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Changes in human skeletal muscle contractile function following stimulated eccentric exercise

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Abstract Indices of human skeletal muscle contractile function were examined in nine subjects for up to 9 days following a single bout of stimulated eccentric exercise. Eccentric muscle actions of the knee extensor muscles were evoked by percutaneous electrical myostimulation (PES). Delayed onset muscle soreness (DOMS), elevated serum creatine kinase activity, chronic force loss, and a decline in the 20:100 Hz force ratio were observed in the days postexercise. The exercised knee extensor muscles demonstrated an impaired ability to respond to PES. This was evident by an increased time delay between the start of 100 Hz PES and the onset of contraction immediately postexercise [22.3 (SD 15.9)%, $P < 0.01$] and 3 days postexercise [14.9 (SD 18.1)%, $P < 0.05$]. Muscle relaxation rates appeared unaffected by the eccentric exercise protocol, where the muscles showed no differences in the time between the end of PES and the onset of relaxation ($P > 0.05$). During the days following the exercise, no significant differences were observed in the time between the start of contraction and attainment of 70% of the mean tetanic force following a single 1-s pulse of PES. Similarly, no significant differences were observed in the time between the start of relaxation and attainment of 70% of the total relaxation during the same time. The increased delay in excitation-contraction coupling observed immediately postexercise and 3 days after the exercise, may reflect a damage-induced delay in action potential propagation. Muscle relaxation rates postexercise remained unchanged, which would seem to indicate normal functioning of the sarcoplasmic reticulum, suggesting this was not the site of failure in excitation-contraction coupling.

Key words Skeletal muscle · Exercise-induced muscle damage · Excitation-contraction coupling

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Parts of this study have already been presented during a conference as preliminary results (Brown et al. 1995)

Introduction

It has been suggested that temporary exercise-induced damage to skeletal muscle is predominantly a phenomenon associated with eccentric muscle contractions, although the causes are not fully understood (for review see Stauber 1989). High muscle tensions associated with eccentric exercise have been suggested as a possible cause of injury during this type of muscle activity (McCully and Faulkner 1985; Newham et al. 1983a; Tiidus and Ianuzzo 1983). Focal myofibril disruption and subcellular Z disc streaming have been shown to typify the structural damage visible following repetitive high force eccentric muscle contractions (Friden et al. 1983; Newham et al. 1983b; Jones et al. 1986). One theoretical model of exercise-induced muscle damage cites cell membrane damage and disturbed Ca^{2+} homeostasis as causative mechanisms in the etiology of muscle fibre necrosis and secondary degradation, due to increased activation of cytosolic phospholipases and proteases (Armstrong 1984). A fully functional sarcoplasmic reticulum (SR) has been found to assist the regulation of intracellular Ca^{2+}, and failure of the integrity of SR, with a subsequent inability to regulate intracellular free Ca^{2+}, may prolong the elevated Ca^{2+} concentration (Byrd 1992). This may present an environment which can stimulate Ca^{2+} sensitive autolytic processes involved in fibril degradation. It has been suggested that chronic low frequency fatigue following unaccustomed eccentric exercise (Jones 1981) indicates structural damage to the T-tubule network or SR, in preference to the prolonged alteration of the kinetics of troponin and calcium binding. However, Friden (1984)

has suggested that SR disruption following high force eccentric exercise is unlikely, since such damage would manifest itself in contraction clots visible in muscle biopsy specimens.

Voluntary eccentric exercise models have been used to induce damage within the contracting muscles (Clarkson et al. 1992; Newham et al. 1983a; Rodenburgh et al. 1993), however, controlling the mass of muscle recruited using percutaneous electrical myostimulation (PES), may remove the effects of central fatigue, motivation, and differing motor unit recruitment patterns during a bout of exercise. The PES models consistently evoke contraction within the same muscle mass, and further help to standardise a submaximal exercise protocol within a subject population. Clarkson et al. (1992) have measured functional impairment of skeletal muscle following eccentric muscle actions, and these authors have demonstrated a progressive recovery of maximal isometric force following the bout. Newham et al. (1987) have measured the ratio of forces produced using 20 and 100-Hz stimulation frequencies posteccentric exercise, and these authors have also demonstrated a progressive recovery following the exercise. These functional measures and new indices of skeletal muscle contraction and relaxation (in particular the temporal dissociation between application of an electrical stimulus and contraction/relaxation) were investigated in the present study. This study used PES at 100-Hz to evoke activation of the knee extensors during eccentric exercise. The indices of human skeletal muscle contractility were used to assess functional impairment following the single bout of stimulated eccentric exercise.

Methods

Subjects

Nine volunteers (five men, four women, age range 18–35 years) signed written informed consent forms prior to participation. Approval was obtained for this study from the University of Wolverhampton Ethics Committee. Each subject attended the laboratory for a familiarisation session.

Exercise bout

Subjects performed one bout of 70 electrically stimulated single leg knee extensor eccentric muscle actions, at an angular velocity of 1.05 rad·s^{-1} through a 1.57 rad range of motion. The exercise was performed on a randomly assigned leg using a Kin-Com II isokinetic dynamometer (Chattecx, Tenn., USA). Initially the subjects performed a single isometric maximal voluntary contraction (MVC) of the knee extensors, at a knee flexion angle of 1.57 rad, when seated on the isokinetic dynamometer. The maximal force achieved was recorded. The PES of the knee extensors was evoked using a Bioscience Type 200 electrical stimulator (Bioscience, Kent, UK) employing a unidirectional 0.5-ms square-wave pulse at 100 Hz via large felt covered copper electrodes soaked in warm water. Electrodes were attached proximally and distally to the knee extensor muscles with elastic strapping The stimulation voltage, at a frequency of 100 Hz, was varied until approximately 50% of the MVC force was produced using PES alone, and this voltage was used throughout the exercise. Each eccentric contraction lasted approximately 1 s, with 10-s rest between contractions.

Force data collection

Measures of muscle function were obtained from each subject using an adapted chair incorporating a strain gauge (calibrated daily) interfaced with a micro-computer. Force measurements were collected before, immediately after, and on days 1, 2, 3, 7 and 9 following the exercise. Force data from the strain gauge was amplified, sent through an analogue to digital converter, and recorded on to a computerised chart recorder. All this equipment and software was supplied by AD Instruments, Hastings, East Sussex, United Kingdom. When PES was applied to the knee extensor muscles (using the same conditions as those described above), the stimulator voltage (at 100 Hz) was always sufficient to induce at least a 50% MVC from the subject on the day of testing. Stimulator output was recorded on to a second channel of the chart recorder via the same analogue to digital converter, in parallel with the force data from the strain gauge. Both signals were sampled at 1000 Hz.

Duplicate measurements of muscle function were collected in randomized order, and consisted of:

1. Knee extension maximal isometric voluntary contraction force at a knee flexion angle of approximately 1.57 rad (MVC).
2. The MVC with superimposed myostimulation was obtained by applying a 1-s pulse of a 100-Hz stimuli during a 3-s MVC at a voltage sufficient to induce at least 50% of the subject's MVC on the day of testing (MVS). The MVS technique has been used to indicate any possible unconscious inhibition of fibre recruitment during the performance of an MVC. The MVS values may more clearly demonstrate the force generating capability of the exercised muscle.
3. The mean tetanic force produced during a 1-s pulse of 100-Hz PES.
4. The mean tetanic force produced during a 1-s pulse of 20-Hz PES.
5. The time difference between activation of stimulation and the onset of contraction and onset of relaxation, obtained from the strain gauge recording in 3. and 4. above. Contraction was assumed to have started when the recorded force was 2 standard deviations (SD) above the mean residual baseline. Relaxation was assumed to have started when the mean tetanic contraction force decreased by 2 SD. Mean values and corresponding SD values were obtained by statistical analysis of 300 data points (equivalent to 0.3 s) of the baseline and tetanic force, using MacLab Chart v3.3.3 software (AD Instruments, Hastings East Sussex, UK). It was assumed that values exceeding 2 SD values of a calculated mean represented data which was outside the expected random variation.
6. The time difference between the onset of contraction and attainment of 70% of the mean tetanic force, and the time difference between the onset of relaxation and attainment of 70% total relaxation. The 70% criteria represented an attempt to quantify the majority of both contraction and relaxation time courses.

Measurements 5. and 6. above may give an indication of any temporal disturbances in muscle excitation-contraction coupling.

Indices of muscle damage

Delayed onset muscle soreness (DOMS) was assessed daily using a questionnaire incorporating a total of eight sites: six sites on the anterior muscles of the upper leg, and two sites on the posterior muscles of the upper leg. The subjects were required to palpate the relaxed muscle and to rate the soreness on a scale of 1 (normal) to 10

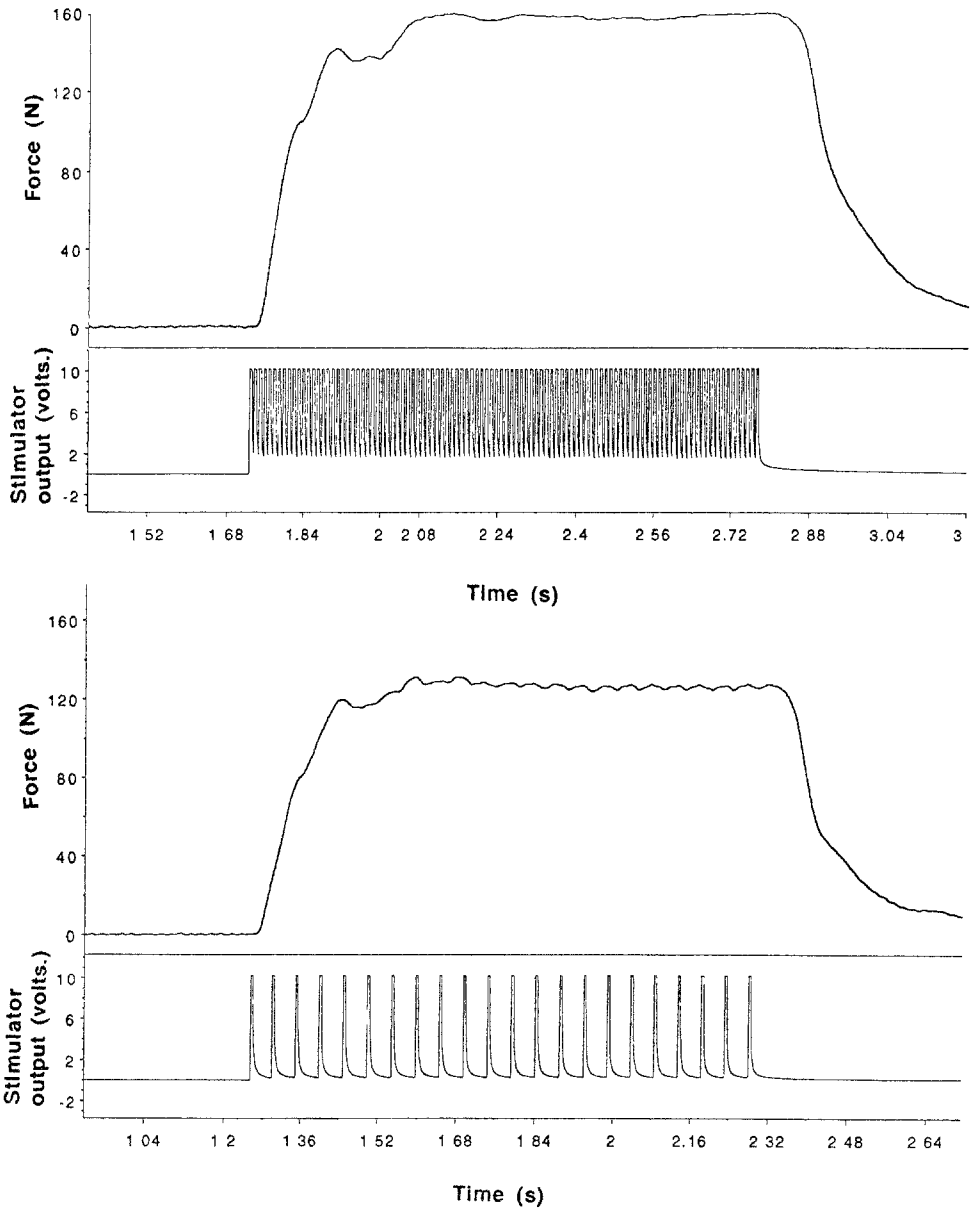

Fig. 1 **a** (*Top*) Force trace and stimulator output during percutaneous electrical myostimulation (PES) at 100 Hz. **b** (*Bottom*) Force trace and stimulator output during PES at 20 Hz

(very, very sore) for each site. The questionnaire allowed a minimal value of 8 to be recorded for a leg exhibiting no soreness, and a maximal soreness rating of 80.

A 10-ml venous blood sample was collected from the subject's antecubital fossa pre-exercise, and on days 1, 2, 3, 7 and 9 following the bout. Blood samples were allowed to clot at room temperature for 30 min prior to centrifugation at 3000 rpm for 10 min thus allowing the serum layer to be recovered. Serum samples were stored at $-20°$ C prior to analysis, in duplicate, for creatine kinase (CK) concentration using an enzymatic kit (no. 47-10, Sigma, Poole, Dorset, UK).

Statistics

Parametric data were analysed using repeated measures analysis of variance (ANOVA) with posthoc analysis using Duncan's multiple range test. Where stated, paired Student's *t*-tests were carried out. Nonparametric data were analysed using a Wilcoxon test for matched pairs.

Results

Examples of the strain gauge and stimulator output are given in Fig. 1a and b. Figure 1a shows a typical force recording using 100-Hz PES and Fig. 1b shows the typical force recording using 20-Hz PES. Complete tetanus did not occur during 20-Hz stimulation and some muscle tremor existed during baseline recording and with 100-Hz stimulation. This variability in strain gauge output necessitated the use of the 2 SD criterion as an indicator of the onset of contraction and relaxation.

The DOMS increased the day after exercise ($P < 0.05$, Wilcoxon test) and peaked on day 3 (Table 1). Soreness remained above baseline until day 7 ($P < 0.05$, Wilcoxon test) but had returned to baseline

Table 1 Upper leg muscle soreness (*DOMS*), and serum creatine kinase (CK) activity following a single bout of stimulated eccentric exercise

	Pre		Day 1		Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 9	
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
DOMS	8	0	19[a]	7	32[a]	11	33[a]	15	27[a]	14	19[a]	9	15[a]	6	11[a]	4	9	2
Serum CK activity IU·l^{-1}	87	43	247	1479	6816[c]	3951	12540[c]	7335	—		—		—		4281[b]	4818	825	971

[a] $P < 0.05$, Wilcoxon test; [b] $P < 0.05$ ANOVA with Duncan post hoc; [c] $P < 0.01$ ANOVA with Duncan post hoc

values by day 8. Serum CK activity (Table 1) increased on days subsequent to the exercise bout ($P < 0.01$, ANOVA), with the highest recorded mean value on day 3 postexercise. Pre-exercise, the mean MVC and MVS values were 360 (SEM 49) N and 356 (SEM 53) N, respectively, and these were not significantly different ($P > 0.05$). The MVC and MVS were reduced after exercise ($P < 0.01$, ANOVA) with maximal force loss 3 days after exercise (Fig. 2a). Immediately following the exercise, the mean decline in MVC was 40.9%, and on day 3 postexercise a 54% loss of force was recorded. The MVS technique consistently increased the force generation by approximately 10% postexercise, and this difference was significant on days 1, 2 and 3 postexercise ($P < 0.05$, t-test).

The ratio of forces produced at 20-Hz and 100-Hz stimulation frequencies (20:100 ratio, data shown in Fig. 2b), was reduced after exercise ($P < 0.01$, ANOVA) and showed a trend toward recovery in days following the bout. Table 2 shows the mean latency values for the onset of contraction and the onset of relaxation using both the 100-Hz and the 20-Hz frequencies. Figure 3a shows the relative time delay, from pre-exercise, between the start of stimulation and the onset of contraction. Following a 1-s pulse of 100-Hz PES, an increase in the time between the start of stimulation and the onset of contraction was observed immediately after exercise ($P < 0.01$, paired t-test between pre-exercise and postexercise), and on day 3 ($P < 0.05$, paired t-test between pre-exercise and day 3 postexercise). A similar trend was observed following a 1-s pulse of 20-Hz PES, although these differences were not significant. No significant differences were observed in the time between the end of PES and the onset of relaxation (data shown in Fig. 3b) following both 1s of 100-Hz and 1s of 20-Hz stimulation.

No significant changes were observed in the time between the onset of contraction and attainment of 70% of the total force produced during a 1-s pulse of 100-Hz stimulation, or in the time between the onset of relaxation and that taken to reach 70% of the total relaxation from the same stimulus. Similarly, no significant changes were observed in these measures during a 1-s pulse of 20-Hz stimulation.

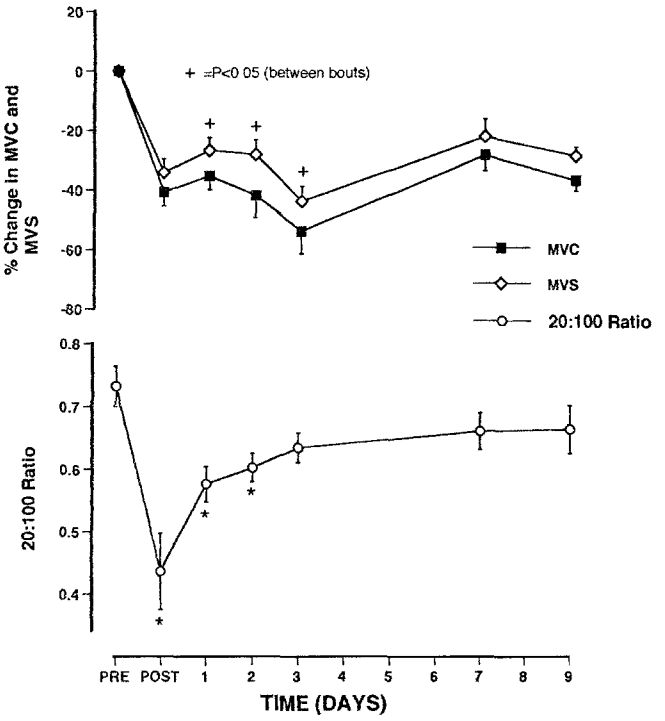

Fig. 2 **a** (*Top*) Percentage change in maximal voluntary contraction (*MVC*) and *MVC* with superimposed percutaneous electrical myostimulation (*MVS*) following a single bout of stimulated eccentric exercise (mean and SEM. *P* values using student's *t*-tests). **b** (*Bottom*) The 20:100 Hz ratios following a single bout of stimulated eccentric exercise (mean and SEM. * $P < 0.01$. ANOVA with Duncan's post hoc test)

Discussion

Muscle soreness is a characteristic of unaccustomed exercise, especially types of activity involving a large eccentric component. The delay in the occurrence of peak soreness has been shown to be 48 h postexercise in some downhill running protocols, and up to 3–4 days postexercise following high force eccentric repetitions (Rodenburg et al. 1993). Also, leakage of muscle specific enzymes into the circulation following eccentric exercise has been used to indicate altered muscle membrane

Table 2 Delay in the onset of muscle contraction and the onset of muscle relaxation using 1 s of stimulation with 100-Hz and 20-Hz frequencies, following a single bout of stimulated eccentric exercise

		Pre mean	SD	Post mean	SD	Day 1 mean	SD	Day 2 mean	SD	Day 3 mean	SD	Day 7 mean	SD	Day 9 mean	SD
100 Hz	Onset of contraction (ms)	17.0	2.1	20.8	3.2	17.8	2.4	18.3	3.5	19.5	2.7	18.8	2.4	19.4	3.7
	Onset of relaxation (ms)	35.9	4.3	33.7	6.1	38.6	9.1	36.0	6.5	34.1	6.9	38.9	14.5	36.4	6.1
20 Hz	Onset of contraction (ms)	17.0	1.8	18.9	2.5	16.7	2.1	17.4	2 0	18.3	2.7	17.5	2.7	18.6	4.3
	Onset of relaxation (ms)	57.6	3.5	59.1	4.4	58.6	2.4	58.3	2.3	58.9	3.2	59.4	3.8	56 2	5.0

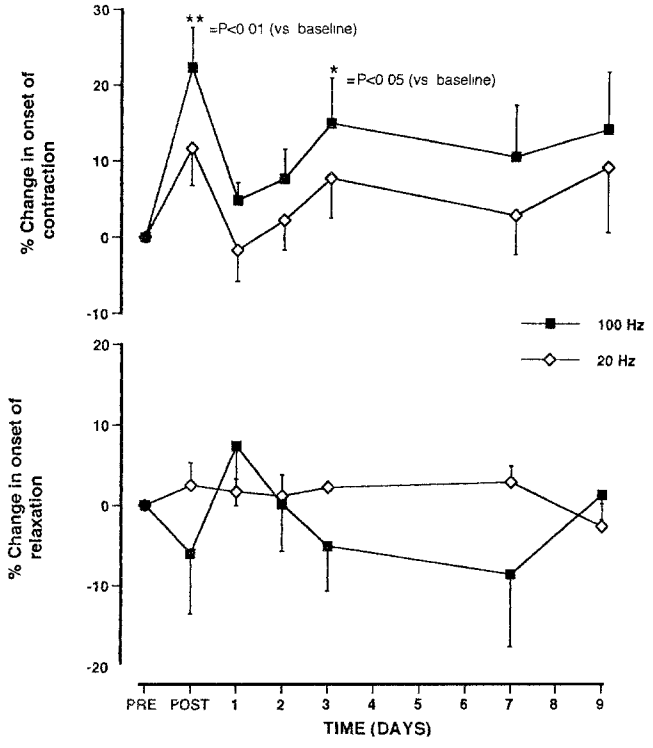

Fig. 3 **a** (*Top*) Percentage change from pre-exercise in the delay to the onset of contraction following a single bout of stimulated eccentric exercise (mean and SEM. *P* values using student's *t*-tests). **b** (*Bottom*) Percentage changes from pre-exercise in the delay to the onset of relaxation following a single bout of stimulated eccentric exercise (mean and SEM)

permeability following this type of activity. In the present study, delayed muscle soreness and elevated serum CK activities have been used as indirect markers of skeletal muscle damage following eccentric exercise.

The force loss immediately postexercise may have reflected the combined effects of muscle fatigue and exercise-induced damage. However, the continuing force loss on subsequent days may have reflected increasing ultrastructural damage which has been observed by previous authors using eccentric exercise models (Friden et al. 1983; Jones et al. 1986). A recovery in force during the days after eccentric exercise has been previously reported (Clarkson et al. 1992; Nosaka et al. 1991), although these authors have acknowledged that progressive ultrastructural damage may develop during the time that strength was recovering. The force difference between the MVC and the MVS possibly indicated the extent of inhibition of motor unit recruitment during performance of isometric strength tests in the days postexercise. The consistent 10% increase in force using the MVS compared to the MVC technique, suggested that it was not a problem in motivation, or a problem associated with soreness. The MVS contraction was still greater than the MVC contraction even when soreness was back to baseline (e.g. on day 9 postexercise). Since the force increases when using the MVS technique were only observed after the exercise, this may indicate the presence of a central limiting mechanism which may have restricted recruitment of partially damaged or vulnerable motor units.

The relative change observed in the time delay associated with the onset of contraction demonstrated a biphasic response, with an extended delay immediately postexercise and again on day 3 postexercise. The transient nature of this response may have reflected the compounding effects of different causes, where the initial increased delay could have been due to fatigue, and the delays observed on day 3 may have reflected progressive muscle fibre degeneration within a susceptible fibre population. Although the delay was significant with the 100-Hz stimulus and not with the 20-Hz stimulus, the similarities in the trends described in Fig. 3a may suggest that similar delaying mechanisms were operating with both stimuli. However, it is unclear as to why the delay with a 100-Hz stimulus was greater than the delay with a 20-Hz stimulus.

Immediately postexercise, a fatigue and/or damage-induced failure of excitation-contraction coupling may have contributed to the extended delay between the application of PES and the onset of muscle contraction. In a fatigued muscle, it has been shown that K^+ accumulation in the T tubules and interfibre spaces would increase the excitation threshold of the muscle membrane, and force production would be compromised if

accumulation of K^+ interfered with the conduction of the action potential (Jones 1981).

The extended delay in the onset of contraction following an external stimulus 3 days postexercise, may indicate that there was a damage-induced delay in excitation-contraction coupling possibly attributable to secondary degradation processes. This could have reflected neuromuscular block, reduced excitability, or defective action potential propagation over the muscle fibre sarcolemma. Failure of the neuromuscular junction to initiate an action potential may have reflected a damage-induced disturbance of the metal ion homeostasis in the neuromuscular region, causing a delay in the transmission of the action potential to the postsynaptic muscle fibre membrane i.e. a similar situation to the fatigued state. However, in the present study, the use of PES could potentially have over-ridden the neuromuscular junction, indicating that this was not the site of the increased latency of contraction. The adenosine triphosphate-dependent K^+ channels in muscle have shown an increase in activity at decreased intracellular pH, and Ca^{2+} dependent K^+ channel activity would be promoted by an elevated intracellular Ca^{2+} concentration (Fitts 1994). The K^+ efflux from the myoplasm, attributable to either accelerated ion-channel activity or cell-membrane leakage, would contribute to resting membrane potential depolarisation and to a reduced action potential amplitude, ultimately perhaps to a depolarisation block of the sarcolemma or T-tubule action potential. These mechanisms could potentially increase the temporal offset between stimuli and the onset of contraction.

A more rapid force loss in muscle stimulated at short muscle lengths has been recorded (Sacco et al. 1994), although this could not be explained in terms of neuromuscular junction failure. These authors have cited T-tubule compression and loss of lumen volume in T-tubules as the cause of the enhanced force loss at short muscle lengths. The swelling and oedema in muscle damaged by eccentric exercise found by Crenshaw et al. (1994) could compress T-tubules and present a similar intramuscular environment to that reported by Sacco et al. (1994). However, the late appearance of swelling following exercise reported by Clarkson et al. (1992) cannot account for the contraction delays observed immediately postexercise. It is proposed that although oedema within the tissue may contribute to the extended delay observed 3 days postexercise, the delay immediately postexercise cannot be attributable to oedema.

Chronic depression of the 20 : 100 ratio suggested the presence of low frequency fatigue (Edwards et al. 1977), and previous work has attributed this to a reduced tetanic intracellular Ca^{2+} concentration rather than to changes in the extracellular ion composition (Westerblad et al. 1993). Low frequency fatigue in single fibres could not be explained in terms of failure of the action potential conductance down the T-tubules, myoplasma buffering, or to changes in SR pumping. Westerblad et al. (1993) have argued that low frequency fatigue is most likely to be caused by reduced Ca^{2+} release from the SR or structural damage to one of the proteins involved in excitation-contraction coupling. However, the same authors have expressed caution in extrapolating these mechanisms to human systems.

Distention of the SR postexercise is thought to be transient (Byrd 1992), which may suggest that ion disturbance in the T-tubule is a more likely explanation of the increased delay in contraction. No significant changes were observed in the time between the onset of contraction and attainment of 70% of the mean tetanic force produced during a 1 s pulse of 100-Hz stimulation, and this may suggest that the rapid Ca^{2+} release and uptake from SR at the 100-Hz frequency were unaffected by this eccentric exercise model. Also relaxation appeared to be unaffected by the stimulated eccentric exercise model, with no significant differences recorded in the time delay between the end of stimulation and the onset of relaxation or the time to reach 70% of the total relaxation. Therefore the SR affinity for Ca^{2+} appeared unaltered, which may suggest this was not the site of failure in excitation-contraction coupling. However, variability in the relaxation data may account for the lack of any detectable trends in relaxation rates postexercise.

In the present study, stimulated eccentric exercise has been used to induce temporary damage to skeletal muscle. The chronic force loss, depressed 20 : 100 ratio, and delayed elevation of serum CK activity recorded in the present study, typify eccentric exercise-induced muscle damage. An increased delay between the start of stimulation and the onset of contraction was observed, without changes in the times for relaxation. It is suggested that the cause of this increased delay in excitation-contraction coupling originated in a slowing of the action potential propagation prior to Ca^{2+} release from the SR, and not altered SR functioning.

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