Prolonged depression of knee extensor torque complexity following eccentric exercise

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Abstract

Neuromuscular fatigue reduces the temporal structure, or complexity, of muscle torque output. Exercise-induced muscle damage reduces muscle torque output for considerably longer than high-intensity fatiguing contractions. We hypothesised that muscle damaging eccentric exercise would lead to a persistent decrease in torque complexity, whereas fatiguing exercise would not. Ten healthy participants performed five isometric contractions (6 s contraction, 4 s rest) at 50% maximal voluntary contraction (MVC) before, immediately after, 10, 30 and 60 minutes, and 24 hours after eccentric (muscle damaging) and isometric (fatiguing) exercise. These contractions were also repeated 48 hours and one week after eccentric exercise. Torque and surface EMG signals were sampled throughout each test. Complexity and fractal scaling were quantified using approximate entropy (ApEn) and the detrended fluctuation analysis α exponent (DFA α). Global, central and peripheral perturbations were quantified using MVCs with femoral nerve stimulation. Complexity decreased following both eccentric (ApEn, mean (SD), from 0.39 (0.10) to 0.20 (0.12), $P < 0.001$) and isometric exercise (from 0.41 (0.13) to 0.09 (0.04); $P < 0.001$). After eccentric exercise ApEn and DFA α required 24 hours to recover to baseline levels, but only 10 minutes following isometric exercise. MVC torque remained reduced (from 233.6 (74.2) to 187.5 (64.7) N.m) 48 hours after eccentric exercise, with such changes only evident up to 60 minutes following isometric exercise (MVC torque, from 246.1 (77.2) to 217.9 (71.8) N.m). The prolonged depression in maximal muscle torque output is therefore accompanied by a prolonged reduction in torque complexity.

Abbreviations: ApEn, approximate entropy; DFA detrended fluctuation analysis; MVC maximal voluntary contraction.

New findings

What is the central question?

Does eccentric exercise leading to prolonged knee extensor torque depression also result in a prolonged loss of knee extensor torque complexity?

What is the main finding of importance?

The recovery of the loss of torque complexity following eccentric exercise took 24 hours, whereas after acute muscle fatigue it took 10 minutes, thus the depression of torque complexity following eccentric exercise was prolonged.

Keywords: eccentric; fatigue; non-linear dynamics; complexity; fractal scaling.

Introduction

Human movement is characterized by inherent variability and fluctuations (Hamilton *et al*., 2004; Stergiou and Decker, 2011). Such fluctuations have typically been quantified according to their *magnitude*, using measures such as the standard deviation (SD) and coefficient of variation (CV; Jones *et al*., 2002; Taylor *et al*., 2003). However, these fluctuations also possess an irregular temporal *structure*, or complexity (Lipsitz and Goldberger, 1992), which refers to the relationship between successive data points and the predictability of a time-series (Pincus, 1991; Slifkin and Newell, 2000). Complex outputs are thought to be a hallmark of healthy physiological systems (Peng *et al*., 2009), and can be observed in, *inter alia*, normal heart rate, gait and muscle torque output (Hausdorff *et al*., 1995; Goldberger, 1996; Slifkin and, Newell, 1999). A loss of physiological complexity appears to be a ubiquitous response to ageing and/or pathology (Lipsitz and Goldberger, 1992).

In the context of muscle torque output, it is thought that complexity reflects the adaptability of the neuromuscular system (i.e., the ability to modulate motor output rapidly and accurately in response to perturbations; Vaillancourt and Newell, 2003). Any loss of muscle torque complexity therefore has the potential to negatively affect co-ordination, impact motor task performance and exercise tolerance (Cortes *et al*., 2014; Pethick *et al*., 2016). We have recently demonstrated that neuromuscular fatigue reduces the complexity of muscle torque output during both maximal and submaximal isometric contractions (Pethick *et al*., 2015). We subsequently demonstrated that this fatigue-induced loss of complexity is only evident during contractions performed above the critical torque (Pethick *et al*., 2016) and that such losses can be slowed by the ingestion of caffeine (Pethick *et al*., 2018a). These studies have demonstrated that the loss of torque complexity is tightly coupled to the fatigue process, with complexity declining in tandem with the loss of force-generating capacity. However, whether this effect is specific to the development of fatigue during high-intensity contractions is not known. If the fatigue-induced loss of torque complexity is related to the loss of force-generating capacity in the neuromuscular system *per se*, then interventions that reduce this capacity independently of metabolite-mediated fatigue should also diminish torque output complexity.

Unaccustomed eccentric exercise, which involves the active lengthening of muscle fibres (Enoka, 1996), has been repeatedly demonstrated to lead to muscle damage, attributed to mechanical disruption of the sarcomeres, in the days after exercise (Asmussen, 1956; Fridén *et*

al., 1981; Clarkson *et al*., 1992; Proske and Morgan, 2001). In contrast, no such damage is typically observed following either isometric or concentric exercise (Newham *et al*., 1983; Lavender and Nosaka, 2006). A consequence of this eccentric exercise-induced muscle damage is a decrease in maximal force generating capacity, which lasts considerably longer than after the performance of either isometric or concentric contractions (Jones *et al*., 1989; Gibala *et al*., 1995; Smith and Newham, 2007). While maximal force typically recovers to >90% of its fresh value within 60 minutes of isometric contractions (Sahlin and Ren, 1989; Allman and Rice, 2001), significant decrements in maximal force following eccentric exercise have been shown to persist for several days and, in some cases, for up to two weeks (Cleak and Eston, 1992; Sayers and Clarkson, 2001). If the loss of torque output complexity during fatigue is directly linked to the decrement in force generating capacity, then the persistent loss of maximal force following eccentric exercise should be accompanied by a persistent loss of torque complexity.

In support of the contention that eccentric exercise may lead to a prolonged decrease in the complexity of muscle torque output, it has been observed that eccentric actions result in an increase in the magnitude of torque fluctuations, as measured by the CV, during subsequent low, moderate and high intensity isometric contractions (Weerakkody *et al*., 2003; Lavender and Nosaka, 2006; Semmler *et al*., 2007; Skurvydas *et al*., 2010). This effect has typically been observed an hour after the cessation of exercise, though in some cases has persisted for 24 hours (Leger and Milner, 2001; Dartnall *et al*., 2008), and has not been observed following isometric or concentric contractions (Lavender and Nosaka, 2007; Semmler *et al*., 2007). Thus, whilst is it known that the magnitude of torque variability can be altered following eccentric exercise, the effect on the structure of these fluctuations over several days has not yet been investigated.

The purpose of the present study was to investigate the effect of muscle damaging eccentric exercise on the complexity of knee extensor torque output. To that end, we aimed to compare the recovery kinetics of torque complexity following eccentric and isometric exercise. The experimental hypothesis tested was that muscle damaging eccentric exercise would result in a persistent loss of torque complexity, quantified by decreased approximate entropy (ApEn) and increased detrended fluctuation analysis α exponent (DFA α), whereas fatiguing isometric exercise would not.

Materials and methods

Participants

Ten healthy participants (8 male, 2 female; mean (SD): age 24.8 (6.2) years; height 1.75 (0.08) m; body mass 69.5 (10.6) kg) provided written informed consent to participate in the study, which was approved by the ethics committee of the University of Kent (Reference Number: Prop_02_2015_2016), and adhered to the Declaration of Helsinki, except for registration in a database. None of the participants had been involved in any lower limb strength training for ≥ 3 months. Participants were instructed to arrive at the laboratory in a rested state (having performed no strenuous exercise in the preceding 24 hours, and not to have consumed any food or caffeinated beverages in the three hours before arrival. Participants attended the laboratory at the same time of day (± 2 hours) during each visit.

Experimental design

Participants were required to visit the laboratory on seven occasions over a four to six-week period. During their first visit, participants were familiarised with all testing equipment and procedures, and the settings for the dynamometer and femoral nerve stimulation were recorded. The second visit involved performance of fatiguing intermittent isometric knee extension contractions ("*Isometric exercise*"; see below); with the third visit, 24 hours later, assessing recovery. The contractions in these visits were performed with the dominant leg (the leg participants would instinctively use to kick a football). At least one week after the third visit, the fourth visit involved performance of intermittent eccentric knee extension contractions ("*Eccentric exercise*"; see below); with the fifth, sixth and seventh visits, 24 hours, 48 hours and one week later, assessing recovery. The contractions in these visits were performed with the non-dominant leg, in order to maximise the damaging effect of the eccentric exercise. In each visit, torque output was sampled continuously to allow the quantification of complexity, muscle activity was measured using the *m. vastus lateralis* electromyogram (EMG), and MVCs with supramaximal femoral nerve stimulation were used to quantify global, central and peripheral fatigue, as detailed below.

Dynamometry

Participants sat in the chair of a Cybex isokinetic dynamometer (HUMAC Norm; CSMi, Stoughton, MA, USA), initialised and calibrated according to the manufacturer's instructions. The leg to be used was attached to the lever arm of the dynamometer, with the seating position adjusted to ensure that the lateral epicondyle of the femur was in line with the axis of rotation of the lever arm. The lower leg was securely attached to the lever arm above the malleoli with a padded Velcro strap, while straps secured firmly across both shoulders and the waist prevented any extraneous movement and the use of the hip extensors during the contractions. The seating position was recorded during familiarisation and replicated during each subsequent visit.

Electromyography and femoral nerve stimulation

During all visits, on arrival at the laboratory participants had the leg to be used in that visit shaved and cleaned using an alcohol swab over the belly of the *vastus lateralis* and on the medial aspect of the tibia, at the level of the tibial tuberosity. Two Ag/AgCl electrodes (Nessler Medizintechnik, Innsbruck, Austria) were placed on the belly of the *vastus lateralis* in line with the muscle fibers, and a single electrode was placed on the medial aspect of the tibia at the level of the tibial tuberosity for EMG acquisition.

For femoral nerve stimulation, the anode (100 mm x 50 mm; Phoenix Healthcare Products Ltd., Nottingham, UK) was placed on the lower portion of the *gluteus maximus*, lateral to the ischial tuberosity. The location of the cathode was determined using a motor point pen (Compex; DJO Global, Guildford, UK), and another Ag/AgCl electrode was placed on that point. The establishment of the appropriate stimulator current (200 μs pulse width) was then determined as described in Pethick *et al*. (2015). Current was incrementally increased until knee extensor torque and the compound motor unit action potential (M-wave) response to single twitches had plateaued and was verified with stimulation delivered during an isometric contraction at 50% MVC to ensure a maximal M-wave was also evident during an isometric contraction. The stimulator current was then increased to 130% of the current producing a maximal M-wave. In all trials, doublet stimulation (two 200 μs pulses with 10 ms interpulse interval) was used, with stimuli delivered 1.5 seconds into MVCs to coincide with maximal torque and assess the maximality of the contraction, and 2 seconds after the contraction to provide a potentiated doublet.

Protocol

Each participant performed two tasks involving isometric contraction of the knee extensors: 1) MVCs, to assess torque generating capacity; and 2) a constant force task at 50% MVC, to assess muscle torque complexity. These measures were taken before, immediately after, 10, 30 and 60 minutes after, and 24 hours after the eccentric and isometric exercise. Additional measures were taken 48 hours and one week after eccentric exercise. Estimates of muscle damage and soreness were also taken prior to and after the eccentric and isometric exercise.

MVC task. Following the instrumentation of the participants, the (re)-establishment of the correct dynamometer seating position and the establishment of the supramaximal stimulation response, participants performed a series of brief (3 second) MVCs to establish the maximum torque of the leg to be used in that visit. These MVCs were separated by a minimum of 60 seconds rest, and continued until three consecutive peak torques were within 5% of each other. Participants were given a countdown, followed by very strong verbal encouragement to maximise torque. The first MVC was used to establish the fresh maximal EMG signal, against which the subsequent EMG signals were normalised ("*Data analysis*"; see below). The second and third MVCs were performed with femoral nerve stimulation.

Constant force task. Following the establishment of maximal torque, participants rested for 10 minutes and then performed a series of five isometric contractions at a target torque of 50% MVC, based on the fresh pre-test MVC recorded in visit 2 or 4. These contractions were 6 seconds long and separated by 4 seconds rest.

Estimates of muscle damage. Participants were asked to rate their muscle soreness and capillary whole-blood was sampled from a fingertip. Muscle soreness was measured using a visual analog scale consisting of a horizontal line 10 cm long, with 0 and 10 marked at each end. On this scale, zero corresponded to no muscle soreness and 10 corresponded to the most intense soreness imaginable. Participants performed a squat down to ~90° of knee flexion and were asked to draw a line marking their subjective soreness, with the distance to the mark (in centimetres) being used to quantify soreness. A fingertip blood sample was then taken, and centrifuged for 10 minutes to obtain plasma. Plasma samples were then stored at –80°C for later analysis of creatine kinase (CK). Plasma CK was determined using a commercially available kit (CKNAC, Randox Laboratories Ltd., Crumlin, County Antrim, UK) and standard spectrophotometric-colorimetric procedures with a Randox Monza (Randox Laboratories Ltd., Crumlin, County Antrim, UK). These measures were obtained prior to exercise, immediately

at task failure, 60 minutes after and 24 hours after both the eccentric and isometric conditions. They were additionally obtained 48 hours and one week after eccentric exercise.

Isometric and eccentric exercise

Isometric contractions (ISO). Participants performed intermittent isometric knee extension contractions at a target torque of 50% MVC until task failure in their second visit to the lab. The target torque of 50% MVC was based on the highest instantaneous torque recorded during the pre-test MVCs. The duty cycle for the contractions was 0.6; with contractions lasting 6 seconds and being followed by 4 seconds rest. The contractions were performed until task failure, the point at which the participant failed to reach the target torque on three consecutive occasions, despite strong verbal encouragement. Participants were not informed of the elapsed time during the trials, but were informed of each "missed" contraction. After the third missed contraction, participants were instructed to immediately produce an MVC, which was accompanied by femoral nerve stimulation.

Eccentric exercise (ECC). Eccentric knee extension actions with the non-dominant leg were used to induce a minimum 40% reduction of isometric MVC torque (Prasartwuth *et al*., 2006; Dartnall *et al*., 2008) in visit four. This protocol was used to induce a similar amount of muscle damage in all participants, compared to the large variation in strength loss that can be seen following a fixed number of eccentric contractions (Hubal *et al*., 2007). Participants were seated with their non-dominant leg strapped to the dynamometer, and raised their leg to an angle of 20° extension (with full extension being 0°). The dynamometer then flexed the participant's knee to an angle of 90° extension, at a constant angular velocity of $60°\cdot s^{-1}$, whilst the participant resisted this motion by attempting to maximally extend their knee. Each eccentric contraction was separated by a minimum of 3 seconds rest. Contractions were performed in sets of 10, followed by a 1 minute rest period. At the start of each 1 minute rest period, participants performed an isometric MVC. The eccentric exercise continued until there was a reduction in isometric MVC torque exceeding 40%. At this point, participants performed another isometric MVC, this time accompanied by femoral nerve stimulation.

Data acquisition and participant interface

Data acquisition was performed in the same manner as described in Pethick *et al*. (2015). All peripheral devices were connected via BNC cables to a Biopac MP150 (Biopac Systems Inc.,

California, USA) and a CED Micro 1401-3 (Cambridge Electronic Design, Cambridge, UK) interfaced with a personal computer. All signals were sampled at 1 kHz. The data were collected in Spike2 (Version 7; Cambridge Electronic Design, Cambridge, UK). A chart containing the instantaneous torque was projected onto a screen placed ~1 m in front of the participant. A scale consisting of a thin line (~1 mm thick) was superimposed on the torque chart and acted as a target, so that participants were able to match their instantaneous torque output to the target torque.

Data analysis

All data were processed and analysed using code written in MATLAB R2013a (The MathWorks, Massachusetts, USA). The analysis focused on three main areas: 1) measures of torque and EMG; 2) measures of global, central and peripheral fatigue; and 3) measures of torque variability and complexity.

Torque and EMG. The mean and peak torque for each isometric contraction at 50% MVC (i.e. from the constant force tasks and isometric fatigue test) were determined. The mean torque was calculated based on the steadiest five seconds of the contraction, identified by MATLAB code as the five seconds containing the lowest standard deviation. To determine task failure in the isometric condition, the mean contraction torque produced in the first minute of contractions was calculated, and task failure deemed to have occurred when participants' mean torque output failed to achieve that in the first minute by more than 5 N·m for three consecutive contractions, with the first of these contractions being the point of task failure (Pethick *et al*., 2015).

The EMG signal from the *vastus lateralis* from the isometric contractions at 50% MVC was filtered (10-500 Hz) and full-wave rectified with a gain of 1000. The average rectified EMG (arEMG) for each contraction was then calculated and normalised by expressing arEMG as a fraction of the arEMG obtained during an MVC from fresh muscle.

Global, central and peripheral fatigue. Global fatigue was assessed as the fall in MVC torque. Measures of central and peripheral fatigue were calculated based on the stimuli delivered to the femoral nerve during and after the pre-test and recovery MVCs. Peripheral fatigue was evidenced by a fall in the peak potentiated doublet torque, and central fatigue by the decline in voluntary activation (VA; Behm *et al*., 1996):

$$Voluntary\ activation\ (\%) = (1 - superimposed\ doublet/potentiated\ doublet) \times 100 \quad [1]$$

where the superimposed doublet was that measured during the contraction of interest and the potentiated doublet was measured 2 seconds after the contraction.

Variability and complexity. All measures of variability and complexity were calculated using the steadiest five seconds of each isometric contraction at 50% MVC, identified as the five seconds containing the lowest standard deviation (SD; Forrest *et al*., 2014). The magnitude of variability in the torque output of each contraction was measured using the SD and the CV. These provide measures of the absolute magnitude of variability in a time series, and the magnitude of variability in a time series normalised to the mean of the time series, respectively.

The temporal structure, or complexity, of torque output was quantified using multiple time domain analyses, as recommended by Goldberger *et al*. (2002). To determine the regularity of torque output, we calculated approximate entropy (ApEn; Pincus, 1991), and to estimate the temporal fractal scaling of torque the detrended fluctuation analysis (DFA) α exponent was used (Peng *et al*., 1994). ApEn and DFA α were calculated as in our previous studies (Pethick *et al*., 2015; Pethick *et al*., 2016; Pethick *et al*., 2018a), with these calculations briefly detailed below.

ApEn quantifies the negative natural logarithm of the conditional probability that a template of length *m* (set at 2) is repeated during a time series (Pincus, 1991). Matching templates that remain arbitrarily similar (i.e. within the tolerance, *r*, set at 0.1SD; Pincus, 1991) are counted, with the number of matches to the *i*th template of length *m* designated B_i. The number of these matches that remain similar for the *m* + 1th point is then counted, with this number for the *i*th template designated A_i. The conditional probability that the template including the *m* + 1th data point matches given the template of length *m* is then calculated for each template match. The negative logarithm of the condition probability is calculated for all templates and the results averaged. If the data are highly ordered, then templates that are similar for *m* points are likely to also be similar for *m* + 1 points. The conditional probability will be close to 1, and the negative log, and therefore the entropy, will be close to zero. This will reflect low complexity and high predictability.

$$ApEn(m,r,N) = \frac{1}{N-m}\sum_{i=1}^{N-m} log\,\frac{A_i}{B_i} \quad [2]$$

where: N is the number of data points in the time series, m is the length of the template, A_i is the number of matches to the ith template of length $m + 1$ data points, and B_i is the number of matches to the ith template of length m data points.

In the DFA algorithm, the time series is first integrated and the vertical characteristic scale of this integrated time series is measured. The integrated time series is then divided into boxes of length n and a least-squares line is fitted, representing the trend in each box. The y co-ordinate of the straight-line segment of length n in the kth box is denoted by $y_n(k)$, and the integrated time series is detrended by subtracting the local trend in each box. For a given box size, n, the characteristic size of fluctuation for the integrated and detrended time series is given by:

$$F(n) = \sqrt{\frac{1}{N}\sum_{k=1}^{N}\left[y(k) - y_n(k)\right]^2} \quad [3]$$

This computation is repeated over all time scales of box sizes to provide a relationship between box size and $F(n)$. We used 57 boxes, ranging from 1250 to 4 data points. The slope of the log-log plot of n and $F(n)$ determines the scaling parameter α. When $\alpha = 0.5$, every value will be completely independent of the values of previous observations. When $\alpha \neq 0.5$, each observation is not completely independent and is correlated, to some extent, with the values of previous observations. When $0 < \alpha < 0.5$ power law anti-correlations are present, and when $0.5 < \alpha < 1$ power law correlations are present. When $\alpha > 1$ correlations exist but cease to be of a power law form. Brownian noise is indicated by $\alpha = 1.5$.

Statistics

All data are presented as means (SD) unless otherwise stated, and results were deemed statistically significant when $P < 0.05$. No comparisons were made between the ISO and ECC conditions due to the fact that the limbs used in each condition were selected rather than randomised. Consequently, the time courses in each condition were analysed. To that end, one-way ANOVAs with repeated measures were used to test for differences between time points for MVC torque, arEMG, potentiated doublet torque, voluntary activation, measures of

variability, measures of complexity, muscle soreness and plasma creatine kinase in ISO and ECC. When main effects were observed, Bonferroni-adjusted 95% confidence intervals were then used to determine specific differences.

Results

Preliminary measures

The contractile properties of the knee extensors, along with muscle soreness and plasma CK, measured prior to ISO and ECC are shown in Table 1. The variability and complexity of torque output prior to ISO and ECC are shown in Table 2. These tables show that there were no significant differences between the conditions prior to exercise for any of the variables.

Plasma creatine kinase and muscle soreness

As shown in Table 1, plasma CK increased in ECC ($F = 19.68$, $P < 0.001$). CK peaked 24 hours after exercise (893 (388) $U.L^{-1}$; 95% paired samples confidence intervals (CIs) 299, 1144 $U.L^{-1}$) and remained significantly elevated 48 hours after exercise (CIs 203, 998 $U.L^{-1}$). It had recovered and was not significantly different from its pre-test value one week after exercise (CIs –161, 71 $U.L^{-1}$). There were no significant differences between time points for plasma CK in ISO.

Self-reported muscle soreness increased following both ECC ($F = 27.48$, $P < 0.001$) and ISO ($F = 13.42$, $P < 0.001$; Table 1). By the end of ECC, soreness had increased from 0.4 (0.4) to 6.9 (3.0) cm (CIs 3.4, 9.7 cm) and remained significantly elevated over the next 48 hours (CIs 3.3, 6.5 cm), before recovering to its pre-test level one week after exercise (CIs –0.7, 0.2 cm). At the end of ISO, soreness had increased from 0.5 (0.4) to 5.0 (2.3) cm (CIs 2.2, 6.8 cm). It decreased over the next 24 hours, though remained significantly elevated at this time point (CIs 0.3, 2.2 cm).

Torque and EMG

Both ECC ($F = 64.37$, $P < 0.001$) and ISO ($F = 93.21$, $P < 0.001$) had significant effects on MVC torque (Figure 1; Table 1). Task end in ECC occurred when an isometric MVC performed at the end of a set had decreased by 40%. This occurred after 182 ± 24 contractions and resulted in a change in MVC torque from 233.6 (74.2) to 137.7 (45.6) N·m; a decrease of

41.0 (5.2)% (CIs –46.7, –35.2%). MVC torque slowly recovered over the next 48 hours, but remained significantly depressed at this time point, by 19.7 (9.4)% (CIs –30.1, –9.3%). MVC torque had recovered and was not significantly different from its pre-test value one week after exercise (CIs –6.1, 10.2%). Task failure in ISO occurred when participants were no longer able to achieve the target torque (123.0 (38.6) N·m) despite a maximal effort. This occurred after 4.3 (1.7) minutes and resulted in a change in MVC torque from 246.1 (77.2) to 130.6 (36.2) N·m; a decrease of 46.2 (4.5)% (CIs –50.9, –41.5%). MVC torque exhibited partial recovery throughout the subsequent 60 minutes, though still remained significantly depressed, by 11.9 ± 2.1%, at the end of this period (CIs –18.8, –5.0%). MVC torque had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –0.3, 4.3%).

The mean arEMG, normalised to a fresh pre-test MVC, during the contractions at 50% MVC changed in ECC (F = 24.59, P < 0.001; Table 1). ECC resulted in an increase in arEMG from 51.2 (6.9) to 66.5 (13.1)% (CIs 1.1, 29.2%). Throughout the subsequent 60 minutes this increased further, reaching 89.7 (7.9)% at the end of this period. arEMG remained significantly elevated after 48 hours (68.4 (11.2)%; CIs 4.5, 30.0%) and had recovered, and was not significantly different, from its pre-test value one week after exercise (CIs –13.2, 4.9%). The mean arEMG also changed in ISO (F = 18.33, P < 0.001; Table 1). ISO resulted in an increase in arEMG from 52.9 (6.4) to 88.3 (18.4)% (CIs 18.5, 52.2%). arEMG decreased over the subsequent 60 minutes, but still remained significantly elevated at the end of this period (66.2 (8.5)%; CIs 7.3, 19.2%). It had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –10.9, 7.1%).

Peripheral and central perturbations

Both ECC (F = 33.22, P < 0.001) and ISO (F = 26.52, P < 0.001) resulted in significant reductions in potentiated doublet torque (Figure 1; Table 1), indicating the presence of peripheral perturbations. In ECC, potentiated doublet torque decreased from 109.2 (28.7) to 84.8 (24) N·m (CIs –34.0, –14.9 N·m) and continued to decrease in the subsequent 60 minutes, reaching 70.8 (18.1) N·m at the end of this period. It had recovered and was not significantly different from its pre-test value 48 hours after exercise (CIs –2.6, 29.1 N·m). In ISO, potentiated doublet torque decreased from 107.9 (26.2) to 63.3 (16.8) N·m (CIs –69.0, –20.2 N·m). Throughout the subsequent 60 minutes it exhibited partial recovery, but still remained significantly decreased at the end of this period (CIs –31.7, –9.0 N·m). It had recovered and

was not significantly different from its pre-test value 24 hours after exercise (CIs –5.8, 13.9 N·m).

Both ECC (F = 16.05, P < 0.001) and ISO (F = 12.70, P < 0.001) also resulted in significant reductions in voluntary activation (Figure 1; Table 1), indicating the presence of central perturbations. In ECC, voluntary activation decreased from 92.0 (2.5) to 68.3 (16.8)% (CIs –37.3, –10.0%). It remained significantly decreased after 30 minutes of recovery (CIs –18.8, –1.2%), but had recovered and was not significantly different from its pre-test value 60 minutes after exercise (CIs –2.4, 8.6%). In ISO, voluntary activation decreased from 91.7 (1.9) to 77.3 (10.2)% (CIs –25.5, –3.3%). It remained significantly decreased after 30 minutes of recovery (CIs –15.4, –0.3%), but had recovered and was not significantly different from its pre-test value 60 minutes after exercise (CIs –1.3, 9.7%).

Variability and complexity

ECC had a significant effect on the amount of variability, as measured by the SD and CV during the contractions at 50% MVC (SD, F = 8.39, P < 0.001; CV, F = 7.88, P < 0.001). In ECC, the SD increased from 3.5 (1.5) to 8.0 (5.0) N·m (CIs 0.09, 9.3 N·m), while the CV increased from 2.8 (0.6) to 7.4 (5.0)% (CIs 0.05, 9.0%). The CV remained significantly higher 10 minutes after exercise (CIs 0.1, 6.0%). ISO also had a significant effect on the SD and CV during the contractions at 50% MVC (SD, F = 19.39, P < 0.001; CV, F = 24.70, P < 0.001; Table 2). In ISO, the SD increased from 3.3 (1.5) to 10.4 (5.4) N·m (CIs 1.9, 12.4 N·m), while the CV increased from 2.6 (0.4) to 9.6 (4.2)% (CIs 2.5, 11.4%). Both had recovered and were not significantly different from the pre-test values after 10 minutes of recovery (SD, CIs –0.7, 0.7 N·m; CV, CIs –0.5, 0.4%).

The torque profiles of contractions in a representative participant in both conditions are shown in Figure 3. Complexity, as measured by ApEn, changed over time in both ECC (F = 17.16, P < 0.001) and ISO (F = 28.27, P < 0.001) for the contractions at 50% MVC (Figure 2; Table 2). In ECC, ApEn decreased from 0.39 (0.10) to 0.20 (0.12) (CIs –0.3, –0.08) and remained significantly depressed 60 minutes after exercise (0.25 (0.13); CIs –0.2, –0.07). It had recovered and was not significantly different from the pre-test value 24 hours after exercise (CIs –0.06, 0.2). In ISO, ApEn decreased from 0.41 (0.13) to 0.09 (0.04) (CIs –0.4, –0.2). It had recovered and was not significantly different from the pre-test value 10 minutes after exercise (0.36 (0.13), CIs –0.1, 0.1).

DFA α changed over time in both ECC ($F = 16.21$, $P < 0.001$) and ISO ($F = 32.45$, $P < 0.001$) for the contractions at 50% MVC (Figure 2; Table 2). In ECC, DFA α increased from 1.43 (0.07) to 1.55 (0.11) (CIs 0.04, 0.2) and remained significantly elevated 60 minutes after exercise (1.56 (0.09); CIs 0.04, 0.2). It had recovered and was not significantly different from its pre-test value 24 hours after exercise (CIs –0.1, 0.03). In ISO, DFA α increased from 1.39 (0.10) to 1.64 (0.07) (CIs 0.2, 0.3). It was still significantly elevated 10 minutes after exercise (1.46 0.09); CIs 0.02, 0.1), but had recovered and was not significantly different from its pre-test value 30 minutes after exercise (CIs –0.1, 0.02).

Discussion

The major novel finding of the present study was that, consistent with our hypothesis, eccentric exercise resulted in a prolonged loss of torque complexity, which was of greater duration than that induced by fatiguing isometric exercise. Both the eccentric and isometric conditions were associated with a loss of MVC torque and the development of significant central and peripheral perturbations, which were accompanied by increasingly Brownian fluctuations in torque output (DFA $\alpha = 1.50$). Importantly, recovery of MVC torque and torque complexity were significantly delayed following eccentric exercise. Torque complexity recovered back to baseline levels after 10 minutes of recovery in the isometric condition, but required 24 hours recovery in the eccentric condition. These results provide the first evidence that eccentric exercise reduces torque complexity during subsequent isometric contractions, demonstrating that such a loss of complexity is not unique to the effects of neuromuscular fatigue. The prolonged depression of complexity following eccentric exercise, which occurred in concert with the prolonged loss of maximal torque-generating capacity, suggests that torque complexity may reflect the functional capacity and adaptability of the neuromuscular system.

Effect of eccentric exercise on torque complexity, MVC torque and EMG

It has long been established that eccentric exercise results in a prolonged decrement in force-generating capacity (Davies and White, 1981; Newham *et al*., 1987; Jones *et al*., 1989). More recently, it has been shown that eccentric exercise also results in a prolonged increase in the magnitude of torque variability (Semmler *et al*., 2007; Dartnall *et al*., 2008). The present study

is the first study to demonstrate that such responses also apply to torque complexity (Table 2). Eccentric exercise resulted in a reduction in isometric knee extension torque complexity, as measured by significantly decreased ApEn (indicating increased signal regularity) and significantly increased DFA α (indicating increasingly Brownian fluctuations). Over the next 60 minutes, complexity exhibited no recovery and remained at the same level as at the cessation of exercise. It was only after 24 hours that complexity had recovered back to its baseline level. Such findings are similar to those investigating the magnitude of variability, which have shown increased CV during the 60 minutes following eccentric exercise (Lavender and Nosaka, 2006; Semmler *et al*., 2007; Skurvydas *et al*., 2010). It has been suggested that the complexity of a physiological output reflects the underlying system's ability to adapt to environmental challenges (Lipsitz and Goldberger, 1992; Goldberger *et al*., 2002; Pethick *et al*., 2017). If so, our results demonstrate that eccentric exercise results in a prolonged narrowing of system responsiveness and loss of adaptability in motor control, which could increase the risk of failing a motor task, such as dropping objects, failing to correct a fall, or, in the present experiments, failing to produce the required joint torque (Pethick *et al*., 2018b).

The present study revealed that the recovery kinetics of both fatigue-related variables and of torque complexity were substantially delayed following eccentric exercise compared to fatiguing isometric exercise. Recovery of MVC torque has been shown to be ~90% complete 60 minutes after isometric exercise (Sahlin and Ren, 1989; Allman and Rice, 2001), but takes several days to recover following eccentric exercise (Jones *et al*., 1989; Sayers and Clarkson, 2001). The present study provides further support for such recovery kinetics: MVC torque reached ~88% of its fresh value after 60 minutes recovery from isometric exercise, but was still decreased after 48 hours recovery from eccentric exercise (Figure 1; Table 1). As previously observed (Pethick *et al*., 2015; Pethick *et al*., 2016; Pethick *et al*., 2018a), torque complexity significantly decreased over the course of isometric exercise performed to task failure. In contrast to the eccentric exercise, recovery of torque complexity following isometric exercise was complete 10 minutes after the cessation of exercise (Table 2). Given that both the eccentric and isometric conditions resulted in significant global, central and peripheral perturbations, it is possible that the losses in complexity in each condition have, to some extent, similar causes. However, that complexity recovers almost immediately upon the cessation of isometric exercise, but takes 24 hours following eccentric exercise suggests a specific effect of eccentric exercise is responsible for this delayed recovery. It has been speculated that the delayed recovery of the magnitude of variability following eccentric exercise is of central origin, and

could be due to increased motor unit recruitment and rate coding to compensate for losses from damaged motor units or due to enhanced motor unit synchronisation (Semmler *et al*., 2007; Dartnall *et al*., 2008); both of which have been associated with the fatigue-induced loss of complexity observed previously (Pethick *et al*., 2015; Pethick *et al*., 2016).

An important and unexpected observation in the present study was that the recovery kinetics of MVC and potentiated doublet torque, in both conditions, differed from those of complexity. Torque generating capacity recovered appreciably more slowly than torque complexity (Table 1; Table 2). Following fatiguing isometric contractions, decrements in MVC and potentiated doublet torque were still evident after 60 min of recovery. In contrast, torque complexity recovered within 10-30 minutes. A similar pattern was seen in the eccentric condition: complexity recovered after 24 hours, but MVC and the potentiated doublet required at least 48 hours to return to control values. Thus, while the loss of torque complexity appears to be tightly coupled to the neuromuscular fatigue process during exercise (Pethick *et al*., 2016; Pethick *et al*., 2018a), the same is not true during recovery from exercise. The cause of this uncoupling of torque complexity from the functional capacity of the muscle is not clear. However, it is possible that in recovery from both fatigue and muscle damage, the restoration of functional capacity reaches a point at which motor control, which complexity measures reflect, is effectively restored even though maximal torque-generating capacity remains depressed. In short, the neuromuscular system's complexity during submaximal contractions appeared to be restored more rapidly than its maximal torque-generating capacity in both of our experimental conditions.

Previous research has indicated that eccentric exercise results in an increase in the amplitude of submaximal EMG during recovery (Semmler *et al*., 2007; Dartnall *et al*., 2008). In the present study, EMG amplitude following isometric exercise was significantly increased at task failure, but decreased throughout the subsequent 60 minutes of recovery. However, following eccentric exercise the EMG amplitude continued to increase throughout that 60 minutes (Table 1) and it was not until 60 minutes after eccentric exercise that EMG amplitude reached its peak. That EMG starts to recover immediately upon cessation of isometric exercise, but continues to increase during the 60 minutes following eccentric exercise may be of importance to the recovery of complexity. Specifically, increased motor unit synchronisation has been observed immediately following eccentric exercise (Dartnall et al., 2008), with this increase lasting as long as one week (Dartnall et al., 2011). Several computer simulation studies have suggested

603 that increased motor unit synchronisation substantially increases EMG amplitude (Yao et al.,
604 2000; Zhou and Rymer, 2004). Moreover, motor unit synchronisation has previously been
605 speculated to be a potential cause of the fatigue-induced loss of torque (Pethick et al., 2016;
606 Pethick et al., 2018a) and EMG (Mesin et al., 2009; Beretta-Piccoli et al., 2015) complexity.
607
608 *Physiological bases for changes in torque complexity with eccentric exercise*
609 Eccentric exercise is well known for impairing neuromuscular function through peripheral
610 mechanisms, i.e. the muscle damage it induces (Allen, 2001). These mechanisms include those
611 directly related to myofibrillar damage, and those related to damage to sarcolemmal
612 membranes (Allen *et al*., 2005). The muscle damage brought about by eccentric exercise results
613 in some muscle fibres contributing little to force production (Proske and Morgan, 2001). Thus,
614 in order to compensate for losses from damaged motor units, increased recruitment and rate
615 coding would be necessary to achieve the target torque (Semmler *et al*., 2007), as indicated by
616 the increasing EMG during the first 60 minutes of recovery (Table 1). Such an increased
617 activation of the motor unit pool may potentially contribute to the observed prolonged
618 reduction in complexity, since knee extensor torque complexity appears to decrease as
619 contractile intensity increases (Pethick *et al*., 2016). However, the muscle damage experienced
620 and decreased force generating capability persist for longer than the decreased complexity.
621 Furthermore, during recovery from isometric exercise the continued presence of peripheral
622 fatigue would likely indicate fibres contributing less to force production, necessitating greater
623 activation of the motor unit pool, yet complexity recovers within 10 minutes of the cessation
624 of exercise. It may be that measures of complexity during contractions at 50% MVC are
625 insensitive to small differences in neuromuscular system adaptability produced by fatigue as
626 the muscle recovers; higher intensity contractions might be required to reveal a closer
627 correspondence between the recovery from fatigue or muscle damage and that of torque
628 complexity.
629
630 Previous studies have observed increased motor unit synchronisation immediately after and 24
631 hours after eccentric exercise (Dartnall *et al*., 2008; Dartnall *et al*., 2011) and this has been
632 speculated to be a cause of the increased EMG amplitude and torque variability seen after such
633 exercise (Saxton *et al*., 1995; Semmler *et al*., 2007; Dartnall *et al*., 2008). The increasing EMG
634 amplitude (Table 1) and increased amount of variability (Table 2) observed in the 60 minutes
635 following eccentric exercise are both typical of increased motor unit synchronisation (Yao *et*
636 *al*., 2000; Zhou and Rymer, 2004) and suggest a role for adjustments in motor unit activation

(Dartnell *et al*. 2008). Common synaptic input to muscles, and motor unit synchronisation, have been proposed to be major determinants of force variability (Dideriksen *et al*., 2012; Farina and Negro, 2015) and have been demonstrated to increase with fatigue (Castronovo *et al*., 2015). We have, therefore, previously speculated a link between motor unit synchronisation and torque complexity (Pethick *et al*., 2016; Pethick *et al*., 2018a). However, direct measurement of individual motor units via high-density surface EMG electrodes will be necessary to confirm a link between motor unit synchronisation and torque complexity, rather than the analysis of motor unit action potential trains recorded using bipolar EMG, as was utilised in the present study.

Two limitations of the present design were the lack of randomisation of the legs used in each condition, and the non-randomised order of conditions themselves. However, there were strong physiological reasons for choosing this design: it was necessary to ensure that there were no spillover effects to or from the eccentric condition, and this meant that the non-dominant leg was chosen for this condition, and the dominant leg for the isometric condition that preceded it. Conducting the isometric condition first was necessary to ensure that any adaptation following the eccentric exercise-induced damage did not affect the response to isometric exercise. Given the rationale for the present design, the most important effect of the lack of randomisation was on the assumptions of the statistical tests used to directly compare the two conditions. Consequently, no such statistical tests were conducted or reported, and we have instead drawn our conclusions from the separate analysis of the time course of the dependent variables in each condition. It is possible that having shown the effect of unmitigated eccentric induced damage in the present study, a future study could be conducted employing randomisation. However, the long washout time that would be required in such a study would, we believe, most likely compromise the between-condition comparison and negatively affect participant compliance.

Conclusion

In summary, the present study has demonstrated that muscle-damaging eccentric exercise results in a decrease in isometric knee extensor torque complexity, as measured using ApEn and DFA α, with this decrement being considerably more prolonged than that resulting from fatiguing isometric exercise. Eccentric exercise was also associated with more prolonged decreases in MVC torque and peripheral perturbations than isometric exercise, which are attributed to the effects of muscle damage. As torque complexity recovered rapidly following

isometric exercise, the prolonged reduction in complexity following eccentric exercise was also likely due to an effect of this muscle damage. Whether this was due to the mechanical disruption itself or due to the mechanical disruption impairing and/or influencing neural drive is yet to be fully elucidated, though adjustments in motor unit activation appear to be a strong candidate mechanism. These results suggest that the effects of eccentric exercise are not limited to the periphery, but also extend to the central nervous system and the ability to control torque output.

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Table 1. Voluntary torque, potentiated doublet torque, voluntary activation, EMG, muscle soreness and plasma creatine kinase responses over the course of the isometric and eccentric tests.

Parameter		Pre	Task end/failure	10 mins post	30 mins post	60 mins post	24 hours post	48 hours post	1 week post
MVC torque,	Iso	100	53.8 (4.5)*	78.0 (11.0)*	79.8 (9.6)*	88.1 (6.6)*	102.0 (2.2)	–	–
% pre	Ecc	100	59.0 (5.2)*	64.6 (8.6)*	68.8 (9.1)*	71.9 (7.4)*	76.1 (9.8)*	80.3 (9.4)*	98.0 (7.4)
Doublet,	Iso	107.9 (26.2)	63.3 (16.8)*	87.2 (22.5)*	87.7 (22.0)*	87.6 (22.0)*	103.9 (26.6)	–	–
N·m	Ecc	109.2 (28.7)	84.8 (24.0)*	73.8 (19.0)*	71.4 (18.7)*	70.8 (18.1)*	91.6 (24.5)*	96.0 (24.2)	100.4 (24.5)
VA,	Iso	91.7 (1.9)	77.3 (10.2)*	82.2 (8.4)*	83.9 (7.1)*	87.5 (5.6)	91.5 (3.9)	–	–
%	Ecc	92.0 (2.5)	68.3 (10.2)*	78.1 (7.4)*	82.0 (8.3)*	84.1 (8.1)	88.9 (5.3)	89.8 (4.1)	91.2 (4.3)
arEMG,	Iso	52.9 (6.4)	88.3 (18.4)*	72.0 (11.3)*	66.4 (11.4)*	66.2 (9.0)*	54.9 (11.1)	–	–
% MVC	Ecc	51.2 (6.9)	66.3 (13.1)*	80.0 (13.1)*	86.7 (18.2)*	89.7 (15.1)*	76.7 (9.5)*	68.4 (11.3)*	55.3 (9.2)
Soreness,	Iso	0.5 (0.4)	5.0 (2.3)*	–	–	3.5 (2.6)*	1.7 (1.3)*	–	–
cm	Ecc	0.4 (0.4)	6.9 ± 3.0*	–	–	6.00 (2.7)*	5.3 (1.4)*	5.3 (1.6)*	0.6 (0.6)
CK,	Iso	166 (108)	168 (110)	–	–	196 (128)	200 (130)	–	–
U/L	Ecc	172 (164)	317 (255)	–	–	378 (202)*	893 (388)*	722 (293)*	217 (101)

Values are means (SD). MVC, maximal voluntary contraction; doublet, potentiated doublet torque; VA, voluntary activation; arEMG, average rectified EMG of the vastus lateralis; CK, plasma creatine kinase; Iso, isometric condition; Ecc, eccentric condition. * indicates a statistically significant difference from the pre-test value.

Table 2. Variability, complexity and fractal scaling responses over the course of the isometric and eccentric tests.

Parameter		Pre	Task end/failure	10 mins post	30 mins post	60 mins post	24 hours post	48 hours post	1 week post
SD, N·m	Iso	3.3 (1.5)	10.4 (5.4)*	3.3 (1.2)	3.2 (1.2)	3.5 (1.2)	3.0 (0.9)	–	–
	Ecc	3.5 (1.5)	8.0 (5.0)*	6.2 (3.3)	5.5 (3.4)	5.0 (2.8)	3.6 (2.0)	3.3 (1.6)	2.9 (1.1)
CV, %	Iso	2.6 (0.4)	9.6 (4.2)*	2.6 (0.5)	2.6 (0.5)	2.9 (0.6)	2.4 (0.3)	–	–
	Ecc	2.8 (0.4)	7.4 (5.0)*	5.9 (3.2)*	5.1 (3.5)	4.4 (2.4)	3.0 (1.5)	2.7 (1.0)	2.4 (0.5)
ApEn	Iso	0.41 (0.13)	0.09 (0.04)*	0.36 (0.13)	0.37 (0.14)	0.35 (0.12)	0.37 (0.09)	–	–
	Ecc	0.39 (0.10)	0.20 (0.12)*	0.19 (0.07)*	0.21 (0.09)*	0.25 (0.13)*	0.33 (0.13)	0.36 (0.15)	0.38 (0.11)
DFA α	Iso	1.39 (0.10)	1.64 (0.07)*	1.46 (0.10)*	1.44 (0.10)	1.45 (0.09)	1.42 (0.07)	–	–
	Ecc	1.43 (0.07)	1.54 (0.11)*	1.56 (0.07)*	1.57 (0.07)*	1.55 (0.09)*	1.49 (0.10)	1.45 (0.10)	1.43 (0.11)

Values are means (SD). SD, standard deviation; CV, coefficient of variation; ApEn, approximate entropy; DFA α, detrended fluctuation analysis; Iso, isometric condition; Ecc, eccentric condition. * indicates a statistically significant difference from the pre-test value.

Figure Legends

Figure 1: Maximal voluntary contraction (panel A), potentiated doublet (panel B), and voluntary activation (panel C) before and following fatiguing isometric contractions and damaging eccentric exercise. Note that recovery from isometric exercise is complete within 24 hours, whereas eccentric exercise requires at least 24-48 hours. Values are mean ± SD.

Figure 2: complexity of torque output in response to isometric and eccentric exercise. Panel A shows the responses of approximate entropy (ApEn), and panel B shows the results of the detrended fluctuation analysis. Note the rapid recovery of complexity following isometric contractions (complete within 10-30 min), but the slower recovery following eccentric exercise (recovery requiring 24 hours). Values are mean ± SD

Figure 3: example contractions from a representative participant in each condition. Note the decrease in complexity at task failure in both conditions. Recovery to, or towards, the complexity observed in a fresh isometric contraction (Before) required 10 minutes (isometric condition) or 24 hours (eccentric condition).

Figure 1

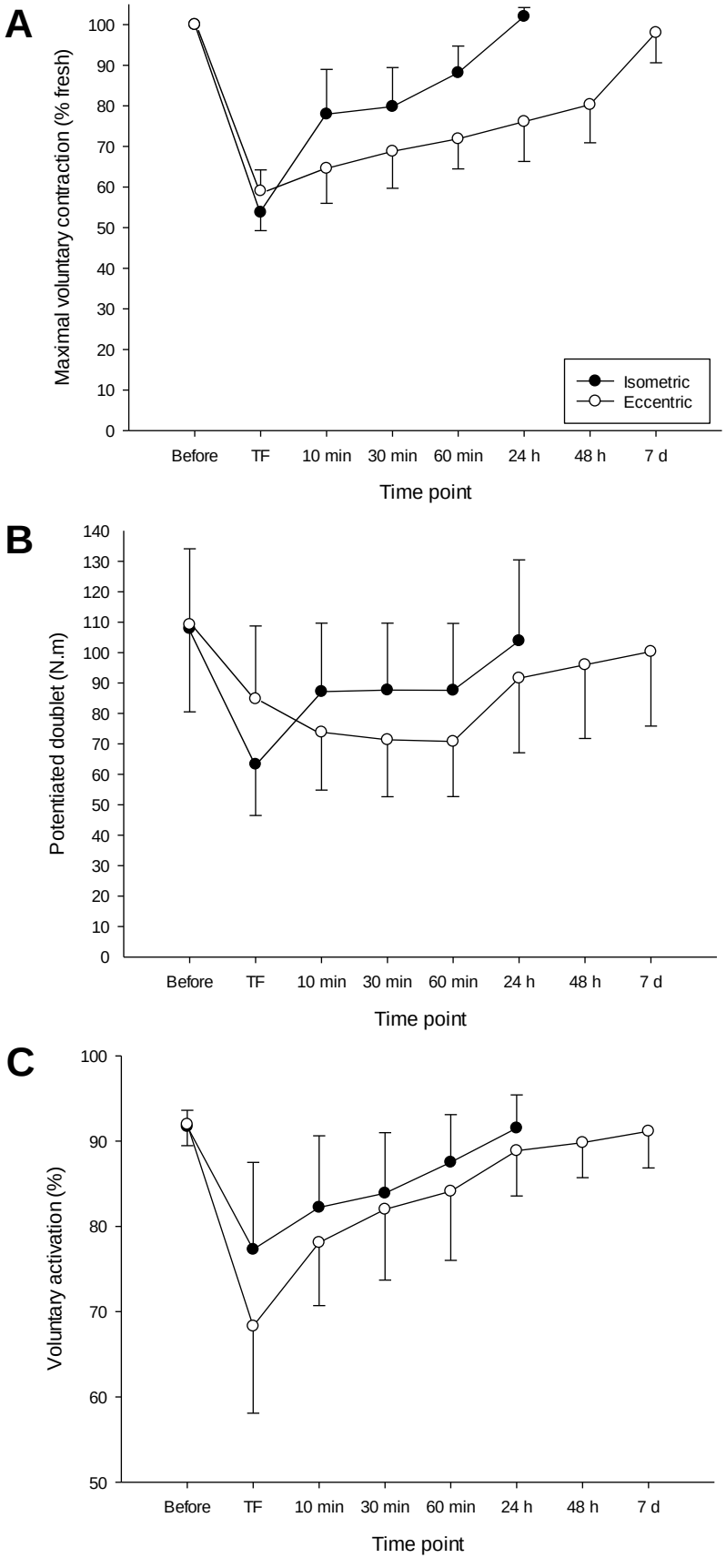

A
Maximal voluntary contraction (% fresh)
100
90
80
70
60
50
40
30
20
10
0
Isometric
Eccentric
Before
TF
10 min
30 min
60 min
24 h
48 h
7 d
Time point
B
Potentiated doublet (N.m)
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
Before
TF
10 min
30 min
60 min
24 h
48 h
7 d
Time point
C
Voluntary activation (%)
100
90
80
70
60
50
Before
TF
10 min
30 min
60 min
24 h
48 h
7 d
Time point

Figure 2

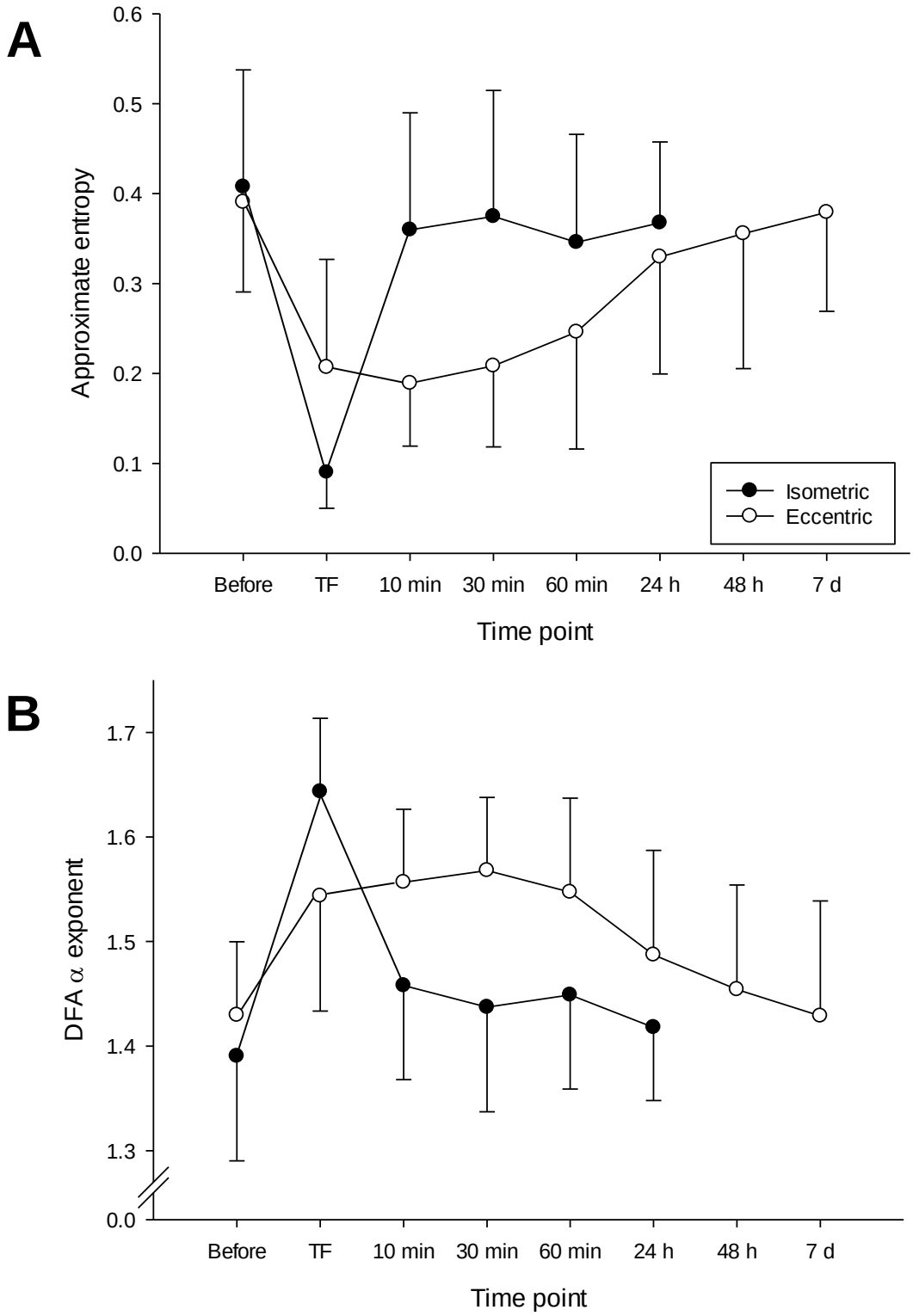
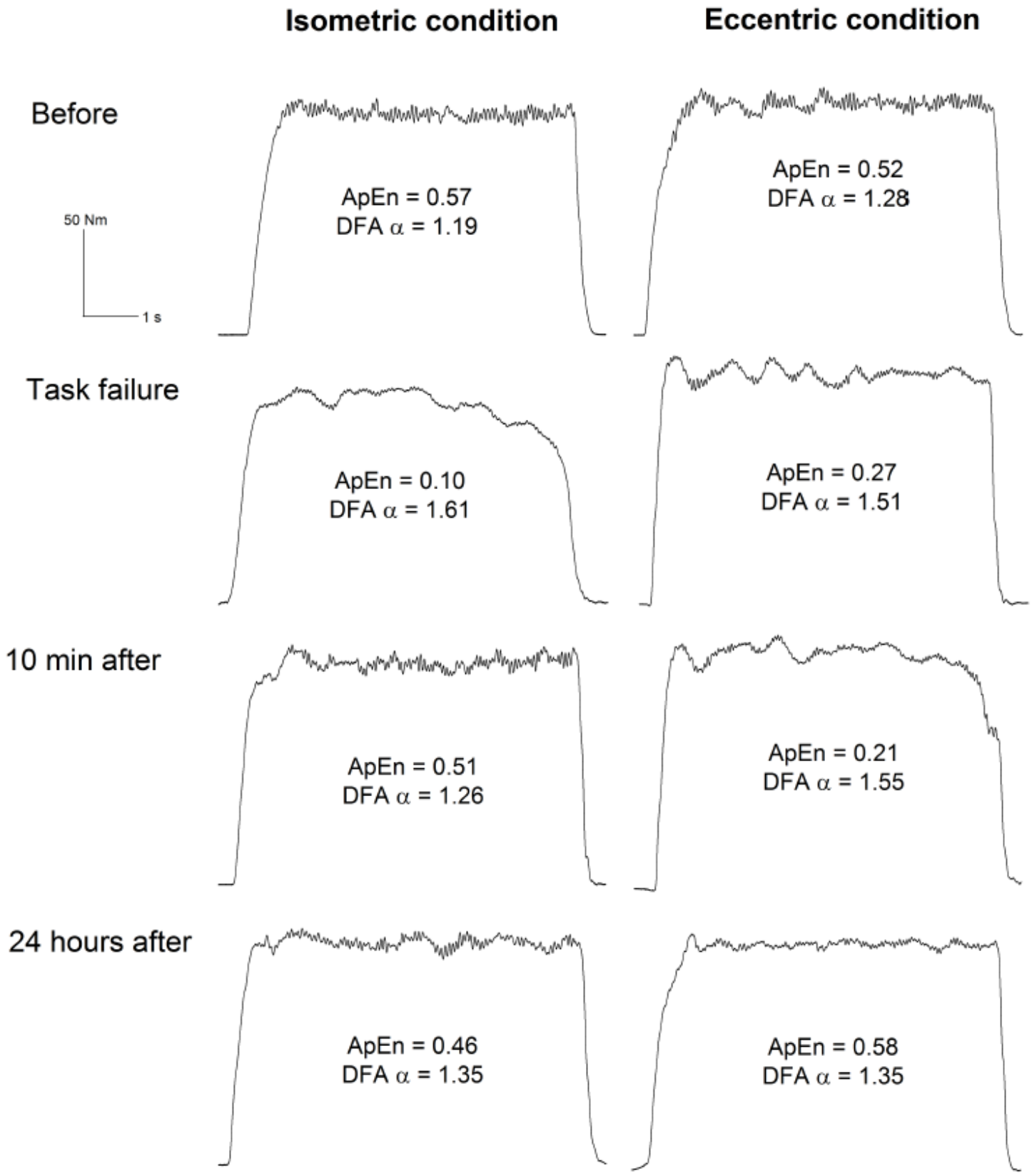

A
Approximate entropy
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Isometric
Eccentric
Before
TF
10 min
30 min
60 min
24 h
48 h
7 d
Time point
B
DFA α exponent
1.7
1.6
1.5
1.4
1.3
0.0
Before
TF
10 min
30 min
60 min
24 h
48 h
7 d
Time point

Figure 3


Isometric condition
Eccentric condition
Before
50 Nm
1 s
ApEn = 0.57
DFA α = 1.19
ApEn = 0.52
DFA α = 1.28
Task failure
ApEn = 0.10
DFA α = 1.61
ApEn = 0.27
DFA α = 1.51
10 min after
ApEn = 0.51
DFA α = 1.26
ApEn = 0.21
DFA α = 1.55
24 hours after
ApEn = 0.46
DFA α = 1.35
ApEn = 0.58
DFA α = 1.35