Title:

Repeated exercise stress impairs volitional but not magnetically-evoked electromechanical delay of the knee flexors

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Running Head:

Fatigue and knee flexor electromechanical delay

Key words: Fatigue, Electromechanical delay, Magnetic stimulation, Females, Knee flexors

ABSTRACT

The effects of serial episodes of fatigue and recovery on volitional and magnetically-evoked neuromuscular performance of the knee flexors were assessed in twenty female soccer players during: (i) an intervention comprising 4x35s maximal static exercise; (ii) a control condition. Volitional peak force (PF_V) was impaired progressively (~16 % vs. baseline: 235.3±54.7 to 198.1±38.5 N) by the fatiguing exercise and recovered to within ~97 % of baseline values following six-minutes of rest. Evoked peak twitch force (P_TF_E) was diminished subsequent to the fourth episode of exercise (23.3 %: 21.4±13.8 vs. 16.4±14.6 N) and remained impaired at this level throughout the recovery. Impairment of volitional electromechanical delay performance (EMD_V) following the first episode of exercise (25.5 % :55.3±11.9 vs. 69.5±24.5 ms) contrasted with concurrent improvement (10.0 %: 24.5±4.7 vs. 22.1±5.0 ms) in evoked electromechanical delay (EMD_E) ($p < 0.05$) and this increased disparity between EMD_E and EMD_V remained during subsequent periods of intervention and recovery. The fatiguing exercise provoked substantial impairments to volitional strength and EMD_V that showed differential patterns of recovery. However, improved EMD_E performance might identify a dormant capability for optimal muscle responses during acute stressful exercise and an improved capacity to maintain dynamic joint stabilty during critical episodes of loading.

INTRODUCTION

Electromechanical delay (EMD) refers the time lag between the onset of electrical activity and the external force production in skeletal muscle (Zhou, Carey, Snow, Lawson, Morrison, 1998) and it represents an important component of neuromuscular performance. For example, EMD is vital in the interpretation of the role and coordination of muscles in a movement (Hug, 2011; Vos, Harlaar, van Ingen Schenau, 1991). It is also an important determinant of successful performance in tasks where the time available for the development of force is limited, such as jumping (Vos, Mullender, van Ingen Schenau, 1990) and although not associated causally with musculoskeletal injury, it is considered to be an important marker of the temporal limitations for protective neuromuscular responses (Blackburn, Bell, Norcross, Hudson, Engstrom, 2008; Minshull, Gleeson, Walters-Edwards, Eston, Rees, 2007; Linford et al., 2006; Chan, Lee Wong, Yeung, 2001; Shultz & Perrin, 1999; Vos et al., 1991).

Electromechanical delay is comprised of several events and mechanisms associated with excitation-contraction coupling and the production of muscle force, including the time course for the propagation of the action potential, the duration of excitation-contraction coupling process and the stretch of the series elastic component (SEC) (Norman & Komi, 1979). As such, changes in EMD performance might coincide with alterations in the functional properties of the muscle and tendon or for example, when there might have been fatigue-induced changes to the number, type and excitation frequency of the motor units recruited in a contraction. Whilst there is a body of research that explores the

electromechanical responses of the knee extensors subsequent to fatiguing exercise (e.g. Chan et al., 2001; Zhou et al., 1998), indicating up to a 70% fatigue-related lengthening of EMD in males (Zhou, McKenna, Lawson, Morrison, Fairweather, 1996), there is limited research into the EMD of the knee flexor musculature following exercise, especially in females. The efficacy of dynamic joint stabilisation during sudden loading is dependent on the temporal parameters related to the initiation and development of muscle force, including EMD and the magnitude of the force response (Blackburn et al., 2008; Linford et al., 2006). As such, these considerations may be important given that females are at considerably greater risk of sustaining some types of knee injury, especially to the anterior cruciate ligament (ACL) compared to male counterparts (Prodromos, Han, Rogowski, Joyce, Shi, 2007). One study has shown that a single acute episode of muscle fatigue in the knee flexors elicited a 19.3% increase in the EMD of females but not males (Minshull et al., 2007). This change in performance was observed at joint angles proximal to full extension, where the knee joint is particularly vulnerable to ACL injury. The timely activation of the knee flexors may be especially important in the provision of dynamic protection to the knee joint (Blackburn et al., 2008) by resisting rotational and anterior translational stresses, which threaten the integrity of the anterior cruciate ligament (Li et al., 1999). As such, the functional consequences of fatigue-related prolongation of EMD responses may present additional risk factors for ACL injury in female populations.

The importance of neuromuscular capabilities to the maintenance of dynamic joint stability and the avoidance of non-contact injuries is widely recognised (Sbriccoli,

Solomonow, Zhou, Lu, Sellards, 2005; Chan et al., 2001; Shultz et al., 2001), especially in female populations and players of team games, (Prodromos et al., 2007; Harmon & Ireland, 2000). Our understanding of the evoked and volitional muscular activation performance associated with knee joint positions that have been shown to be vulnerable to injury (Johansson, 1991) is currently limited, especially during serial episodes of fatiguing exercise. Furthermore, there is limited information in the literature on the recovery process of EMD after a fatiguing exercise. In situations where where is a limited time frame for the most rapid and protective responses of the neuromuscular system to counteract potentially harmful dynamic joint forces, sub-optimal performance may present additional injury risk (Shultz et al., 2001; Mercer, Gleeson, Claridge, Clement, 1998; Gleeson, Reilly, Mercer, Morris, Rees, 1998; Huston & Wojtys, 1996).

Spontaneous or reflexive responses to emergency scenarios including 'fight or flight' responses would be expected to reflect an individual's true maximal performance capability. It is conceivable that factors such as autogenic neuromuscular inhibition associated with waning motivation, conditioning status, responses to exercise stress, and injury may affect the measurement of an individual's true EMD under routine voluntary conditions of assessment (Gleeson, 2001; Hopkins & Ingersoll, 2000). As such, methods of evoked muscle activation, such as magnetic stimulation, might have the potential to better examine maximal performance capability; (Minshull, Gleeson, Eston, Bailey, Rees, 2009; O'Brian, Reeves, Baltzopolous, Jones, Maganaris, 2008; Polkey et al., 1996).

The aim of this study was to investigate the effects of repeated episodes of maximal exercise and subsequent recovery on the volitional and magnetically-evoked performance of the knee flexors in females.

METHODS

Participants

Twenty female soccer players (age: 21.3 ± 2.3 yrs; height 1.68 ± 0.06 m; body mass 68.0 ± 6.3 kg [mean ± SD]) who were free from injury provided written informed consent to participate in this study. Participants were instructed to refrain from strenuous physical activity for the 24-hours prior to the test. Assessment protocols were approved by the Institutional Ethics Committee for Human Testing.

Experimental Procedures

Following habituation, participants were secured in a prone position on a custom-built dynamometer (modified from Gleeson, Rees, Rakowski, Reilly, 1995) and the bi-lateral lever-arms were configured to achieve a functionally-relevant knee flexion angle of 25 degrees (0.44 rad) (figure 1), which is also associated with the greatest mechanical strain on key ligaments (Li et al., 1999). The experimental design comprised two treatment conditions: (i) a fatiguing intervention condition that required participants to perform repeated sustained static MVMAs (maximal voluntary muscle activations) of 4 x 35-seconds of the knee flexors of the preferred leg and; (ii) a control condition of equivalent duration to the fatiguing task consisting of no exercise. Episodes of fatiguing exercise were separated by 15-seconds and each episode consisted of 30-seconds sustained effort followed by a 5-second rest period and a further episode of 5-seconds effort. This intermittent pattern of exercise was selected to enable multiple estimates of magnetically-evoked performance to be obtained. Assessments of volitional and magnetically-evoked neuromuscular performance were obtained prior to (0s); following each episode of exercise (35s;

70s; 105s; 140s); 1, 3 and 6-minutes following the cessation of exercise (200s; 320s; 500s, respectively), and at corresponding periods throughout the control condition (figure 1). Magnetic stimulation was performed first on each test occasion in order to minimise the possible potentiating effects of prior MVMA associated with the volitional testing. The two conditions were presented in sequence (control; fatigue) and were separated by 20 minutes. Fatigue was defined as a loss in maximal force generating capacity (Kent-Braun 1990).

**** Figure 1 near here ****

Assessment of Neuromuscular Performance

Supra-maximal magnetic stimulation of the sciatic nerve (L4-L5) and associated activation of the knee flexors was achieved by means of a double wound coil (120 mm) that was powered by a Magstim 200 stimulator (Magstim Co. Ltd., Whitland, Dyfed, Wales). The protocol deployed to elicit and verify supra-maximal stimulation was in accordance with the methodology described previously (Minshull, Rees, Gleeson 2011; Minshull et al., 2007). Briefly, the magnetic coil was placed over the L4-L5 region and small iterative positional changes of the coil were made that were commensurate with increasing size of responses during a series of discrete stimulations. The optimum site for stimulation of the nerve was defined as the site that elicited the largest twitch force and M-wave amplitude and this optimised coil position was maintained manually throughout the remainder of the test. Supra-maximal stimulation was verified by contemporaneous visual inspection of the data during a sequence of seven discrete stimulations of increasing intensity that commenced at 40 % of the Magstim 200's maximal capacity output with subsequent

increments of 10 % to 100 % of capacity and by retrospective analyses of M-wave and peak twitch force data. Sequential stimulations were separated by at least 10-seconds (Moore & Kulka, 1991). All stimulations to assess evoked neuromuscular performance were performed at 100% of the Magstim's capacity.

Following a series of sub-maximal warm-up muscle activations an auditory signal was given randomly within 1-4 seconds that instructed the participants to flex the knee joint as rapidly and forcefully as possible against the immovable restraint offered by the apparatus (load cell: 615, Tedea-Huntleigh, Cardiff, UKl). Another auditory signal was given to the participant after 3-seconds of MVMA to cue neuromuscular relaxation. Intra-trial MVMA replicates were each separated by at least 10-seconds to enable neuromuscular recovery (Moore & Kulka, 1991).

Indices of Neuromuscular Performance

Volitional static peak force (PF_V) and magnetically-evoked peak twitch force (P_TF_E) were recorded as the mean of the highest force response during two intra-trial replicates. Electromyographic activity (EMG) was recorded from the m. biceps femoris (long head) during estimates of PF_V and P_TF_E using bipolar surface electrodes (self-adhesive, silver-silver chloride, 10 mm diameter) that were applied longitudinally over the muscle belly. The m. biceps femoris was selected as an important contributor to the avoidance of ACL injury (Noyes, Mooar, Matthews, Butler, 1983). The raw unfiltered EMG signals, which incorporated minimal intrusion from induced currents associated with external electrical and electromagnetic sources and noise inherent in the remainder of the recording instrumentation were passed through a differential amplifier, input impedance 10,000

MΩ, CMRR 100 dB, gain of 1000 (Cambridge Electronic Design, UK)) were analogue-to-digitally converted at 2.5 kHz sample rate, ensuring a significant margin of reserve between the highest frequency expected in the EMG signal and the Nyquist frequency (Gleeson, 2001). The inter-electrode distance was 30 mm and a reference electrode was placed 30 mm lateral and equidistant from the recording electrodes. Standardised skin preparation techniques yielded inter-electrode impedance of less than 5 kΩ.

Volitional and magnetically-evoked electromechanical delay (EMD_V and EMD_E, respectively) were computed as the mean response of two intra-trial muscle activations in which the time delay between the onset of electrical activity and the onset of force was recorded. Onset of electrical activity and muscle force were defined as the first point in time where the recorded signals exceeded consistently the 95 % confidence limits of the background electrical noise amplitude in quiescent muscle (Minshull et al., 2007) (figure 2).

**** Figure 2 near here ****

Statistical Analysis

The performance indicators were described using ordinary statistical procedures (mean ± SD). The effect of the fatiguing exercise intervention was assessed for each index of performance using separate two (condition: control; intervention) by five (time: 0s; 35s; 70s; 105s; 140s) fully repeated-measures ANOVAs. Recovery was investigated for each index of performance using separate two (condition: control; intervention) by four (140s; 200s; 320s; 500s) fully repeated-measures ANOVAs. *A*

priori (Simple [reference: group mean scores at 0s]) contrasts were used to examine any significant differences between means. The assumptions underpinning the use of repeated measures ANOVA were checked and violations corrected by the Greenhouse-Geisser adjustment of the critical F-value, as indicated by $_{GG}$. Statistical significance was accepted at $p < 0.05$.

The experimental design offered an approximate .80 power of avoiding a Type-II error when employing a least detectable difference of 16 N and 8 ms for PF_V and EMD_V and 3.13 N and 3.5 ms for P_TF_E and EMD_E, respectively.

RESULTS

Significant two-factor interactions associated with analyses of PF_V and EMD_V scores ($F_{[2.4, 44.8\ GG]} = 3.2$, $p < 0.05$ and $F_{[4, 76]} = 2.7$, $p < 0.05$, respectively) showed that while performance was preserved over the control period, the exercise intervention induced significant impairments in performance. *A priori* tests of PF_V values showed a progressive increase in fatigue up to the cessation of the third episode of exercise (35s-105s) ($F_{[1, 19]} = 5.9, 14.9, 6.1$, $p < 0.05$) with corresponding decrements to performance of 10.3 %, 15.9%, 15.8 % compared to pre-exercise levels, respectively (figure 3) A 25.5 % impairment to EMD_V performance was observed following the first episode of exercise (35s), and was maintained at this level throughout the remainder of the exercise. A significant two-factor interaction associated with recovery measures of PF_V ($F_{[3, 57]} = 17.1$, $p < 0.001$) indicated a restoration of PF_V performance following the cessation of exercise. A significant main effect associated with the analysis of recovery measures for EMD_V ($F_{[1, 19]} = 10.0$, $p < 0.05$), showed that the impairment of EMD_V performance was maintained throughout the 6-minute recovery period (figure 4). *A priori* tests showed that recovery of PF_V performance commenced at 1-minute following cessation of exercise (200s) reaching 96.6 % of pre-exercise values at 6-minutes following exercise (500s) ($F_{[1, 19]} = 4.5, 28.7, 17.1$, respectively, $p < 0.05$).

**** Figure 3 near here ****

**** Figure 4 near here ****

Significant two-factor interactions associated with analyses of P_TF_E and EMD_E scores ($F_{[2.3, 44.5\ GG]} = 3.2$, $p < 0.05$ and $F_{[2.6, 49.6\ GG]} = 3.18$, $p < 0.05$, respectively) showed that the exercise intervention induced a significant impairment in P_TF_E performance (23 % following the fourth episode of exercise [140s] ($F_{[1, 19]} = 6.4$, $p < 0.05$)) and a significant improvement in EMD_E performance (10 % following the first episode of exercise ($F_{[1, 19]} = 5.9$, $p < 0.05$)) that was maintained throughout the remainder of the exercise. A significant main effect associated with the analysis of P_TF_E recovery measures ($F_{[1,19]} = 20.2$, $p < 0.001$) showed that the impairment of P_TF_E performance was sustained throughout the 6-minute recovery period (figure 5). Analysis of EMD_E recovery scores revealed no significant main or interaction effects, implying EMD_E performance scores were similar between treatment conditions throughout the recovery period (measurement occasions 140s-500s) (figure 4).

**** Figure 5 near here ****

DISCUSSION

The exercise intervention induced fatigue in the knee flexors, characterised by a progressive decrease in PF_V from pre- to immediately following the third episode of exercise (up to 15.8 % loss at 105s vs. baseline [235.3±54.7 to 198.1 ± 38.5 N, respectively]). In addition, EMD_V was substantively impaired following the first episode of exercise (25.5 % increase in latency vs. baseline [55.3 ± 11.9 vs. 69.5 ± 24.5 ms, respectively]) and remained impaired at this level across the consecutive episodes of exercise and the recovery period. The magnitude of PF_V and EMD_V performance decrements observed in the current study is congruent with that of previous studies subsequent to similar fatiguing exercise at extended knee joint angles (Minshull et al., 2007; Chan et al., 2001), and is similar to that observed following simulated soccer match-play (Gleeson et al., 1998).

The sustained increase in the time needed to initiate muscle force demonstrated that repetitive episodes of high-intensity exercise had adversely affected the capability for dynamic activation performance of the musculature for several minutes. Volitional muscle electromechanical response times that are no longer optimal and which may be coupled with the effects of increased knee laxity brought about by cyclical loading activities on connective tissues (Solomonow, 2004) may result in an excessively compliant system that is less capable of restraining high joint loads rapidly enough to prevent ligamentous injury (Sbriccoli et al., 2005). These mechanisms could particularly afflict the female athlete whose specific anatomical and bio-physiological capacities may already predispose them to increased risk of serious ligamentous injury during team sports compared to male counterparts (Harmon & Ireland, 2000).

It was interesting that there was a noticeable differential in the temporal restoration of volitional strength and electromechanical delay performance. For example, while PF_V performance was almost fully-restored within 6-minutes of the cessation of fatiguing exercise, the recovery of EMD_V to baseline performance was not achieved during the same time period. The differential patterns of restoration to estimates of PF_V and EMD_V performance throughout exercise and acute recovery may reflect varying degrees of adaptation and interactions within conductive (Zhou et al., 1998; O'Leary, Hope, Sale, 1997), contractile, and elastic properties (Rassier & MacIntosh, 2000; Stone, 1992). Alongside fatigue-related limitations to the peripheral processes involved in the conversion of excitation into muscle force (Kent-Braun, 1999), increased intra-muscular temperature with correspondingly greater tissue compliance and increased time to stretch the series elastic component (Zhou et al., 1998) may have been an important determinant of the sustained and increased EMD_V in this study. Potential alterations in muscle firing synchrony between the individual hamstring muscles (m biceps femoris vs. m semimembranosus and m semitendinosus) and thus the detection of the onset of EMG, may also have contributed minimally to the observed changes in EMD_V.

Peak twitch force (P_TF_E) was reduced by up to 23 % compared to baseline performance following the fourth episode of exercise (200s) (21.4 ± 13.8 vs. 16.4 ± 14.6 N) and remained impaired at this level throughout the cessation of exercise and recovery (500s). The extent of this impairment is likely to reflect a reduced capacity to produce force given that magnetic stimulation of a nerve root activates even the fastest motor units (Kiers & Chiappa, 1989). Whereas P_TF_E was impaired by serial episodes of stressful exercise, the rapidity of evoked muscle activation was enhanced

concomitantly: EMD_E was improved by 10.0 % from baseline to following the first episode of exercise (24.5 ± 4.7 vs. 22.1 ± 5.0 ms, respectively) and appeared to have remained improved during subsequent episodes. These differential exercise-related changes might suggest different relative influences of the physiologic determinants of performance capacity. It is conceivable that impairment to the contractile capacity of the muscle (P_TF_E and PF_V) might be mediated by the metabolically-driven consequences of the fatiguing exercise on excitation-contraction coupling (Kent-Braun, 1990). Electromechanical delay is comprised of several events and the primary determinant of this latency is thought to comprise the time taken to stretch the SEC (Norman & Komi, 1979; Sasaki, Sasaki, Ishii, 2011). The aponeurosis and tendon has been estimated recently to account for approximately 48% of EMD when measured by means of very high frame rate ultrasound (Nordez et al., 2009). Thus the capability to generate contractile force might only partially influence EMD and it would seem that the interaction of exercise-related stress and the different assessment characteristics associated with evoked and volitional EMD might account for the disparate responses in the latter indices of the time needed to initiate muscle force. A net decrease in compliance within the knee joint system and a shortening of the evoked latency (EMD_E) might have been facilitated by the particular impulse of the force associated with evoked activation and maximal recruitment of all motor units during the stretching of stress-relaxed viscoelestic structures, combined with exercise-related hyperaemia and distension of the muscle (McComas 1996). Whilst speculative and perhaps contrary to the presumed effects of exercise-related increases in intra-muscular temperature and in tissue compliance that would tend to prolong EMD (Zhou et al., 1998), weak associations between volitional and evoked indices of performance (Minshull et al., 2011) would tend to underscore the potential for

differing influences under each mode of neuromuscular assessment. Further research is required to explore the specific mechanisms underpinning the differential fatigue-related responses of EMD_V and EMD_E.

Impairment (25.5 % compared to baseline scores) in EMD_V following the first episode of exercise contrasted with the concurrent potentiation (10.0 % compared to baseline scores) in EMD_E ($p < 0.05$). How the former might threaten the capability for rapid stabilisation of a joint system and injury avoidance during episodes of excessive mechanical stress is worthy of future investigation, particularly in high-risk female populations. It was noteworthy that the this level of disparity between evoked and volitional muscle activation performance remained during subsequent periods of intervention and recovery and constituted systematically longer latencies associated with EMD_V (e.g. 25.5 % of 69.4 ms) by comparison to EMD_E (e.g. 10 % of 22.1 ms). The findings of improved EMD_E performance might identify a dormant capability for optimal muscle responses during acute stressful exercise endeavours that might otherwise pose threats to knee joint stability and a corresponding temporal capacity to maintain dynamic joint stabilty during critical eposodes of loading. The efficacy of the preservation of EMD_E performance capacity in the avoidance of synovial joint injury, however, would depend entirely on the achievement of optimal neuromuscular recruitment strategies and the down-regulation of autogenic inhibitory mechanisms.

CONCLUSION

Prolonged impairment to both the speed (EMD_V) and force (PF_V) of volitional knee flexor neuromuscular performance subsequent to high intensity exercise may have implications for optimal athletic performance and recovery and, importantly, these performance decrements may contribute to an increased and prolonged risk of knee injury in particularly vulnerable populations. However, the enhanced responses of EMD_E performance subsequent to a single episode of exercise in the current study might suggest that in some circumstances, an episode of fatiguing exercise may actually facilitate rather than impede neuromuscular performance in the knee flexors and ultimately enhance the capability of the joint to rapidly resist injury in emergency situations.

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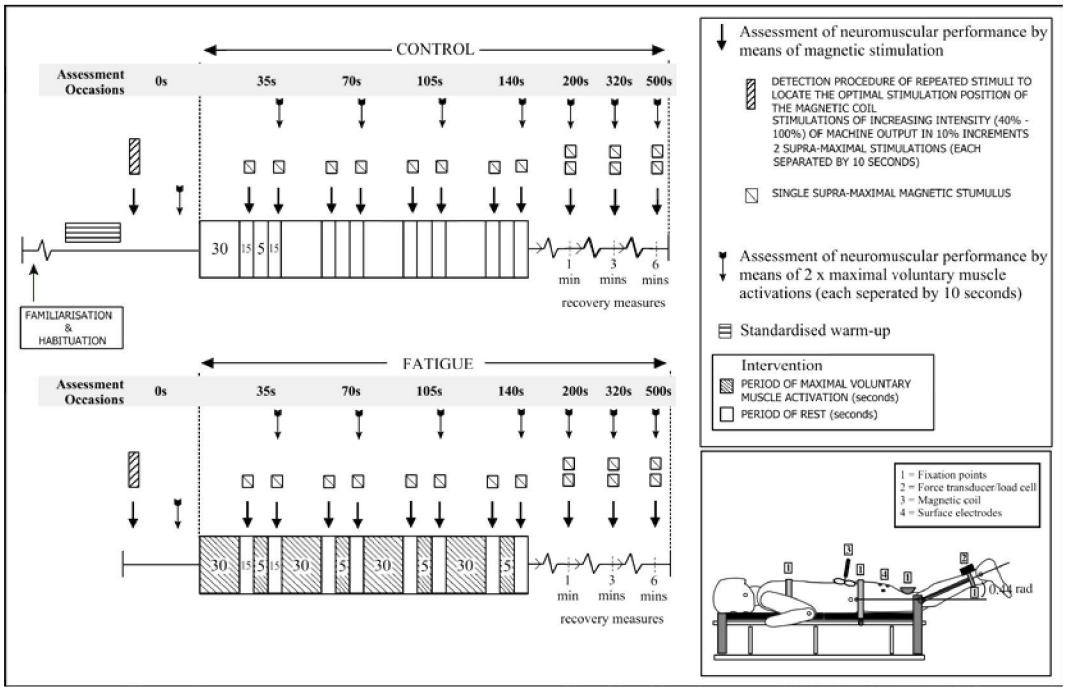

Figure 1. Schematic of the protocol for the assessment of the effects of serial fatiguing tasks and acute recovery on indices of knee flexor volitional and magnetically-evoked neuromuscular performance.

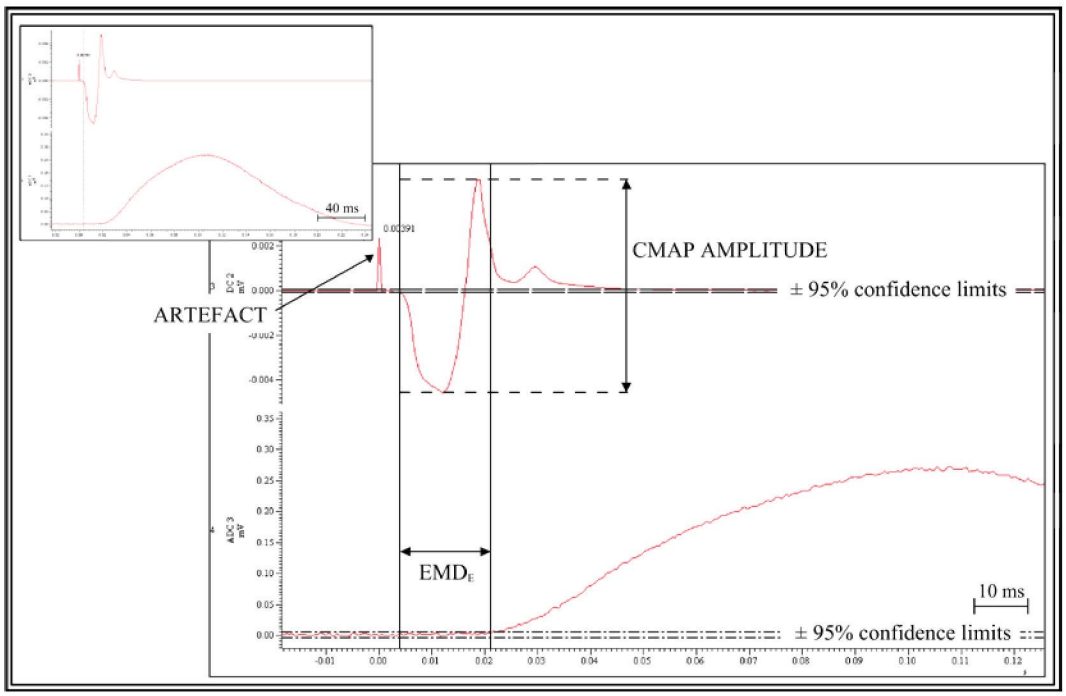

Figure 2. Example data showing: upper trace: example data of force and EMG associated with a single magnetic stimulus; lower trace: magnification of muscle activation to show representative calculation of magnetically-evoked electromechanical delay.

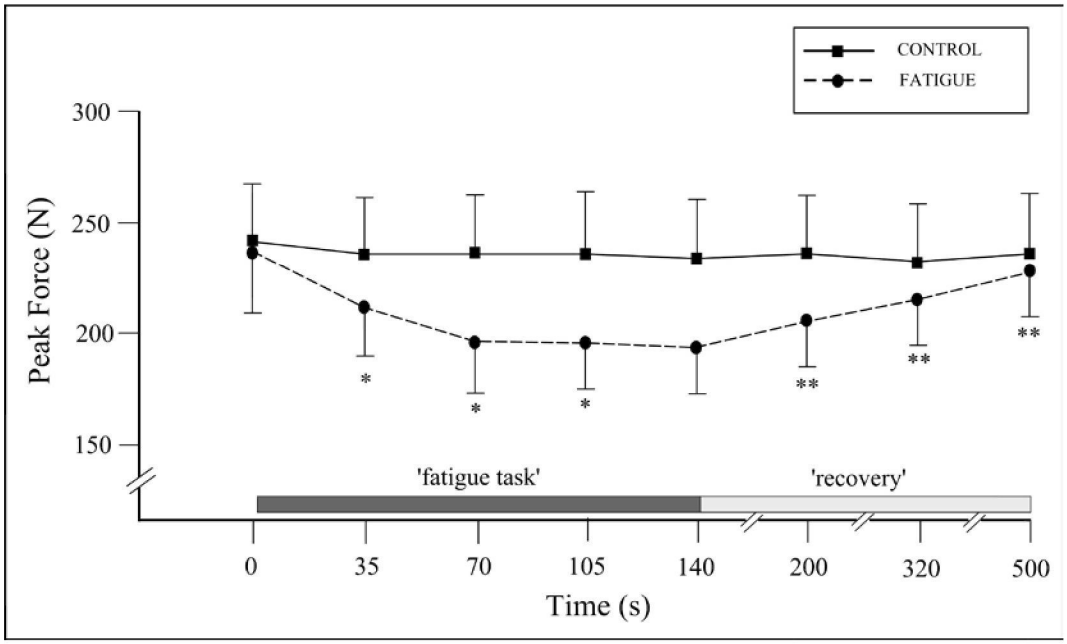

Figure 3. The effects of four episodes of maximal static exercise and subsequent recovery on the volitional peak force performance (PFV) of the knee flexors (group mean ± SD). (* = significantly different to pre exercise values).

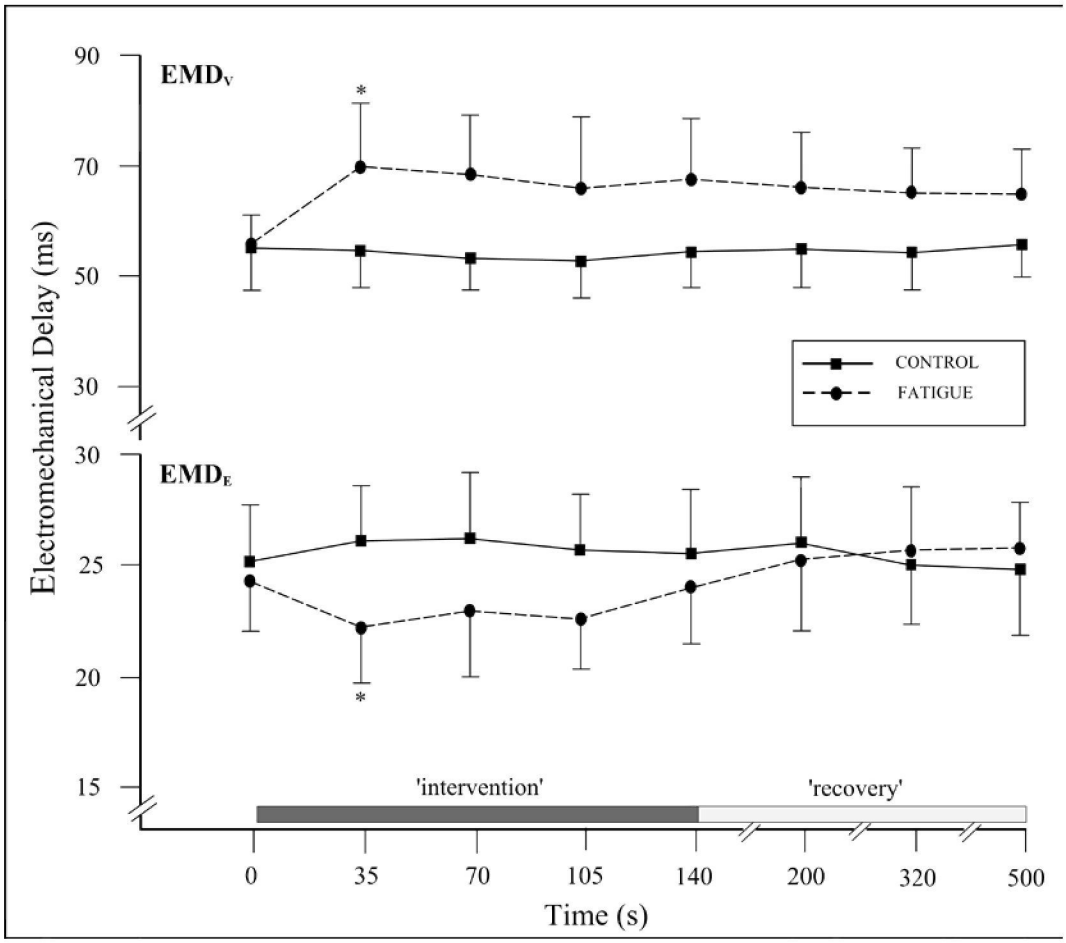

Figure 4. The effects of four episodes of maximal static exercise and subsequent recovery on the volitional and magnetically-evoked electromechanical delay performance (EMDV, EMDE, respectively) of the knee flexors (group mean ± SD). (* = significantly different to pre exercise values; ** = significantly different from 140s).

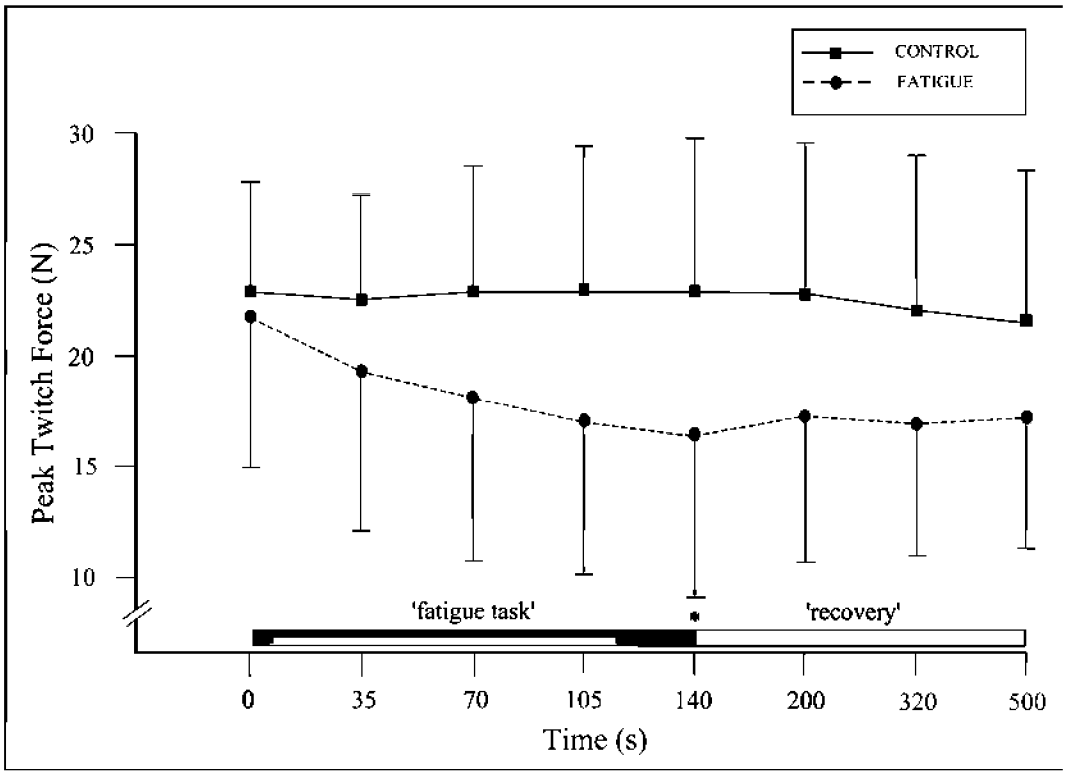

Figure 5. The effects of four episodes of maximal static exercise and subsequent recovery on the magnetically-evoked peak twitch performance (PTFE) of the knee flexors (group mean ± SD). (* = significantly different to pre exercise values).