significant two-way interactions for time × group and time × limb ($P < 0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed across limb) indicated that 1-RM increased from pre- to post-training assessment 1 in both limbs for the DCER group only, and remained elevated during post-training assessments 2 and 3 ($P < 0.05$) (Fig. 4). There were no differences in 1-RM strength for the DCER group among post-training assessments 1, 2 and 3 ($P > 0.05$). In addition, post-hoc pairwise comparisons for the marginal means (collapsed across group) indicated that 1-RM was higher for the dominant leg than non-dominant leg ($P < 0.05$). No other changes were found for the ISOK training or CONT groups ($P > 0.05$).

Table 2 Means (± SE) for anthropometric and body composition data

Parameters	Pre-training assessment 1	Post-training assessment 1	Post-training assessment 2	Post-training assessment 3
BM (kg)				
DCER	77.9 ± 3.7	77.5 ± 3.7[a]	78.0 ± 3.6	77.7 ± 3.6
ISOK	79.4 ± 3.6	78.8 ± 3.4[a]	78.9 ± 3.5	78.8 ± 3.7
CONT	77.6 ± 5.1	76.8 ± 5.1[a]	77.0 ± 5.2	76.8 ± 5.3
BF%				
DCER	24.4 ± 2.5	24.0 ± 2.5	24.1 ± 2.1	24.3 ± 2.4
ISOK	20.6 ± 2.0	20.5 ± 2.0	20.2 ± 2.0	20.4 ± 1.9
CONT	24.8 ± 2.9	24.7 ± 2.9	24.5 ± 2.8	24.3 ± 2.8
TF%				
DCER	30.3 ± 2.7	30.3 ± 2.8	30.5 ± 2.6	30.2 ± 2.7
ISOK	25.4 ± 1.7	25.0 ± 1.9	24.8 ± 1.8	25.1 ± 1.9
CONT	28.1 ± 2.9	28.3 ± 2.9	28.7 ± 2.9	28.0 ± 2.9
TLM				
DCER	6570.3 ± 298.5	6582.1 ± 300.0	6595.5 ± 285.9	6494.3 ± 310.9
ISOK	7064.5 ± 284.6	7072.8 ± 305.6	7046.3 ± 285.1	7046.3 ± 360.6
CONT	6329.6 ± 278.0	6315.9 ± 260.3	6257.0 ± 263.7	6310.8 ± 254.3
TMCSA				
DCER	152.1 ± 9.5	150.9 ± 12.4	148.7 ± 12.1	151.0 ± 11.3
ISOK	171.0 ± 9.6	173.4 ± 10.2	170.1 ± 9.9	175.4 ± 11.3
CONT	151.4 ± 9.9	151.8 ± 8.4	153.0 ± 9.3	153.8 ± 7.8

BM body mass, *BF%* body fat percentage, *TF%* thigh fat percentage, *TLM* thigh lean mass, *TMCSA* thigh muscle cross-sectional area, *ISOK* isokinetic, *CONT* control

[a]Denotes significant change from pre- to 1

Table 3 Means (±SE) for isokinetic strength testing

		Pre-training assessment 1	Post-training assessment 1	Post-training assessment 2	Post-training assessment 3
PT (N m)					
DCER					
Dominant	90°/s	184.0 ± 11.4	188.3 ± 13.1	185.3 ± 11.0	186.4 ± 10.2
	180°/s	131.5 ± 10.1	139.2 ± 11.6	133.9 ± 11.8	142.1 ± 8.4
Non-dominant	90°/s	173.0 ± 11.7	168.1 ± 13.7	171.9 ± 13.5	168.3 ± 12.3
	180°/s	131.8 ± 10.0	133.7 ± 12.1	137.2 ± 11.9	133.3 ± 13.4
ISOK					
Dominant	90°/s	196.1 ± 13.3	207.0 ± 13.7	197.9 ± 14.5	200.8 ± 15.2
	180°/s	136.9 ± 8.1	154.0 ± 9.3	145.5 ± 9.7	184.7 ± 11.6
Non-dominant	90°/s	187.0 ± 10.6	185.1 ± 12.5	183.1 ± 11.3	145.6 ± 10.5
	180°/s	141.5 ± 8.1	144.2 ± 7.6	139.5 ± 7.5	144.8 ± 7.2
CONT					
Dominant	90°/s	159.6 ± 9.2	159.1 ± 10.5	152.9 ± 11.6	155.2 ± 9.5
	180°/s	127.9 ± 7.9	128.7 ± 11.9	114.6 ± 8.5	121.8 ± 7.9
Non-dominant	90°/s	157.8 ± 9.6	146.5 ± 8.9	139.2 ± 7.4	134.7 ± 4.9
	180°/s	124.3 ± 7.2	125.2 ± 9.4	117.8 ± 9.5	116.6 ± 6.8

PT peak torque, *DCER* dynamic constant external resistance, *ISOK* isokinetic, *CONT* control

Table 4 Means (±SE) for dynamic constant external resistance 1-RM

	Pre-training assessment 1	Post-training assessment 1	Post-training assessment 2	Post-training assessment 3
1RM (kg)				
DCER				
Dominant	43.2 ± 2.7	52.4 ± 3.4[a]	50.3 ± 3.1[a]	50.8 ± 3.0[a]
Non-dominant	42.3 ± 2.5	48.9 ± 3.8[a]	48.9 ± 3.5[a]	48.9 ± 3.2[a]
ISOK				
Dominant	46.4 ± 4.2	49.5 ± 4.3	49.7 ± 4.3	48.0 ± 4.7
Non-dominant	45.5 ± 4.0	47.7 ± 4.0	48.4 ± 4.1	46.5 ± 4.0
CONT				
Dominant	40.5 ± 2.3	40.8 ± 2.1	40.7 ± 2.3	41.7 ± 1.7
Non-dominant	40.7 ± 2.3	40.3 ± 2.2	40.2 ± 2.3	41.1 ± 1.8

1RM 1 repetition maximum, *DCER* dynamic constant external resistance, *ISOK* isokinetic, *CONT* control

[a]Denotes significant change from pre- to post-assessment 1

Isometric Assessments

There was no interaction for time × group and no main effect for times or group ($P > 0.05$). Table 5 displays the means (± SE) for isometricathle MVC for all groups.

Surface Electromyography

There was no four-way interaction for time × group × muscle × velocity, no three-way interactions for time × muscle × velocity, time × group × velocity, time × group × muscle, time × muscle × velocity, or group × muscle × velocity, and no two-way interactions for time × muscle, time × group, time × velocity, group × muscle, velocity × muscle, or velocity × group for the EMG amplitude of the RF and VL ($P > 0.05$). However, there was a significant main effect for velocity ($P < 0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed across time, group and muscle) indicated EMG amplitude was significantly higher at 180°/s than 90°/s ($P < 0.05$). Figure 5 displays the means (± SE) for EMG amplitude collapsed across groups, quadriceps muscles, and velocity.

For the BF, there was no three-way interaction for time × group × velocity, and no two-way interactions for time × velocity, time × group, or group × velocity ($P > 0.05$). However, there were significant main effects for time and main effect for velocity ($P < 0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed

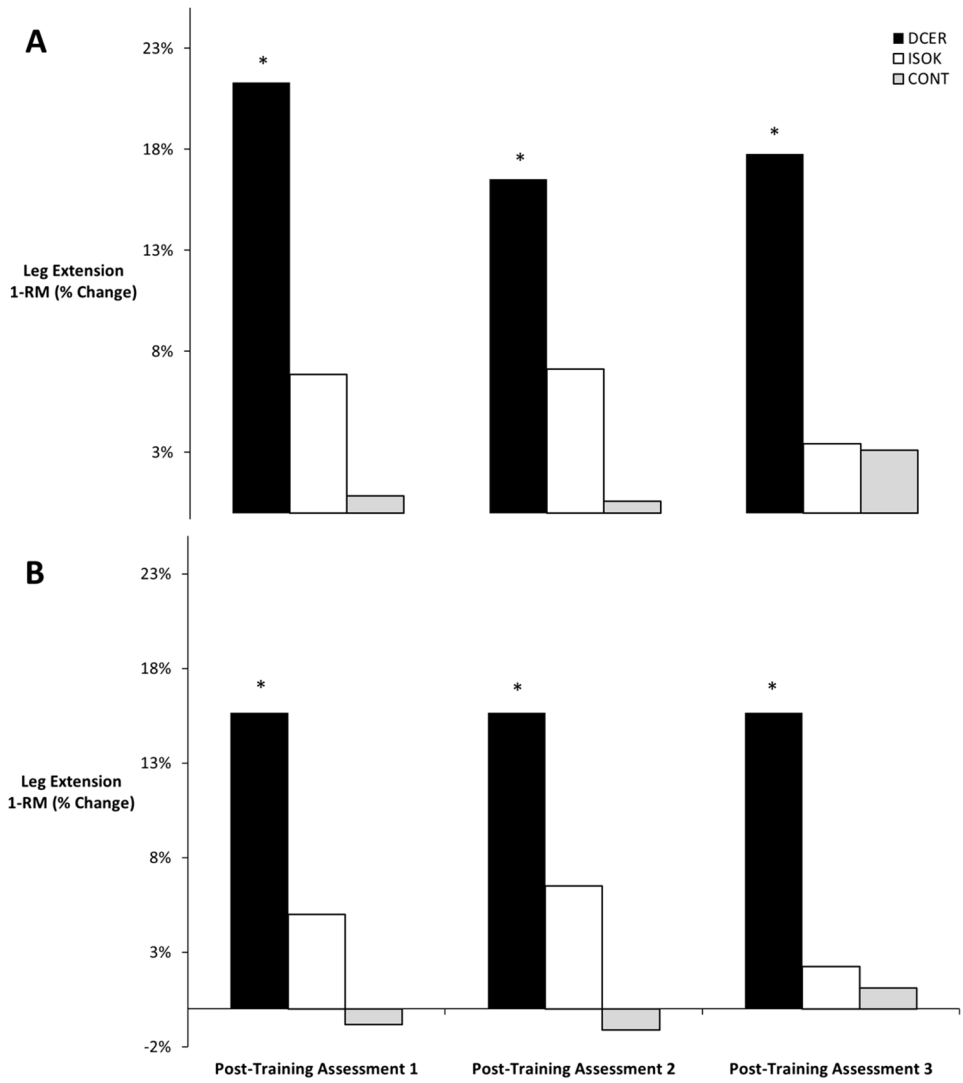

Fig. 4 Means of percent change for leg extension (kg) 1-RM for the dominant (**A**) and non-dominant (**B**) leg. *Denotes significant difference from the pre-test for the DCER group. *DCER* dynamic constant external resistance, *ISOK* isokinetic, *CONT* control

across group and velocity) indicated that EMG amplitude for the BF at post-training assessment 3 was significantly greater than the pre-training assessment, post-training assessment 1, and post-training assessment 2 ($P < 0.05$). In addition, post-hoc pairwise comparisons for the marginal means (collapsed across group and time) indicated that EMG amplitude for the BF was significantly greater at 180°/s than 90°/s ($P < 0.05$). Figure 6 displays the means (± SE) for EMG amplitude collapsed across groups and velocities for the BF muscles.

Table 5 Means (± SE) for isometric maximal voluntary contraction

	Pre-training assessment 1	Post-training assessment 1	Post-training assessment 2	Post-training assessment 3
MVC (N)				
DCER	643.2 ± 44.2	668.2 ± 44.1	654.5 ± 42.8	667.8 ± 51.2
ISOK	664.2 ± 52.5	657.7 ± 55.7	698.0 ± 47.9	734.3 ± 56.9
CONT	463.3 ± 32.9	479.4 ± 35.6	493.7 ± 28.6	502.8 ± 27.9

1RM 1 repetition maximum, *DCER* dynamic constant external resistance, *ISOK* isokinetic, *CONT* control, *MVC* maximal voluntary contraction

Twitch Interpolation

There was no significant three-way interaction for time × group × intensity, and no two-way interactions for time × intensity, group × intensity, or time × group ($P > 0.05$). However, there was a significant main effect for intensity ($P < 0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed across group and time) indicated voluntary activation significantly increased from 30% to 100% of MVC intensity ($P < 0.05$). Figure 7 displays the means (± SE) for voluntary activation collapsed across groups and time.

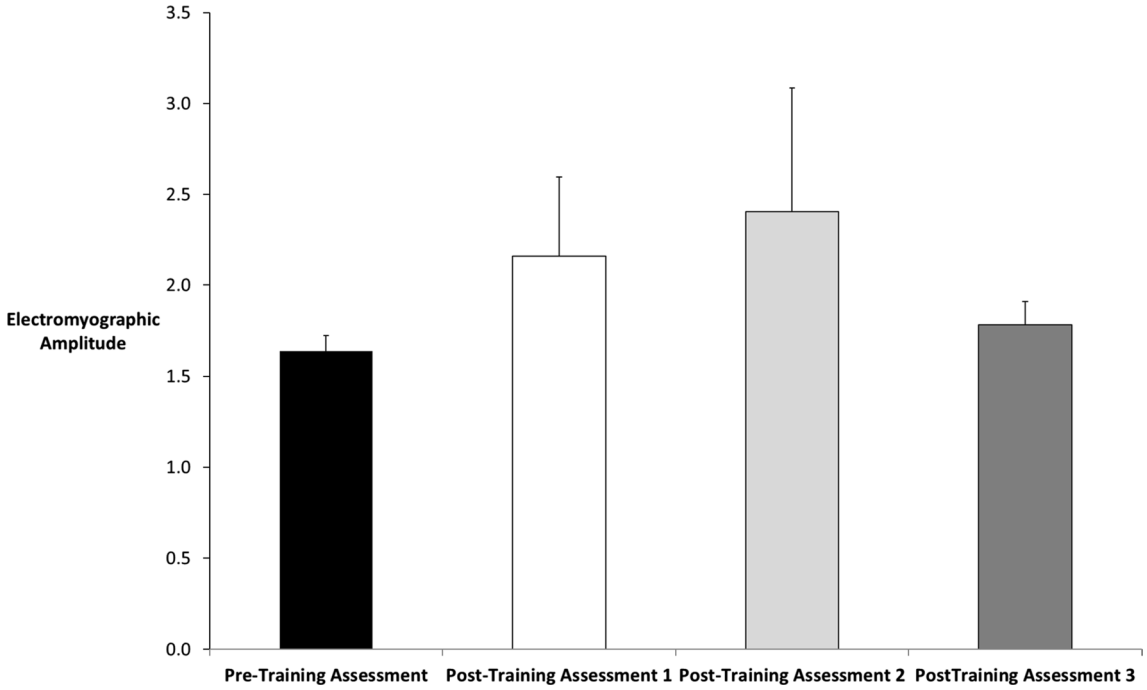

Fig. 5 Means (±SE) for electromyographic amplitude collapsed across group, muscle, and velocity

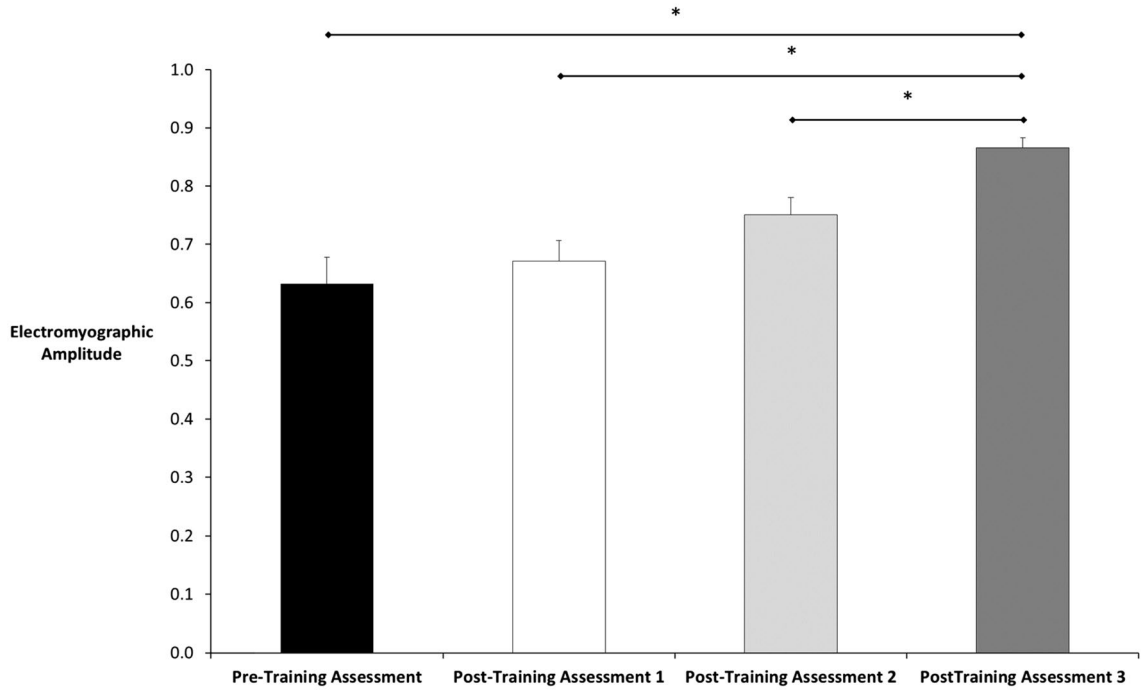

Fig. 6 Means (±SE) for electromyographic amplitude collapsed groups and velocities for the BF muscles. *Denotes significant difference between tests

Mechanomyography

There was no four-way interaction for time × group × muscle × velocity, no three-way interactions for time × muscle × velocity, time × group × muscle, time × group × velocity, or time × muscle × velocity, and no two-way interactions for time × muscle, time × group, time × velocity, group × muscle, muscle × velocity, or group × velocity ($P > 0.05$). However, there was a significant

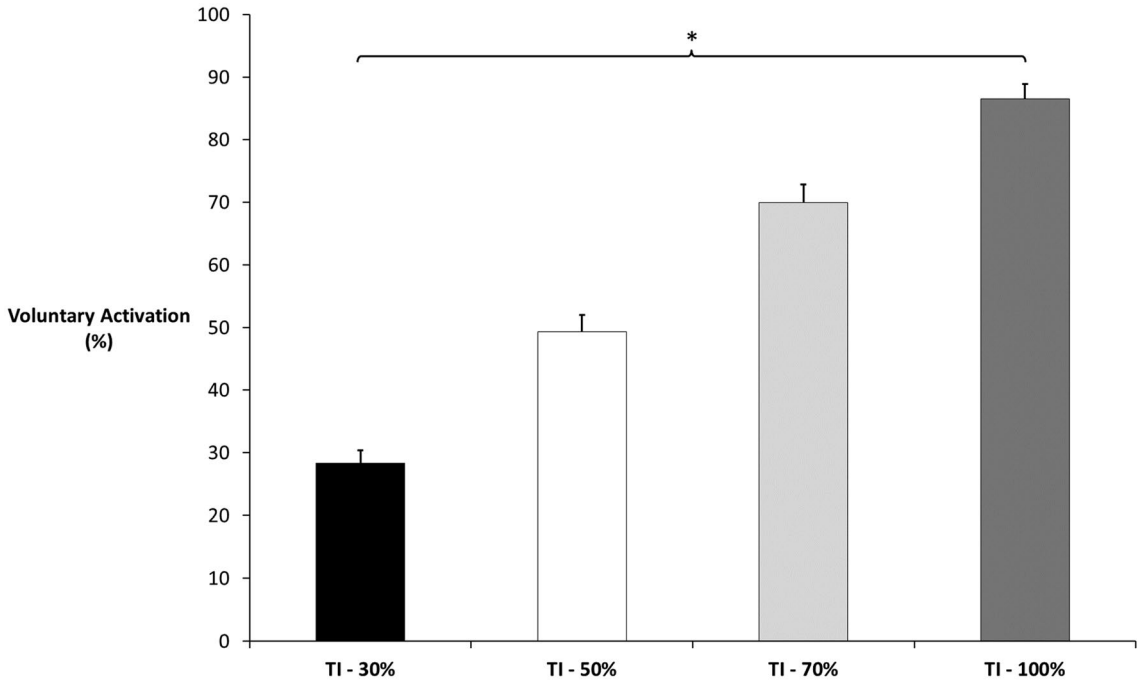

Fig. 7 Means (±SE) for voluntary activation collapsed across group and time. *Denotes significant difference between percentages of maximum voluntary contraction. *TI* twitch interpolation

three-way interaction for muscle × velocity × group ($P<0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed across time) indicated a significant main effect for velocity ($P<0.05$). MMG amplitude was significantly higher at 180°/s than 90°/s for all groups ($P<0.05$). Figure 8 displays the means (±SE) for MMG amplitude collapsed across groups, quadriceps muscles, and velocity.

Rating of Perceived Exertion

There was no three-way interaction for time × group × set, and no two-way interactions for time × set or time × group for set RPE ($P>0.05$). However, there was a significant set × group interaction for training ($P<0.05$). Post-hoc pairwise comparisons for the marginal means (collapsed across time) indicated a significant main effect for set RPE ($P<0.05$) (Fig. 9). RPE increased significantly ($P<0.05$) from the first until the last set within all sessions for both training groups. For session RPE, there was no two-way interaction for time × group and no main effect for time or main effect for group ($P>0.05$) (Fig. 10).

Discussion

Perhaps the most important finding of the present study was the increases in DCER strength acquired in the DCER group. DCER strength increased similarly in both limbs for the DCER groups. In addition, the DCER group retained the strength gains during post-training assessments 2 and 3 over a two-week period. Typically, increases in strength obtained in longer resistance training programs are diminished after several weeks of training [13, 28, 29, 35, 50, 66]. Colliander and Tesch [13] compared the effects of resistance training and detraining using concentric-only and combined concentric and eccentric muscle actions of the leg extensors and reported that the group performing coupled concentric and eccentric muscle actions had a greater overall increase in PT after training and detraining than the concentric-only group [13]. These authors suggested a resistance training program incorporating combined concentric and eccentric repetitions of leg extension can retain more of the obtained strength gains than the training program with concentric-only repetitions [13]. Likewise, Farthing found eccentric muscle action training elicited greater strength gains than concentric training [24]. Similarly, Knight et al. [41] suggested isotonic muscle actions may be more effective at increasing torque because isokinetic resistance is accommodating, hence, it decreases with fatigue [41]. These findings [13, 24, 41], along with the findings of the current study may indicate an advantage of DCER over ISOK resistance training programs when conducted over a relatively short period of time.

No training-related PT changes were observed for any of the groups. The unique aspect of the present study was that both DCER and ISOK modes of training were used and neither resulted in increased ISOK strength. Overall, these results are consistent with studies that have reported

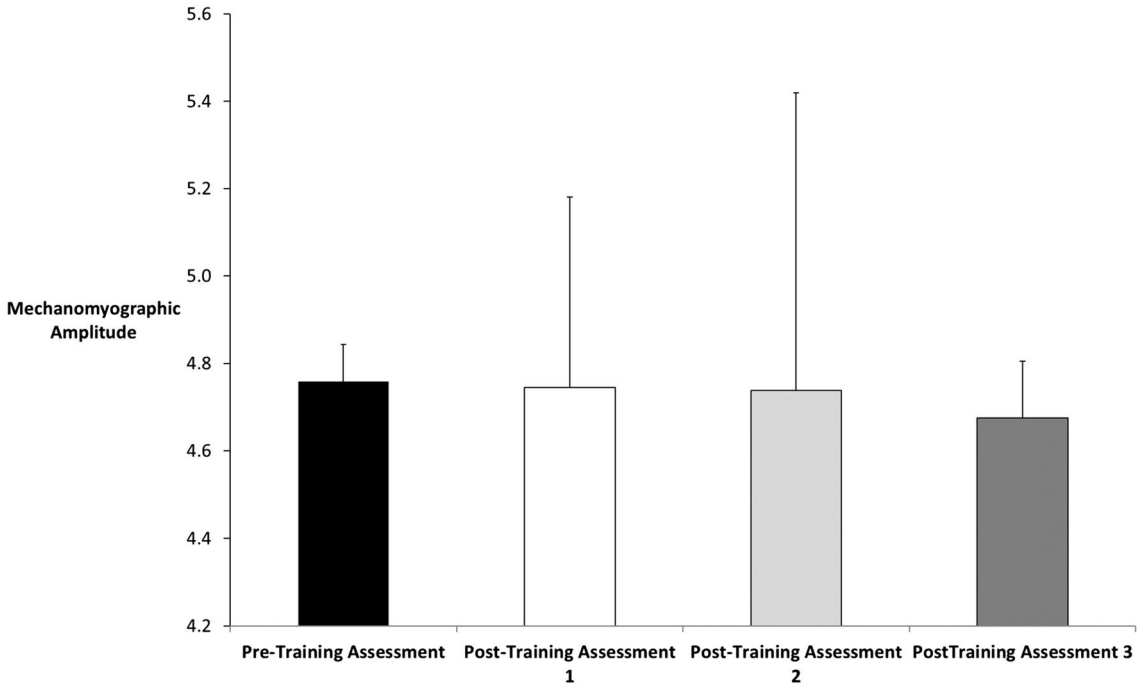

Fig. 8 Means (±SE) for MMG amplitude collapsed across groups, quadriceps muscles, and velocity

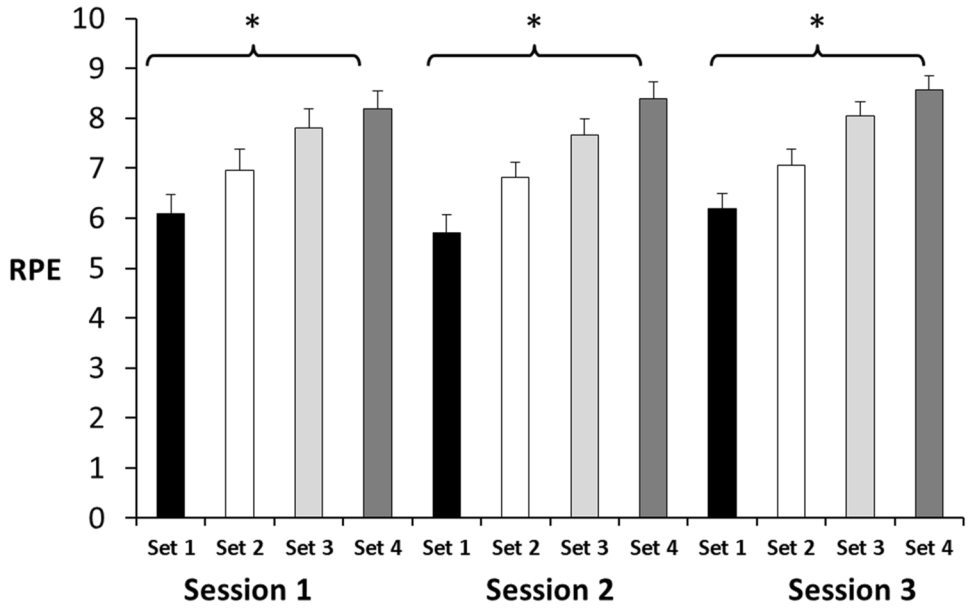

Fig. 9 Mean (±SE) for set rating of perceived exertion collapsed across both training groups.*Denotes significant change over sets within each training session

no increases in PT after short-term ISOK training [3, 5, 55]. Brown and Whitehurst [5] reported no changes in PT after two days of leg extensors ISOK training [5]. Likewise, Beck et al. [3] reported no changes in PT after two days of forearm flexors and extensors ISOK training [3]. The results of the current study are partially in agreement with the findings of Prevost et al. [55], who reported no increases in PT after two days of ISOK training at the velocities of 30 and 150°/s [55]. However, these findings are in contrast with previous studies reporting ISOK PT increases after short-term ISOK training [11, 15].

The non-dominant leg increased strength, despite training only the dominant leg. Unilateral resistance training of a limb can increase the strength of the contralateral limb in a concept termed cross-education [25, 39, 43, 49]. Increases in the contralateral, untrained limb, have been extensively

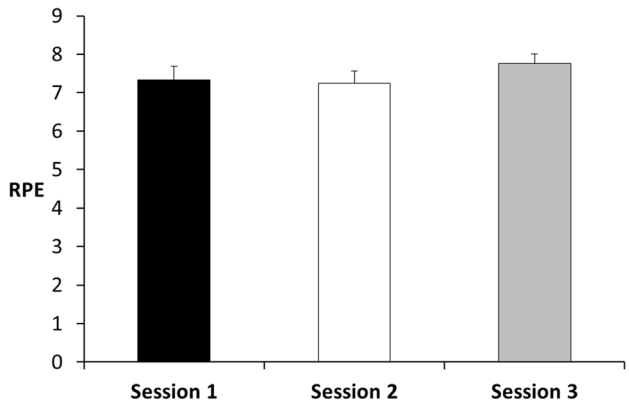

Fig. 10 Mean (±SE) for session rating of perceived exertion collapsed across both training groups

reported in the literature [9, 14, 39, 43, 49, 53]. Perhaps an important finding of the current study is that short-term resistance training also elicits this cross-education effect. This has important implications for injury rehabilitation [25, 54, 70], in particular muscle strains and knee-related sprains, where in the initial period post-injury strength gains on an injured limb can possibly be obtained with short-term contralateral resistance training. Contralateral strength gains have been hypothesized to be attributed to central neural adaptations (i.e., excitation of the cortex), increased motoneuron output, and improved postural stabilization [9, 39, 49, 53, 56]. In addition, eccentric muscle actions as seen with the DCER training group have been shown to cause reduced intracortical inhibition and increased corticospinal excitability compared to concentric muscle actions from the ISOK training group, suggesting eccentric muscle actions provide an unique stimulus for contralateral training [39].

Strength gains were also maintained during the two-week detraining period in the DCER group. Although subjects were untrained in the present study, these findings were similar to those of Hortobagyi et al. [34], who reported two weeks of detraining in resistance-trained athletes did not cause a significant decrease in maximal bench press, squat, isometric, or concentric isokinetic strength [34]. In addition, Ogasawara et al. [51] demonstrated strength was maintained following a three-week detraining period in previously untrained men [51]. Similarly, Shaver [61] found that recently acquired strength can be maintained in both trained and untrained limb for up to one week [61]. To our knowledge, the current study is the first to demonstrate short-term DCER is superior to ISOK training in increasing and maintaining strength for a two-week period in trained and untrained limbs. In contrast, other authors have suggested strength gains that have been recently acquired may diminish faster than in more experienced strength-trained athletes [48, 61]. Therefore, the history experience with resistance training (novice versus well-trained athletes) should be considered when interpreting the results of a short-term resistance training program and its potential lasting effects.

Isometric MVC force did not change for any of the groups in the present study. These results are similar to those found by Beck et al. [3] who reported no changes in isometric PT after two days of forearm ISOK training [3]. These results are in contrast with those of Christie and Kamen [10], who compared the effects of short-term training in young and older subjects [10]. These authors reported increases in MVC force after six sessions of isometric dorsiflexor exercises [10]. The discrepancies between their findings and the results of the current study are likely due to exercise specificity and/or the number of training sessions, as participants trained and tested in the same mode of muscle action (isometric) compared to the current study in which participants engaged in either DCER or ISOK muscle actions. The specificity principle was also demonstrated in the present study by the increase in DCER strength in the DCER training group only. Rutherford et al. also demonstrated the specificity principle [56]. The authors reported large improvements in training load for dynamic leg extensions that were not reflected by a comparable magnitude of increases in isometric strength [56]. In addition, Christie and Kamen [10] trained their subjects for six training sessions while in the present study, participants only took part in three training sessions [10].

No changes in RF or VL EMG amplitude occurred in the present study. These results are in agreement with previous short-term resistance training studies reporting no change in muscle activation [3, 11, 15, 33]. For example, Beck et al. [3] reported no changes in EMG amplitude after two days of forearm flexors and extensors ISOK training [3]. Similarly, Coburn et al. [11] found increases in PT, but no changes in EMG amplitude after three days of short-term isokinetic training [11]. The authors suggested gains in PT after training were not caused by increases in muscle activation [11]. Further, Holtermann et al. [33] reported increases in isometric MVC strength but no increases in EMG after five days of isometric training [33]. Likewise, Cramer et al. [15] found no changes in EMG amplitude, but reported increases in PT [15]. Coburn et al. [11] suggested increases in PT without accompanying increases in EMG can be possible because of a decrease in the antagonist's muscle coactivation induced by the training program and/or an increased ability in coordinating stabilizing/synergistic muscles [11]. The results of the current study along with the findings of previous studies [11, 15] suggest a greater duration of training may be necessary to induce detectable increases in muscle activation. Thus, an increase in PT without a concomitant increase in EMG amplitude could perhaps be due to other neural changes, such as synchronization of motor units and/or optimization of motor unit firing rates [33], or a decrease in reciprocal coactivation of the antagonist muscles [15].

Beck et al. [3] reported no changes in EMG amplitude of the antagonist muscles after two days of forearm flexors and extensors training ISOK [3]. However, EMG amplitude for the BF muscles increased at the post-training assessment 3 in the current study. Other studies have also found an increase in antagonist coactivation as a result of training [18, 63, 67]. A decreased coactivation could theoretically increase force production, but evidence for mechanisms of increased agonist activation and decrease antagonist activation is equivocal [67]. An increase in reciprocal coactivation of the BF after training could be interpreted as a protective mechanism in an effort to maintain, rather than compromise, joint stability [18, 67]. Future studies should further investigate the effects of antagonist muscle coactivation from short-term resistance training.

There were no changes in %VA activation in the present study as determined by the TI technique. These results are similar to those demonstrated by Tillin et al. [67], who reported no changes in %VA after four weeks of isometric training [67]. Likewise, although using a different method to determine muscle activation, Christie and Kamen [10] also found no changes in central activation after six days of isometric training [10]. In addition, the authors reported all subjects were able to fully activate their muscles voluntarily and thus, suggested increases in MVC isometric force were not due to an increased ability to activate their muscles fully [10]. The results of the current study are also in agreement with studies reporting no changes in percent of voluntary activation as assessed by the TI technique following resistance training [4, 7, 8, 16, 27, 30, 31, 37, 59, 67]. In fact, Brown et al. [6] reported slight decreases in %VA activation following eight weeks of resistance training after an initial increase in the first four weeks [6]. Lack of changes in muscle activation are hypothesized to be attributed to complete voluntary muscle activation prior to training, training and testing conducted using different muscle actions (i.e., dynamic and isometric), and some muscles displaying only a slight deficit in voluntary activation leaving only a small window of improvement [62]. The mean %VA in the present study ranged from 85%–87% for all groups over all assessments. Perhaps longer training and/or higher training volume (i.e., more exercises) are necessary to increase %VA.

No changes in mechanomyography were found in the present study. These findings are similar to those demonstrated by Cramer et al. [15]. In that study, the authors reported no changes in MMG amplitude after two days of isokinetic short-term resistance training with and without eight days of creatine supplementation [15]. In addition, the results in the current study indicate MMG amplitude was greater at 180°/s than 90°/s for all groups. These findings are also consistent with those of Cramer et al. [15], who reported MMG amplitude increased from 30°/s to 150°/s to 270°/s [15]. Since the authors reported increases in PT but no increases in EMG or MMG amplitude, it was suggested these increases in strength from training were due to increases in motor unit firing rate rather than motor unit recruitment [15].

There was a minor decrease in BM for all groups from pre-training assessment to post-training assessment 1. No other changes in anthropometric or body composition occurred. These findings were in agreement with short-term resistance training studies, in which no significant hypertrophy or other body composition changes have been reported [1, 5, 11, 15, 55]. For example, Akima et al. [1] reported no increases in muscle CSA or fiber area after two weeks of resistance training. In contrast, DeFreitas et al. [19] reported increases in muscle CSA after six resistance training sessions [19]. However, in addition to taking part in six training sessions compared to three in the present study, subjects trained in three lower body exercises involving agonist and antagonist muscle groups [19], which may explain the difference between the present findings and those of DeFreitas [19].

Some of the present study's limitations were the use of DXA and anthropometric measures to assess lean mass and muscle CSA, respectively. In addition, the present study used apparently healthy but minimally-active participants, which may not replicate the results of injured patients. Furthermore, the findings of the current study with the quadriceps may not generalize to other muscle groups.

Conclusions

The primary finding of this study was that DCER strength can be increased with three days of training. Another important result from the present study is that short-term resistance training induces a cross-education effect increasing the strength in the untrained limb. This has important applications for injury rehabilitation, where in the initial period post-injury strength gains on an injured limb can possibly be obtained with short-term contralateral resistance training. The neuromuscular system undergoes numerous adaptations following a resistance training program [1, 23, 42, 46, 47, 57, 64]. However, the specific mechanisms of such adaptations in short-term training have not been fully understood [11]. Nevertheless, evidence has shown improvements in muscle performance can be observed in a shorter period than what is typically used in longer traditional training programs [1, 15, 46]. Although increases in strength following resistance training have been attributed to neural and hypertrophic factors [23, 42, 46, 57, 58, 64], no increases in TMCSA, TLM, EMG and MMG amplitude, or %VA, were found in the current study. Therefore, strength gains observed after DCER resistance training were due to an unidentified factor. Future studies should investigate the precise physiological components responsible for short-term strength gains. The

findings of the current study may indicate an advantage of DCER over ISOK resistance training programs when conducted over a relatively short period of time. When compared to ISOK training, traditional DCER resistance training appears to be a better and more cost-effective option when rapid increases in strength are desired and a longer period of time is not available. These findings have important implications in clinical rehabilitation settings, sports injury prevention, as well as in other allied health fields such as physical therapy, occupational therapy, and athletic training. Therefore, future studies should examine the effects of short-term resistance training on injury prevention and injury rehabilitation. Furthermore, muscle imbalance has also been associated with injury risk. Thus, future studies should also investigate the effects of short-term training to minimize muscle imbalances.

Authors' contributions PC and JC conceived and designed the research. PC and AH conducted experiments. TH wrote the analyses programs and contributed to data analyses. PC and JC analyzed and interpreted the data. PC wrote the manuscript and all authors contributed to editing and proofreading. All authors read and approved the manuscript.

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Availability of data and material Not applicable.

Code availability Software used are either widely available, or custom-written.

Declarations

Conflicts of interest None of the authors have a conflict of interest to declare.

Ethics approval This study was approved by the university's Institutional Review Board for the protection of human subjects.

Informed consent Prior to any testing, all subjects read and signed an Informed Consent form.

Consent for publication All authors consent to the publication of this work.

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