Myofibre damage in human skeletal muscle: effects of electrical stimulation *versus* voluntary contraction

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Disruption to proteins within the myofibre after a single bout of unaccustomed eccentric exercise is hypothesized to induce delayed onset of muscle soreness and to be associated with an activation of satellite cells. This has been shown in animal models using electrical stimulation but not in humans using voluntary exercise. Untrained males ($n = 8$, range 22–27 years) performed 210 maximal eccentric contractions with each leg on an isokinetic dynamometer, voluntarily (VOL) with one leg and electrically induced (ES) with the other leg. Assessments from the skeletal muscle were obtained prior to exercise and at 5, 24, 96 and 192 h postexercise. Muscle tenderness rose in VOL and ES after 24 h, and did not differ between groups. Maximal isometric contraction strength, rate of force development and impulse declined in the VOL leg from 4 h after exercise, but not in ES (except at 24 h). In contrast, a significant disruption of cytoskeletal proteins (desmin) and a rise of myogenic growth factors (myogenin) occurred only in ES. Intracellular disruption and destroyed Z-lines were markedly more pronounced in ES (40%) compared with VOL (10%). Likewise, the increase in satellite cell markers [neural cell adhesion molecule (N-CAM) and paired-box transcription factor (Pax-7)] was more pronounced in ES *versus* VOL. Finally, staining of the intramuscular connective tissue (tenascin C) was increased equally in ES and VOL after exercise. The present study demonstrates that in human muscle, the delayed onset of muscle soreness was not significantly different between the two treatments despite marked differences in intramuscular histological markers, in particular myofibre proteins and satellite cell markers. An increase in tenascin C expression in the midbelly of the skeletal muscle in both legs provides further evidence of a potential role for the extracellular matrix in the phenomenon of delayed onset of muscle soreness.

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Animal models that have used electrical stimulation to induce skeletal muscle contractions have consistently demonstrated a substantial and rapid reduction of peak isometric force, followed by a loss of cytoskeletal desmin immunostaining, a marked infiltration of inflammatory cells, a transient increase in membrane permeability and an increased expression of muscle-specific genes (Lieber *et al.* 1996, Lieber & Friden, 2002; Peters *et al.* 2003; Barash *et al.* 2004). The disruption of cytoskeletal proteins within the myofibre has been proposed to result in the development of delayed onset of muscle soreness (DOMS) observed 24–96 h postexercise (Lieber & Friden, 2002).

In contrast to these findings, it has been difficult to confirm the above described sequelae seen in animal experiments using human lower limb models (Crameri *et al.* 2004*b*; Malm *et al.* 2004; Yu *et al.* 2004). Although a significant increase in circulating creatine kinase (CK), a loss of muscle strength and a reduction in joint range of motion have been observed following repetitive eccentric muscle contractions in most human studies (Nosaka *et al.* 2002; Crameri *et al.* 2004*b*, Malm *et al.* 2004; Clarkson *et al.* 2006), little evidence is provided to link these events to intracellular alterations in human skeletal muscle (Nosaka *et al.* 2002). Firstly, the loss of the desmin protein postexercise has been limited in human models and, at most, a remodelling of desmin has been reported (Yu & Thornell, 2002; Yu *et al.* 2002, 2004; Crameri *et al.* 2004*b*). Secondly, in the lower limbs of human subjects who reported DOMS (visual analog scale 7.8 ± 1.4) after a bout of eccentric exercise, the intramuscular damage to Z-lines was moderate. These Z-line disruptions were found to lead to an increase in F-actin and desmin rather

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than to any significant loss of desmin as reported in animal models (Yu *et al.* 2004). Thirdly, in humans it has been shown that an inflammatory process in the epimysium of the exercised skeletal muscle occurs in the days following a single bout of eccentric exercise and that this, rather than or in combination with myofibrillar damage, is suggested to cause the DOMS (Clarkson *et al.* 1992; Crameri *et al.* 2004*b*; Malm *et al.* 2004). Fourthly, unaccustomed eccentric exercise was found to induce an increased expression and synthesis of extracellular matrix (ECM) collagen within skeletal muscle in the absence of myofibre damage, which was positively associated with an increase in the expression of markers for satellite cells (Crameri *et al.* 2004*b*,*c*). While it is likely that the intensity of the eccentric exercise bout will alter the intramuscular changes that are reported, no lower limb biopsy study in humans has been able to replicate the findings of the lower limb animal studies even though significant muscle tenderness has been reported. Elbow extensor muscles have been reported to experience greater muscular damage than the lower limb musculature following eccentric exercise; however, this suggestion is based on indirect markers and not on direct intramuscular alterations and therefore no interpretation can be made as to whether a different DOMS mechanism occurs in the upper body (Jamurtas *et al.* 2005).

The major question regarding the mechanism(s) leading to DOMS in the lower limbs, loss of muscle force and a rise in satellite cell activation following intensive eccentric muscle loading remains unsolved; however, from the available data, it is clear that animal and human data do not readily agree. At present it remains unknown what differences in experimental models, or perhaps inborn species differences, can explain this diversity in obtained results and conclusions. The aim of the present study was to investigate whether different ways of eliciting muscular contractions (electrical *versus* voluntary) could explain the contradictory findings in animal and human experiments.

Based on previous findings, it was hypothesized that voluntary eccentric contractions, by involving a non-uniform pattern of muscle activation (Aagaard *et al.* 2000), would distribute intramuscular strain and corresponding stress forces laterally between adjacent muscle fibres via the ECM and thereby reduce the magnitude of intracellular stress forces, resulting in very little, if any, intracellular myofibre damage. In contrast, electrical muscle stimulation, which involves highly uniform contraction of all muscle fibres in the stimulated region, was hypothesized to result in elevated intracellular strain and correspondingly elevated stress forces during eccentric contraction, in turn leading to myofibre damage and subsequent regeneration and satellite cell activation. To monitor this, cytoskeletal, myofibrillar and extracellular protein expression and damage were evaluated by electron microscopy and immunohistochemical methods, as were markers for satellite cell activation and differentiation. In addition, muscle force and tenderness were measured for a period up to 8 days following the acute exercise bout.

Methods

Subjects and experimental design

Eight healthy sedentary male subjects [age, 23 ± 1 years (range, 22–27 years); height, 182 ± 2 cm (range, 172–191 cm); weight, 83.5 ± 2.8 kg (range, 75.0–94.6 kg)] gave informed written consent to participate in this study and followed the experimental design shown in Fig. 1. All subjects were unaccustomed to high-intensity eccentric exercise and were not participating in any regular exercise regime. The Ethics Committees of the Municipalities of Copenhagen and Frederiksberg approved this study, and all procedures conformed to the Declaration of Helsinki.

Exercise protocol

Two exercise protocols were used. One leg was randomly assigned to perform a voluntary (VOL; heterogenic myofibre activation) exercise bout. A total of four subjects used their dominant kicking leg for this exercise protocol. The exercise protocol consisted of two exercise phases: (i) 100 maximal eccentric quadriceps contractions (10 sets of 10

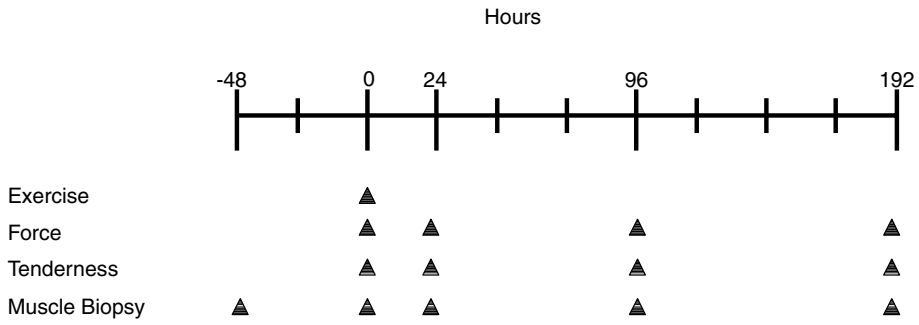

Figure 1. Time line of the protocol followed

repetitions) at slow contraction speed (knee joint angular velocity 30 deg s^{-1}); followed by (ii) 110 maximal eccentric quadriceps contractions (11 sets of 10 repetitions) at high contraction speed (180 deg s^{-1}) using an isokinetic dynamometer (KinCom KC125AP, Chattanooga group Inc., Harrison, TN, USA). Range of motion was from 90 to 10 deg (0 deg = full extension). At the completion of each eccentric contraction, the leg was immediately returned passively to the starting position (10 deg) by the motor of the dynamometer (angular velocity, 60 deg s^{-1}), and another eccentric contraction was immediately initiated. A 30 s rest phase was used between each set, and a 5 min rest period was used between exercise phases (i) and (ii). A total of 210 maximal eccentric muscle contractions were performed. A similar protocol has previously been used in our laboratory, and resulted in significant DOMS (Crameri *et al.* 2004*c*).

The contralateral leg was exercised using percutanous electrical stimulation (ES). Impulse trains (300 μs single pulse duration; 35 Hz; maximal current, 300 mA) were delivered under microprocessor control over the motor points of the vastus lateralis using a constant-bicurrent stimulator (ELFA 2000, Biofina, Odense, Denmark; Crameri *et al.* 2004*a*). After careful preparation of the skin, two electrodes (Bio-Flex, 50 mm × 89 mm, Biofina A/S, Odense, Denmark) were placed over the vastus lateralis muscle. The subjects were carefully instructed not to produce any voluntary muscle contraction during this phase of the exercise protocol. Electrical stimulation was commenced at the initiation of downward movement of the dynamometer lever arm (i.e. at 10 deg knee joint angle), and it was turned off at the end of this downward movement (i.e. at 90 deg knee joint angle). The exercise protocol consisted of two exercise phases, identical to that described above for the voluntary exercise leg. Thus, a total of 210 eccentric muscle contractions were also performed in the electrically stimulated leg. Mechanical muscle output was analysed in all eccentric loading contractions (see 'Force measurements' below). The stimulation pulse train was similar to that previously used in both able-bodied and spinal cord-injured individuals (Crameri *et al.* 2004*a*). The stimulation pulse train was chosen to reduce any unpleasant sensory input from the electrical impulse that would limit the intensity of the muscle contraction that was tolerable by the subjects.

Muscle tenderness

Muscle tenderness was assessed on both legs prior to and at 0, 24, 96 and 192 h after the exercise bout: (i) using a blunt probe applied at an identical pressure; and (ii) during a maximal voluntary contraction (MVC) performed each testing day. The subjects visually recorded the perceived pain on a visual analog scale (VAS) of 1–100 (ranging from 1, no pain to 100, extremely painful). The blunt probe was placed a minimum of 3 cm from any biopsy site to avoid any potential pain resulting from the biopsy procedure. This methodology has been used previously, and no association between previous biopsy sites and increased muscle tenderness was noted in a control leg (Crameri *et al.* 2004*c*).

Force measurements

Maximal isometric muscle strength and rate of force development. To evaluate mechanical muscle function, maximal isometric muscle strength (MVC: maximal voluntary contraction) and rate of force development (RFD) were obtained as described in detail elsewhere (Aagaard *et al.* 2002). Briefly, maximal isometric quadriceps MVC was obtained during static knee extension at a knee joint angle of 70 deg (0 deg = full knee extension) performed in the isokinetic dynamometer also used for the exercise (see 'Exercise protocol' above).

Pre-exercise recordings. After 10 min of warm-up on a cycle ergometer at 50 W, followed by a number of submaximal and maximal preconditioning trials, each subject performed four static knee extensions at maximal voluntary effort. Subjects were carefully instructed to extend the knee 'as fast and forcefully as possible'.

Postexercise recordings. Four static knee extensions were performed at maximal voluntary effort, also with the instruction to extend the knee 'as fast and forcefully as possible'. During all trials, on-line visual feedback of the dynamometer force was provided to the subjects on a PC screen. Trials with an initial counter-movement (identified by a visible drop in the force signal) were always disqualified and a new trial was performed.

The strain gauge signal and lever arm position signals of the dynamometer were sampled at 1000 Hz analog-to-digital conversion rate using an external A/D converter (DT2801-A, Data Translation, Marlboro, MA, USA). During later off-line analysis, the dynamometer signals were smoothed by a digital fourth order, zero-lag Butterworth filter, using a cut-off frequency of 15 Hz (Aagaard *et al.* 2002). Subsequently, the strain gauge signal was converted to newtons and multiplied by the individual lever arm length to calculate the moment of force. All recorded moments were corrected for the effect of gravity on the lower limb according to procedures previously described (Aagaard *et al.* 1994).

In accordance with previous procedures (Aagaard *et al.* 2002), contractile rate of force development (RFD) and impulse were determined from the trial with maximal isometric moment of force (MVC). In brief, RFD was derived as the average slope of the moment–time curve (Δmoment/Δtime) in the initial phase (0–50 ms relative to the onset of contraction) and later phase (0–100 ms) of rising muscle force. Likewise, contractile impulse was

determined as the area under the moment–time curve ($\int$moment dt) at 0–50 and 0–100 ms relative to the onset of contraction (Aagaard *et al.* 2002). Finally, relative RFD was determined as RFD normalized to MVC (RFD/MVC) in the initial phase (at 1/6 MVC) and the later phase (at 1/2 MVC) of rising muscle force (Aagaard *et al.* 2002).

Contractile muscle force, power and work during eccentric loading contractions. In order to evaluate total mechanical contractile activity in the two contraction modes, complete muscle moment–joint angle curves were recorded (100 Hz; DT2801-A, Data Translation) for every single eccentric loading trial (i.e. a total of 210 data sweeps for each leg). All moment–angle curves were analysed for peak moment, mean moment, peak power, mean power and contractile work generated in the range of motion that fulfilled the criterion of true constant angular velocity (preset velocity ± 10%; Aagaard *et al.* 2000). To reflect quadriceps contractile activity only, negative moment signal values (i.e. reflecting net knee flexor action) were set to zero in the calculation of mean moment, mean power and work.

Muscle biopsy and sampling

Muscle biopsies were obtained under local anaesthetic (1% lignocaine: AstraZeneca, Södertälje, Sweden) at a constant depth from the mid-portion of the vastus lateralis muscle in accordance with the needle biopsy technique of Bergstrom (1962). Muscle samples were taken randomly from one leg 48 h prior to the exercise bout (baseline) and from both legs 5, 24, 96 and 192 h after the exercise bout. Biopsy sites were at least 1 cm from previous biopsy sites. Previous studies in our laboratory using identical procedures have shown that repeated biopsies do not interfere with the parameters we were measuring (Crameri *et al.* 2004*b*,*c*). Immediately after removal, the biopsy sample was sectioned, with a small portion being processed for transmission electron microscopy, and the remaining sample was frozen in isopentane that had been precooled in liquid nitrogen and was stored at −70°C.

Transmission electron microscopy

Following three rinses in 0.15 M sodium cacodylate buffer ($Na(CH_3)_2AsO_2 \cdot 3H_2O$; pH 7.2), the specimens were post-fixed in 1% OsO_4 in 0.15 M sodium cacodylate buffer (pH 7.2) for 2 h. The specimens were dehydrated in a graded series of ethanol, transferred to propylene oxide and embedded in Epon (Hexion, Houston, USA) according to standard procedures. Sections were cut with a Reichert–Jung Ultracut E microtome. Ultrathin sections were collected on one-hole copper grids with Formvar (SPI, West Chester, USA) supporting membranes and stained with uranyl acetate and lead citrate. The sections were examined using a Philips CM 100 transmission electron microscope equipped with a Kodak Slow Scan camera and operated at an accelerating voltage of 80 kV. Digital images were obtained with the analySIS (SPSS, Illinois, USA) software package.

A simple, random sample of 10 digitized electron microscopy images was obtained from each biopsy. The Z-lines were counted and measured at a final magnification of 210 000 using Adobe Photoshop (Adobe Systems, USA). On average, 1687 ± 44 Z-lines were counted per biopsy sample. The sample identity was concealed from the technician until all counting was completed. The Z-lines were categorized by a trained laboratory technician as intact, disrupted (Z-lines still present but showing morphological changes from the intact Z-lines) or destroyed (Z-lines not intact), and the percentage of each was calculated.

Muscle histology

Transverse serial sections of the muscle biopsy samples were cut at 5 μm thickness using a cryostat microtome (Microm, Walldorf, Germany) at −22°C and mounted on slides. Serial sections were fixed in cold acetone and then blocked with serum. Serial sections were immunohistochemically stained with the relevant primary antibodies at their predetermined optimal dilution in 0.01 M phosphate-buffered saline (PBS; 50 mM potassium phosphate; 150 mM NaCl, pH 7.2) with 0.1% bovine serum albumin for: (i) indicators of myofibre damage, namely desmin (Zymed 18-0016, San Francisco, CA, USA), dystrophin (Novocastra NCL-DYS 2, New Castle, UK) and vimentin (DAKO M7020, Copenhagen, Denmark); (ii) inflammatory marker, CD68+ cells (DAKO M0718, USA); (iii) extracellular matrix protein, tenascin C (DAKO M0636, USA); (iv) markers of satellite cells, N-CAM (DAKO M0779, USA) and Pax-7 (Hybridoma bank, Iowa, USA); and (v) cell proliferation/differentiation markers, myogenin (DAKO M 3359, Copenhagen, Denmark) and myogenic differentiation factor (MyoD) (DAKO M3512, USA). Sections were washed with 0.01 M PBS and then incubated with a predetermined dilution of the appropriate secondary antibody (DAKO, USA) in 0.01 M PBS with 0.1% bovine serum albumin. Visualization of antibodies was performed with an ABComplex (DAKO, K0376, USA) and a fuschin substrate chromogen (DAKO, K0624, USA). Negative and positive controls were included in each staining batch for all antibodies assessed. A routine Haematoxylin and Eosin stain was also performed to assess for morphological changes.

For quantification of all histological parameters, the sample identity was concealed from the technician. For desmin-negative myofibres, dystrophin-negative myofibres and vimentin-positive myofibres, between 250 and 400 myofibres were counted from two individual sections per biopsy, and the percentage of affected

fibres was calculated. Tenascin C was quantified using Image J software (National Institutes of Health, USA) as the percentage of immunoreactivity per total biopsy area. For quantification of N-CAM-positive cells, the cell was required to be surrounded by a separate cell membrane, and all counting was performed using Image J software. Four hundred myofibres were counted from two individual sections per biopsy (200 muscle fibres per section), and the percentage of positive cells for each antibody tested was calculated as the number of positive cells/(positive cells + myonuclear number) × 100 as previously described (Kadi *et al.* 1999). The expression of Pax-7, myogenin and MyoD was calculated in the same way.

Statistics

The results for the quantification of all data are represented as means ± S.D. A repeated measures ANOVA with Dunn's *post hoc* test was performed. The level of significance was set at $P < 0.05$.

Results

Muscle tenderness

A significant increase in the perceived amount of muscle tenderness was found in both legs at 24 and 96 h after the exercise bout when compared with baseline, assessed with either the blunt probe or during an MVC. No significant difference between the two treatments was noted (Fig. 2*A*:, blunt probe; Fig. 2*B*, MVC).

Force data

Muscle moment, power and work produced during eccentric contractions. Recordings of representative eccentric loading trials are illustrated in Fig. 3. Quadriceps knee extension moment of force (peak moment, average moment in total range of motion), power (peak power, mean power) and contractile work during the 210 eccentric loading contractions were substantially greater for the voluntarily activated leg (VOL) than the electrostimulated leg (ES; $P < 0.0001$; Table 1).

Data on the mechanical moment, power and work produced during the eccentric loading trials are shown in Table 1. When all eccentric loading trials at slow speed (30 deg s^{-1}) were averaged, quadriceps moment, power and work in ES amounted to 11–13, 6–13 and 11–12%, respectively, of that generated during VOL (Table 1). When all eccentric loading trials at fast speed (180 deg s^{-1}) were averaged, quadriceps moment, power and work in ES amounted to 14–27, 19–27 and 16–17%, respectively, of that generated during VOL (Table 1).

Changes in mechanical muscle function induced by eccentric contraction. The temporal changes in contractile muscle function (MVC, RFD and impulse) induced by the eccentric loading contractions are shown in Table 2. The corresponding relative changes are shown in Fig. 4.

Maximal isometric quadriceps strength (MVC) was 16, 25 and 8% reduced in the VOL leg at 4, 24 and 96 h, respectively. In contrast, MVC remained unchanged in the ES leg, except at 24 h, when MVC decreased by 13.3% in ES (Table 2 and Fig. 4).

Rapid muscle force capacity (RFD_{50}) was 20 and 45% reduced at 4 and 24 h in VOL, respectively, but unaffected in ES (Table 2 and Fig. 4). Likewise, RFD_{100} was suppressed at 4, 24 and 96 h in VOL, with no change in ES (Table 2 and Fig. 4).

In the VOL leg, the magnitude of contractile impulse in the most initial contraction phase (IMP_{50}) was 25 and 40% reduced at 4 and 24 h (Table 2), respectively, and also reduced at 24 h when the latter phase of rising muscle force (IMP_{100}) was included (Table 2). In contrast, contractile

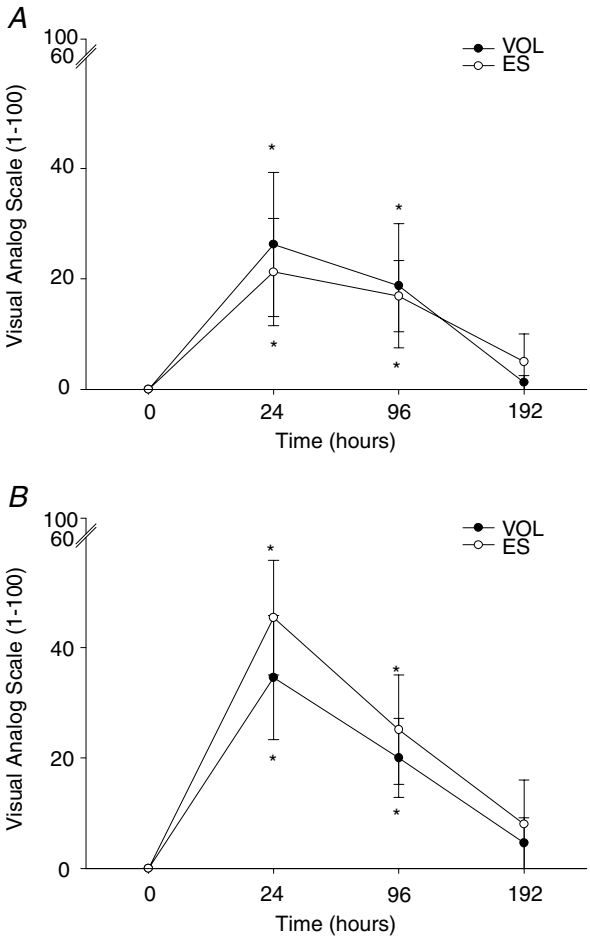

Figure 2. Muscle tenderness assessed by pressure probe placed over the vastus lateralis muscle (*A*) or during a maximal voluntary contraction (*B*)
No significant difference was found between the two treatments; however, a significant time effect was noted ($P < 0.05$). *$P < 0.05$ different from pre-exercise.

Table 1. Contractile force, power and work (group means ± S.D.) generated during the voluntary (VOL) and electrically stimulated (ES) eccentric loading contractions

	VOL leg	ES leg	ES/VOL
Slow eccentric trials (30 deg s^{-1})			
Peak moment (N m kg^{-1})	3.57 ± 0.47	0.47 ± 0.23	13.2%
Mean moment (N m kg^{-1})	2.27 ± 0.25	0.26 ± 0.14	11.5%
Peak power (W kg^{-1})	1.99 ± 0.27	0.26 ± 0.12	13.1%
Mean power (W kg^{-1})	1.18 ± 0.12	0.14 ± 0.07	5.9%
Work (J kg^{-1} per contraction)	2.78 ± 0.31	0.32 ± 0.16	11.5%
Total work (J kg^{-1})	266.7 ± 34.4	29.5 ± 15.1	11.1%
Fast eccentric trials (180 deg s^{-1})			
Peak moment (N m kg^{-1})	2.79 ± 0.46	0.75 ± 0.33	26.9%
Mean moment (N m kg^{-1})	2.29 ± 0.36	0.33 ± 0.24	14.4%
Peak power (W kg^{-1})	9.16 ± 1.60	2.45 ± 1.06	26.7%
Mean power (W kg^{-1})	5.56 ± 0.79	1.04 ± 0.75	18.7%
Work (J kg^{-1} per contraction)	2.29 ± 0.36	0.39 ± 0.30	17.0%
Total work (J kg^{-1})	237.9 ± 39.0	39.1 ± 27.8	16.4%

A total of 210 contractions were performed (100 slow + 110 fast). Values are expressed relative to body mass. VOL was greater than ES for all parameters ($P < 0.00001$). Time course patterns are shown in Fig. 3.

impulse was not significantly different in ES, except for IMP_{50} which curiously increased (by ∼10%) at 96 h.

Relative RFD was 18 and 24% reduced in the VOL leg at 24 and 48 h, respectively (Fig. 4). In contrast, relative RFD remained unchanged in ES except at 96 h, when it increased (by ∼10%).

Electron microscopy

In the VOL leg, a significant decrease in intact Z-lines was observed at 5, 24 and 192 h postexercise when compared with baseline (Fig. 5*A*). There was a corresponding significant increase in the percentage of Z-lines disrupted at 5 and 24 h postexercise (Fig. 5*B*) and a small but significant increase in fully destroyed Z-lines at 24 h postexercise (Fig. 5*C*). In the ES leg, there was a significant decrease in intact Z-lines at 5, 24 and 96 h postexercise when compared with baseline and at 96 h when compared with the VOL leg (Fig. 5*A*). The percentage of disrupted Z-lines was significantly higher at 24 and 96 h postexercise when compared with baseline and at 96 h postexercise when compared with the VOL leg (Fig. 5*B*). Similarly,

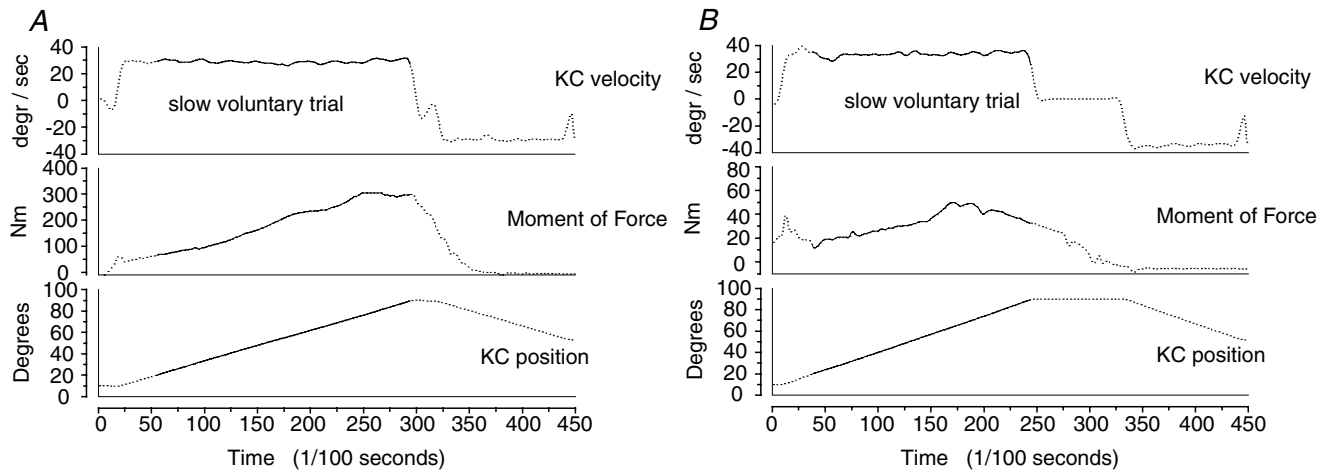

Figure 3. Recordings of representative eccentric loading trials
Knee joint angular velocity (KC velocity), quadriceps extensor moment of force (moment of force) and knee joint angle (KC position) during maximal voluntary eccentric contractions at slow contraction speeds (*A*) and when electrostimulation was applied to the vastus lateralis muscle (*B*). Continuous line traces represent the range of motion that fulfilled the criterion of true constant angular velocity. Note the differences in force scale between voluntary and stimulated trials. Power is not shown because these curves were identical in shape to the moment curves owing to the isokinetic contraction task (constancy in joint angular speed).

Table 2. Change in contractile muscle function induced by 210 eccentric muscle contractions using either VOL or ES muscle fibre activation

	0 h	4 h	24 h	96 h	192 h
MVC (N m kg^{-1})					
VOL	3.35 ± 0.34	2.83 ± 0.44*	2.56 ± 0.29*	3.31 ± 0.39	3.14 ± 0.30*
ES	3.13 ± 0.32	3.01 ± 0.14	2.71 ± 0.50	3.05 ± 0.59	2.97 ± 0.49
RFD_{50} (N m kg^{-1})					
VOL	23.1 ± 4.3	17.8 ± 5.3*	13.1 ± 7.4*	20.4 ± 5.5	18.0 ± 9.4
ES	18.4 ± 4.3	17.6 ± 5.3	18.9 ± 7.4	18.5 ± 5.5	20.5 ± 9.4
RFD_{100} (N m kg^{-1})					
VOL	18.6 ± 3.1	15.6 ± 1.9*	12.2 ± 4.0*	16.1 ± 2.9	14.0 ± 4.8*
ES	16.0 ± 4.6	15.2 ± 2.9	14.5 ± 4.8	14.9 ± 4.1	15.9 ± 4.8
IMP_{50} (N m kg^{-1} s × 10^{-3})					
VOL	26.6 ± 5.8	19.5 ± 4.8*	16.6 ± 8.2*	23.5 ± 7.1	22.1 ± 11.1
ES	20.8 ± 8.1	20.0 ± 5.2	22.8 ± 7.3	22.3 ± 11.1	24.6 ± 9.1*
IMP_{100} (N m kg^{-1} s × 10^{-3})					
VOL	109.0 ± 17.4	91.6 ± 14.6	72.4 ± 26.1*	96.1 ± 21.9	87.8 ± 32.9
ES	92.9 ± 27.8	89.9 ± 18.7	90.6 ± 27.9	92.6 ± 31.0	98.1 ± 30.2
$RFD_{1/6}$ (%MVC s^{-1})					
VOL	524 ± 96	426 ± 71*	380 ± 186*	487 ± 123	463 ± 193
ES	444 ± 116	434 ± 87	467 ± 146	494 ± 200	516 ± 168*
$RFD_{1/2}$ (%MVC s^{-1})					
VOL	524 ± 96	426 ± 71*	380 ± 186*	487 ± 123	463 ± 193
ES	562 ± 280	559 ± 223	582 ± 263	579 ± 294	674 ± 245*

Abbreviations: MVC, maximal isometric contraction strength; RFD, rate of force development; IMP_{50} and IMP_{100}, contractile impulse at 50 and 100 ms, respectively, relative to contraction onset; and $RFD_{1/6}$ and $RFD_{1/2}$, relative RFD at 1/6 and 1/2 MVC, respectively. Data were obtained at 0 h (pre-exercise), 4 h (15 min postexercise), and at 24, 96 and 192 h after obtaining the pre-exercise muscle biopsy sample. All data are expressed relative to body mass. *$P < 0.05$, post < pre-exercise. The MVC tended to decrease in ES at 24 h ($P = 0.06$).

the percentage of fully destroyed Z-lines in the ES leg was significantly greater at 24 and 96 h postexercise when compared with baseline and the VOL leg (Fig. 5*C*). Figure 6 shows representative transmission electron microscopy images of longitudinal sections taken 24 h postexercise.

Myofibre proteins

No evidence of desmin-negative, dystrophin-negative or vimentin-positive myofibres was evident in the voluntarily activated leg at any time point tested (Figs 7 and 8). Furthermore, no positive staining of CD68+ reactive macrophages was noted within the myofibres of the VOL leg (data not shown). In contrast, the stimulated leg showed gross myofibre protein changes, with significant increases in desmin-negative, dystrophin-negative and vimentin-positive myofibres. Figures 7 and 8 show changes in desmin staining only; however, the dystrophin and vimentin staining provided identical results and are therefore not shown in detail. In addition, myofibre necrosis was evident in the ES leg but not in the VOL leg, as reflected by an increase in CD68+ reactive macrophages within the desmin-negative myofibres of the ES leg (data not shown).

Extracellular matrix

The percentage of the total muscle area immunoreactive for tenascin C was significantly increased (VOL, 2.0% and ES, 1.4%) in both legs from 24 h postexercise. No significant difference was noted between the two legs at any time point measured (Fig. 9). Interestingly, two individuals showed tenascin C staining as early as 5 h postexercise in the VOL leg.

Significant increases in CD68+ reactive macrophages were noted in the endomysium and perimysium in both contraction modes. A significant difference was noted between the two groups at 192 h postexercise; however, this can be attributed to the marked muscle fibre necrosis noted in the ES leg at this time (VOL: −48 h, 0.54 ± 0.15; 4 h, 1.41 ± 0.29; 24 h, 6.36 ± 1.47*; 96 h, 5.33 ± 0.88*; 192 h, 4.52 ± 2.19; and ES: −48 h, 0.54 ± 0.15 immunoreative cells/mm^2; 4 h, 1.08 ± 0.20; 24 h, 3.26 ± 0.20; 96 h,

8.53 ± 1.96*; 192 h, 38.75 ± 10.58*†. *$P < 0.05$ different from control; †$P < 0.05$ treatment groups different).

Satellite cell markers

A significant increase in cells positive for N-CAM was found in the histological sections taken from all subjects, at 96 and 192 h postexercise in both legs (Fig. 10). In addition, the ES leg showed a significantly higher expression of N-CAM, 192 h postexercise.

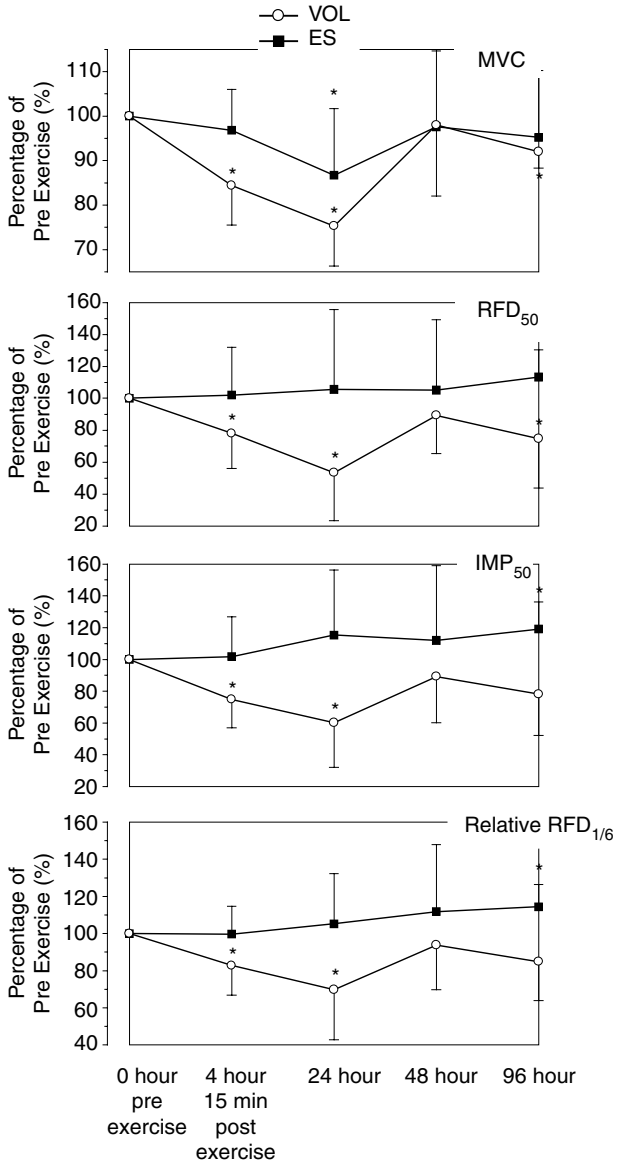

Figure 4. Relative changes in MVC and in rapid muscle strength characteristics (rate of force development, RFD; impulse, IMP; and relative RFD, $RFD_{1/6}$) in the very initial contraction phase (0–50 ms)
Data are expressed relative to pre-exercise values (100%). *$P < 0.05$ different from pre-exercise.

The pattern of Pax-7 expression did not resemble that of N-CAM expression. A significant increase in Pax-7 expression was only observed at 96 h postexercise in the VOL leg. The ES leg showed a significantly elevated expression of Pax-7 at 192 h only when compared with the control sample and the VOL leg (Fig. 11).

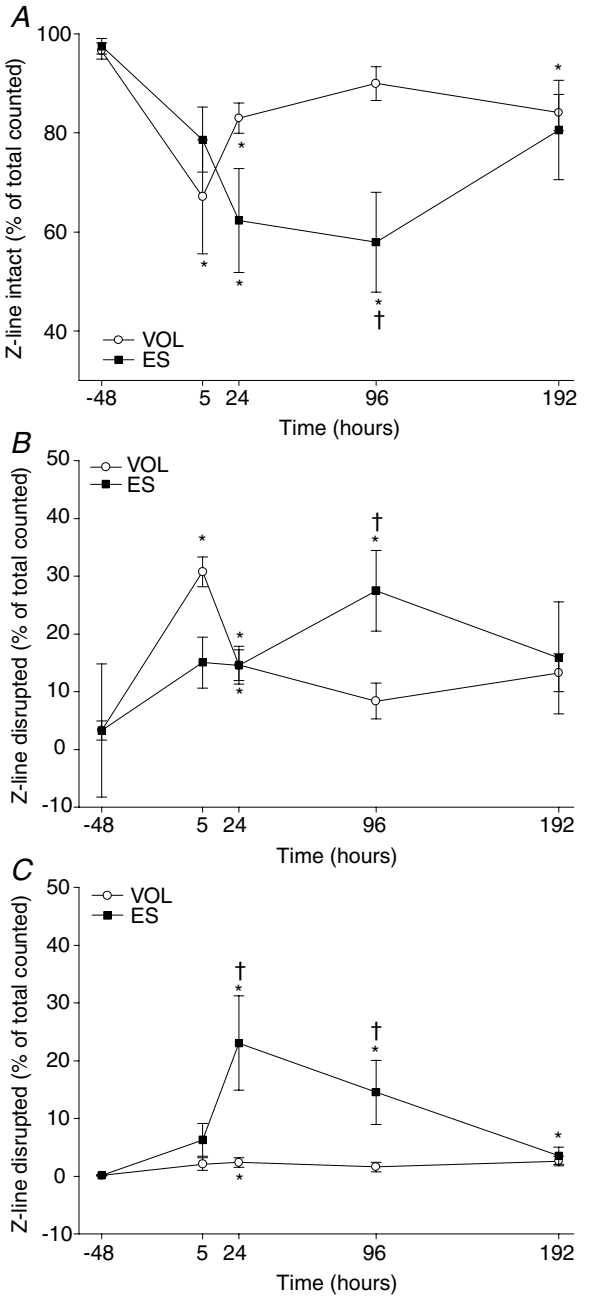

Figure 5. Significant differences in the percentage of Z-lines intact (*A*), disrupted (*B*) and destroyed (*C*) noted at various time points throughout the testing period
Most interesting was the significantly higher percentage of Z-lines destroyed in the ES leg when compared with the VOL leg and the significant increase in disrupted Z-lines in the voluntary leg at 5 and 24 h postexercise. *$P < 0.05$ different from baseline; †$P < 0.05$ treatment groups different.

No significant increase above baseline was noted in the MyoD expression in the VOL and ES leg at any time point analysed (data not shown). Similarly, no significant increase in myogenin expression was noted in the VOL leg at any time point tested. In contrast, the ES leg demonstrated a significant increase in myogenin expression at 192 h postexercise when compared with the baseline sample and the corresponding time point in the VOL leg (Fig. 12).

Discussion

The present study is the first to examine the magnitude of eccentric contraction-induced muscle damage during electrical muscle stimulation (representing that of previous animal studies) in comparison to that evoked by maximal voluntary muscle activation. Several notable findings emerged. Firstly, it was demonstrated that significant damage to the Z-lines and myofibre proteins was present in the skeletal muscle biopsies taken from the ES leg but not the VOL leg. Secondly, acute eccentric muscle loading can activate myogenic satellite cells (both ES and VOL); however, it appears that a degeneration–regeneration process is required for amplified terminal differentiation of these activated satellite cells (ES leg). Thirdly, the present study showed that despite differences in the response of the myofibre proteins to the electrical stimulation and voluntary exercise, no significant differences were noted in the expression of tensacin C within the extracellular matrix. This adds further evidence to the existing literature of a potential role for the extracellular matrix in inducing

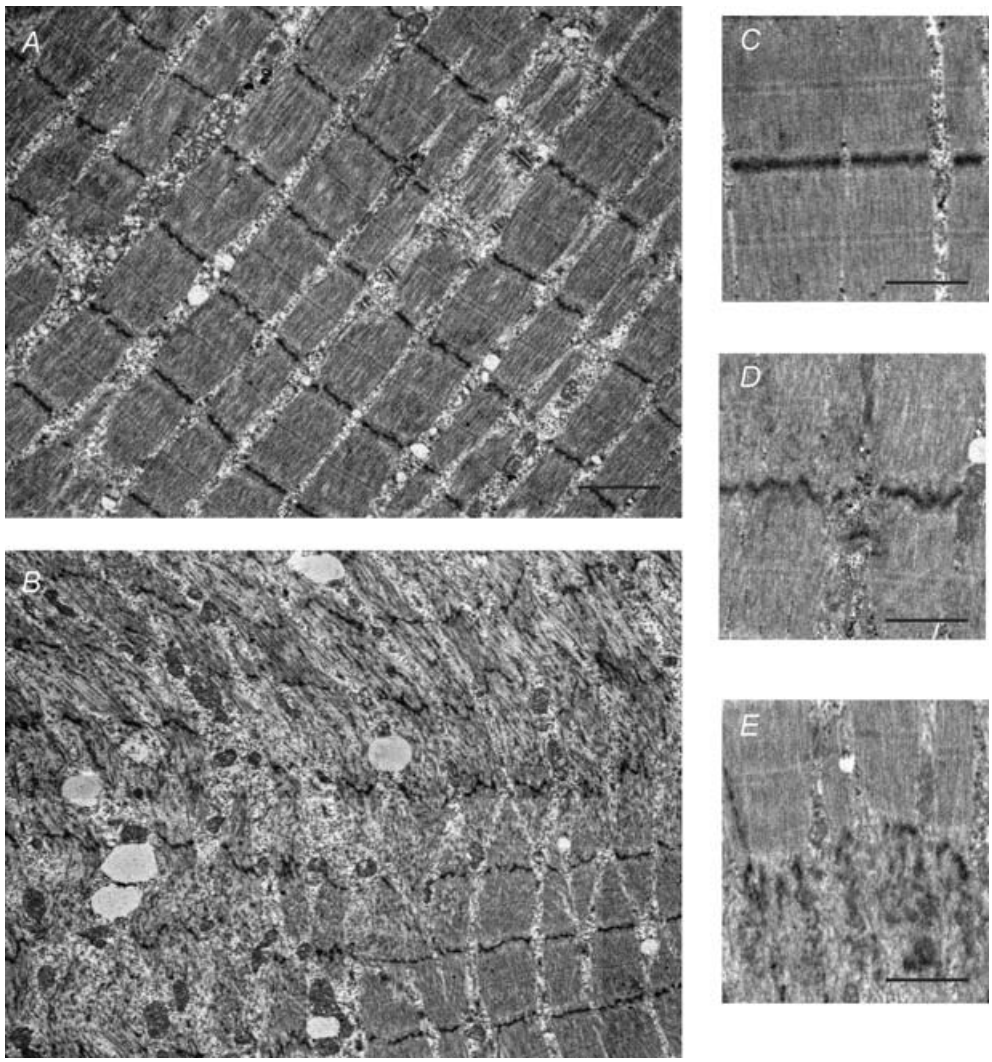

Figure 6. Transmission electron microscopical images of longitudinal sections taken 24 h postexercise
A and *B* show predominantly sarcomeric disruption in the VOL muscle (*A*) and sarcomeric damage in the ES muscle (*B*). Magnified images of Z-lines taken at 24 h postexercise show representative images of Z-lines intact (*C*); Z-lines distrupted (*D*) and Z-lines destroyed (*E*). Scale bar for *A* and *B* represents 1 μm; scale bars in *C*–*E* represent 0.5 μm.

symptoms associated with the delayed onset of muscle soreness observed after unaccustomed intense eccentric exercise.

During eccentric muscle contractions, the effect that the specific muscle activation pattern has on function and recovery has not been fully established, particularly with reference to the phenomenon of DOMS. Previous animal studies have shown that following a single bout of unaccustomed eccentric exercise, disruption to the contractile proteins early in the exercise bout leads to myofibre necrosis in the ensuing days (Barash *et al.* 2002; Peters *et al.* 2003). The contractile stimulus for these studies has been based on muscle contractions elicited by use of artificial electrical stimulation (ES). While the recruitment pattern was not determined in the present study, a plethora of research has suggested that the myofibre recruitment pattern is significantly different between the electrical stimulation-induced contractions and those produced during a voluntary physiological muscle contraction. It is believed that electrical stimulation either reverses the recruitment pattern of the myofibre types or produces a non-selective, spatially fixed and temporally synchronous stimulation of the myofibres (Kim *et al.* 1995; Gregory & Bickel, 2005). In contrast, during voluntary contractions Henneman's size principle of voluntary motor unit recruitment is followed (Gregory & Bickel, 2005). It has previously been suggested that animal studies may not correlate well to those in humans, since the amount of exercise is typically more severe than that seen in human studies (Wackerhage, 2003). An additional confounding factor is the potential activation of at least a proportion of type IIB fibres (type IIX fibres in humans) at the beginning of the electrical stimulation exercise bout, thereby exposing the early recruited type IIX fibres to myofibre protein disruption. Histological (myosin ATPase) and immunohistological staining (anti-myosin heavy chain (MHC) antibodies) was performed on all muscle biopsies in this study to determine whether the affected fibres were of type IIX. Unfortunately, the staining within the disrupted fibres was not consistent with the myosin ATPase stain to enable the affected fibre to be classified, and we were not confident that the epitope was intact to enable accurate determination of the fibre type through immunohistochemistry. Indications from both staining techniques did suggest a preferential type II fibre disruption, but this cannot be confirmed. It has been shown that as the type IIX fibres become exhausted of their glycogen supply, stretch-induced stiffness is increased (Madsen & Bagger, 2004). The proteins that maintain the cellular integrity of these mechanically stiff myofibres

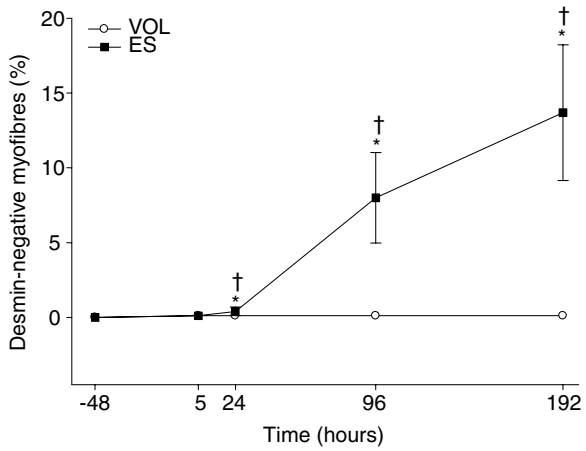

Figure 7. A significant increase in the number of desmin-negative myofibres was noted in both the ES and the VOL leg at 24, 96 and 192 h postexercise when compared with the baseline sample
$^*P < 0.05$ different from baseline; †$P < 0.05$ treatment groups different.

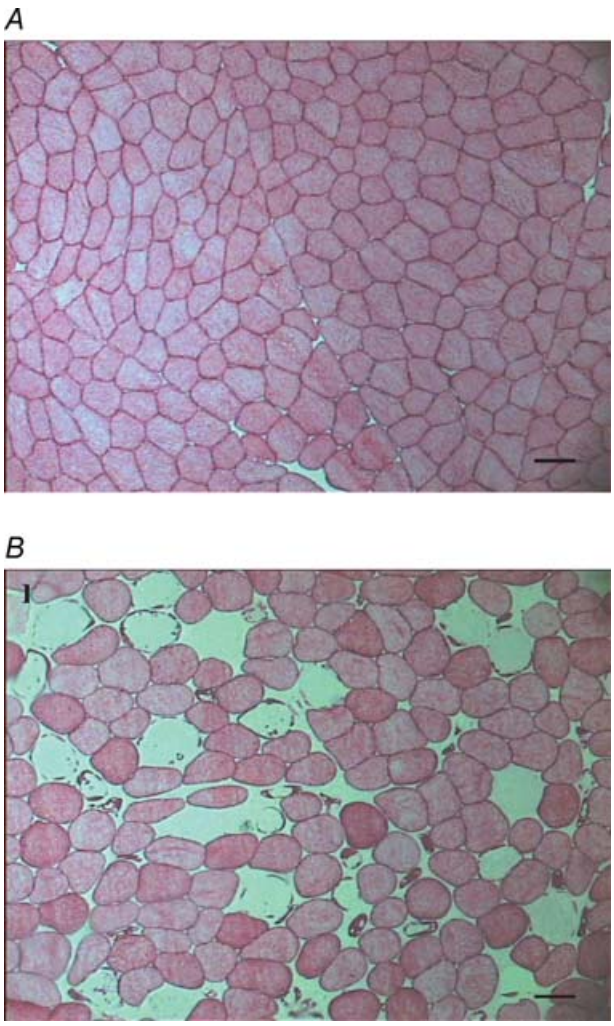

Figure 8 Detection of gross disturbance to the myofibre
No desmin-negative myofibres were noted in the VOL muscle (*A*; 192 h postexercise) at any time point measured. In contrast, there was a significant increase in the number of myofibres in the ES leg that were not immunoreactive to desmin and showed classic signs of myofibre necrosis (*B*; 192 h postexercise). Scale bars represent 50 μm.

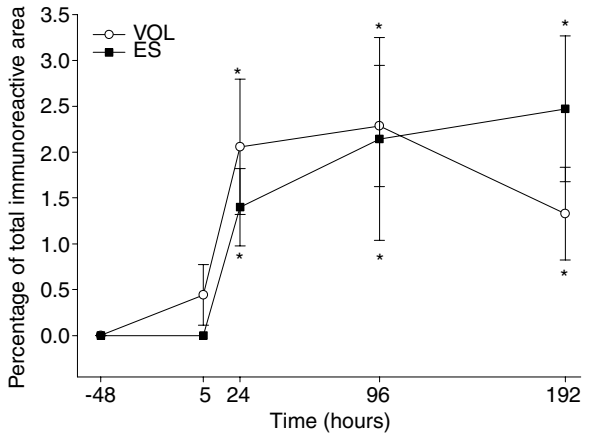

Figure 9. A significant increase in the area immunoreactive for tenascin C is seen when compared with baseline for both treatment groups
No significant difference was noted between the two groups. *$P < 0.05$ different from baseline.

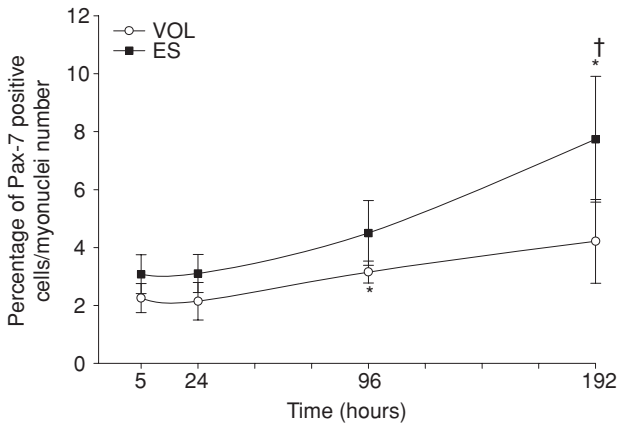

Figure 11 Expression of PAx-7 in mononuclear cells in the satellite cell position
A significant increase in the number of mononuclear cells expressing Pax-7 was noted in the VOL leg at 96 h postexercise. A significant difference was noted in the number of mononuclear cells expressing Pax-7 in the ES leg at 192 h postexercise when compared with both baseline and the VOL leg. *$P < 0.05$ different from baseline; †$P < 0.05$ treatment groups different.

subsequently are more vulnerable to myofibre damage during eccentric contractions owing to the continual stretch of the muscle (Morgan, 1990; Lieber *et al.* 1996; Proske & Morgan, 2001). Therefore, if the recruitment pattern during the electrical stimulation causes an early activation of type IIX fibres there is a greater potential for myofibre damage, as was seen in the present study in the ES muscle biopsies.

The results obtained in the present study are in keeping with previously published data using electrical stimulation in small animals, although the magnitude of the changes is somewhat different (Barash *et al.* 2002; Peters *et al.* 2003). For the human vastus lateralis muscle stimulated electrically in the present study, $13.7 \pm 4.5\%$ of the myofibres were desmin negative, with the peak observed at 192 h postexercise. In contrast, the tibialis anterior of the rat showed 1.7% desmin-negative fibres and the peak was 12 h postexercise. The different timings in the appearance of desmin-negative fibres are likely to be due to species-related differences in protein turnover, with rodents having a much higher protein synthetic rate than humans (Lewis *et al.* 1984; Miller *et al.* 2005). It is unknown why there was a difference in the number of desmin-negative fibres between the two species; however, this might result from the different protocols used, with

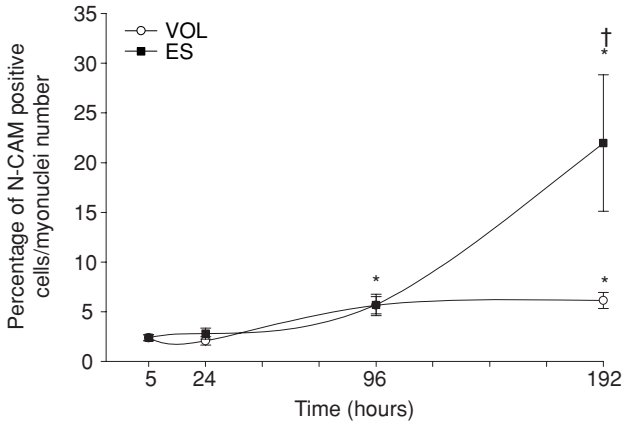

Figure 10 Expression of N-CAM in mononuclear cells in the satellite cell position
A significant increase in the number of mononuclear cells expressing N-CAM was noted in the VOL leg at 96 and 192 h postexercise. A significant difference was noted in the number of mononuclear cells expressing N-CAM in the ES leg at 192 h postexercise when compared with both baseline and the VOL leg. *$P < 0.05$ different from baseline; †$P < 0.05$ treatment groups different.

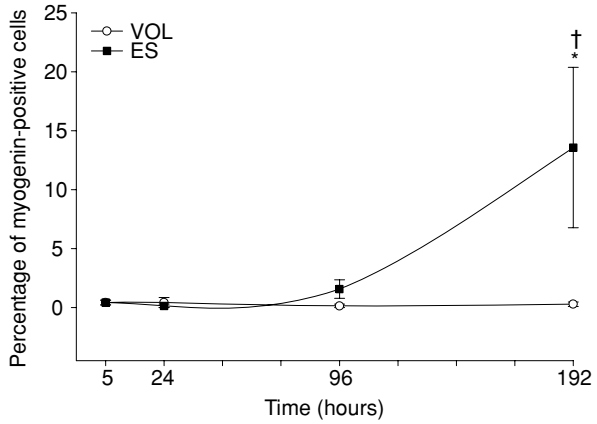

Figure 12 Expression of myogenin positive cells
Terminal differentiation of satellite cells was not noted at any time point in the VOL leg. In contrast, a significant increase in myogenin expression, hence terminal differentiation of satellite cells, was displayed in the ES leg at 192 h when compared with both baseline and the VOL leg. *$P < 0.05$ different from baseline; †$P < 0.05$ treatment groups different.

30 contractions occurring in the rats *versus* 210 contractions in our human study. Despite these differences, the available animal data are similar with regard to the ES leg while being in stark contrast to the results obtained using voluntary activation of myofibres during *in vivo* eccentric muscle exercise in humans (Aagaard *et al.* 2000).

In the present study, no gross morphology changes within the myofibre could be found in the voluntarily (VOL) activated muscle at any time point analysed. This confirms the results of previous studies that have used similar exercise protocols in humans (Yu & Thornell, 2002; Yu *et al.* 2002, 2003, 2004; Crameri *et al.* 2004*b*). These results suggest that the pattern of recruitment plays a significant role in the amount of myofibre protein damage and subsequent necrosis that occurs after a single bout of unaccustomed eccentric exercise. Similar to reports by Yu *et al.* (2004), we observed changes at the subcellular level, with a significant reduction in the number of intact Z-lines. The disruption in the Z-lines did occur earlier than previously reported by Yu *et al.* (2004); however, this is most likely due to the different collection times used in both studies. In the present study, the increases in Z-line streaming, smearing and disruption are morphologically different from the segmental muscle fibre lesions shown by Friden & Lieber (1998). In this model, in a whole cross-section of the fibre, the Z-lines can appear damaged and the plasma membrane is not intact; there is a subsequent loss of cell integrity and an invasion of inflammatory cells while proximally and distally to this site the Z-lines appear intact. In the present study, the Z-line disruptions in the VOL muscle samples were intermittently spread throughout the fibre and were not localized to specific regions within the myofibre. Most importantly, a loss of cell integrity did not occur and an inflammatory cell invasion was not evident. Consequently, the present data confirm recent findings by Yu *et al.* (2004) that there is an adaptive remodelling of the myofibril proteins rather than a segmental myofibril damage as previously shown (Friden & Lieber, 1998). A number of studies have shown that following a single bout of either eccentric or concentric exercise there is an increase in the myofibrillar protein synthesis rate in the first 24 h postexercise (Miller *et al.* 2005; Moore *et al.* 2005). Our electron microscopy data support these recent findings that after a single bout of maximal voluntary eccentric contractions there is a requirement within the muscle firstly to repair the disrupted myofibrillar proteins and possibly to increase the protein content within the muscle to strengthen it against further high-intensity exercise bouts, as shown by the increase in desmin content reported by Yu *et al.* (2004).

At present there is no consensus concerning the exact mechanism for the delayed onset of muscle tenderness and the corresponding drop in maximal force. Despite the markedly different necrotic response in the myofibres observed between the VOL and ES contractions, there was no significant difference between the legs in the level of muscle tenderness experienced, despite obvious differences in active force exertion capacity, particularly for rapid muscle force capacity (RFD, impulse). Oedema has been suggested to cause the increase in muscle tenderness; however, oedema within the myofibre itself would require an increase in myofibre cross-sectional area, which was not noted in this study. Furthermore, changes in the ultrastructure of the myofibre would be expected if an increase in intracellular fluid was present. It has previously been hypothesized that the ECM may play a role in the onset of the muscle tenderness after a single bout of eccentric exercise (Stauber *et al.* 1990; Crameri *et al.* 2004*c*; Malm *et al.* 2004). We hypothesize that the eccentric contractions induced tears within the ECM and resulted in an increase in interstitial inflammatory mediators and that these triggered the nociceptor response within the muscle. Disruptions to the ECM were noted in this study, as evidenced by an increase the number of CD68+ immunoreactive macrophages in the ECM in both legs, with a slightly higher response seen in the VOL muscle at 24 h postexercise. Both legs showed a significantly upregulated expression of CD68+ cells at 96 h postexercise, suggesting that earlier events had included a release of inflammatory mediators needed to attract the inflammatory cells to the site where the disruption had taken place. Previous reports has shown a significant increase in blood and interstitial bradykinin and/or prostaglandin in human skeletal muscle and the Achilles tendon early after a single bout of exercise (Blais *et al.* 1999; Langberg *et al.* 2002, 2003). It is known that bradykinin and other inflammatory mediators can interact with nociceptors in the muscle to trigger the pain response (Graven-Nielsen & Mense, 2001; Rosendal *et al.* 2005). We are currently investigating the release of potential mediators in this process.

Tenascin C is an adhesion-modulating ECM molecule that is not normally produced by fibroblasts in the endomysium along muscle fibres, except for those fibres close to the myotendinous junction (Järvinen *et al.* 1999, 2000). It has been shown that tenascin C expression in skeletal muscle is regulated by mechanical stress, inflammation, or a combination of the two (Mackie *et al.* 1988; Mackie & Tucker, 1999; Järvinen *et al.* 2000, 2003; Chiquet *et al.* 2004; Sarasa-Renedo & Chiquet, 2005). While the specific stimulus for *de novo* synthesis of tenascin C is unclear, the upregulation of this protein observed in the present study, in combination with the increase in the number of CD68+ macrophages, suggests that a disruption within the ECM or between the ECM and myofibres had occurred postexercise. Interestingly, in the present study, despite gross morphological changes only being present within the myofibres of the electrically stimulated leg, the ectopic tenascin C immunoreactivity increased to a similar extent in muscle biopsies taken from both legs. Furthermore, in

two subjects, detection of tenascin C was seen as early as 5 h postexercise, with the significant increase in expression commencing 24 h after the exercise bout. Tenascin C expression is known to alter the actin cytoskeletal organization in cells by modulating Rho GTPase activity and is expressed prior to wound contraction to allow for cell migration of macrophages, fibroblasts and endothelial cells, while also allowing for a greater flexibility within the ECM (Midwood *et al.* 2004). Since the ECM is known to transfer up to 70% of muscle force produced by the myofibre, disruptions in the cell-to-matrix interaction, as indicated by the expression of tenascin C, would suggest an inability of the muscle to efficiently distribute the force produced. In addition, the expression of tenascin C as early as 5 h postexercise in two subjects suggests that the protein may play a role in allowing a greater flexibility within the ECM to limit further disruption should another exercise bout occur before the ECM and/or myofibre are repaired. Both the VOL and ES legs showed significant reductions in MVC, 4 and 24 h postexercise, which also corresponds to the increase in tenascin C expression, suggesting a possible role of the ECM (not exclusively tenascin C) in the decrement of force. The role played by the disruption to the ECM in the decrement in force is, however, unlikely to be controlled by the expression of the tenascin C because force had recovered by 96 h postexercise while the tenascin C protein remained elevated 192 h postexercise. Rather, we interpret these data, in combination with the increase in the inflammatory cells within the ECM, to mean that there is a disruption to the ECM and/or ECM–cell border that reduces the lateral transfer of force. These data further strengthen the possibility that the ECM plays a role in the decrement of force and muscle tenderness. It is unlikely that force decrement and muscle tenderness are caused by the same mechanism within the ECM because the two do not follow the same time course.

Mechanical muscle function was markedly impaired in response to the eccentric voluntary contractions, while electrical stimulation only affected the relative MVC measurements. In the VOL leg, the contraction-induced suppression of rapid contractile strength characteristics was most pronounced in the very initial phase of muscle contraction (0–50 ms) compared with the later phase of rising muscle force (100 ms) and at MVC. The decrease in relative RFD further indicates that rapid force characteristics were more affected than maximal contraction strength. This preferential decrease in rapid muscle force characteristics (RFD, impulse) further suggests that the stiffness of cellular mechanotransduction pathways (serial and/or lateral) was reduced following the eccentric exercise, possibly owing to changes in cytoskeletal integrity (discussed above). The difference between the two treatment groups in the mechanical function is potentially masked by the protocol used to induce both muscle contractions. All muscle groups responsible for a lower limb eccentric contraction would have been activated in the VOL exercise conditions and would therefore have experienced DOMS. However, only those muscles lying under the electrodes would have been contracted during the ES exercise, thus sparing other muscle groups from DOMS and enabling them to participate fully in the mechanical tests postexercise. The vastus lateralis muscle has been shown to comprise about 34% of the total anatomical cross-sectional area of the quadriceps muscle (Lieber, 1992). This is also supported by the work and moment data recorded during the loading sessions in our present study, where the ES moments and contractile work were approximately 17–22% of that produced by the VOL contracted leg. Given that the mass distribution of 34%, the fibre pennation angle and the fibre length are about the same for all the quadriceps compartments, the fraction of

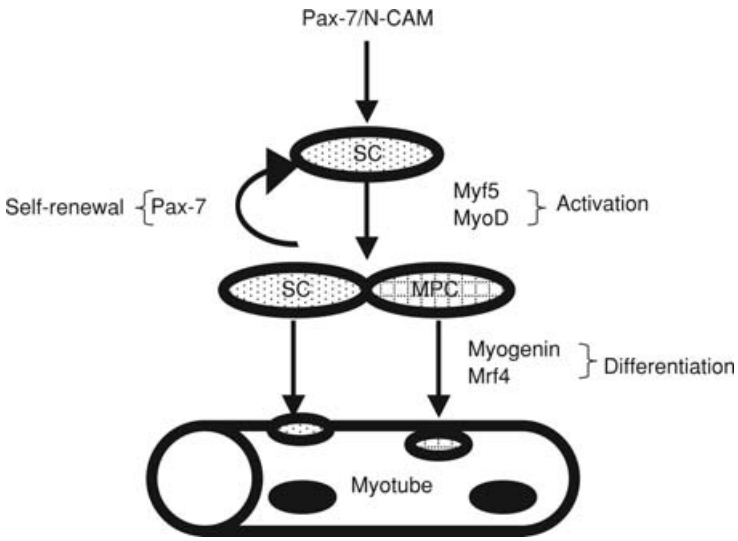

Figure 13. Schema depicting the stages of expression of various markers associated with satellite cells (SC) and myogenic progenitor cells (MPC) in human skeletal muscle
The Pax-7 and N-CAM are expressed in activated SC; however, the Pax-7 appears to be switched off in the satellite cells that continue through to terminal differentiation (MPC). The growth factors myogenic factor 5 (Myf5), MyoD, myogenin and myogenic regulatory factor 4 (Mrf4) are then expressed at varying stages throughout the activation and differentiation cycle as depicted. Schema derived from Zammit *et al.* (2004).

physiological cross-sectional area is approximately 30% for the vastus lateralis muscle. This means that the overall contractile capacity of the quadriceps probably was more severely affected in the VOL leg compared with the ES leg, and therefore the decrements during the maximal voluntary test contractions were most compromised in that leg. This would have masked the effect of DOMS from the ES leg when observing whole-limb mechanical changes.

Previously, we have shown that a single bout of high-intensity exercise was sufficient to increase the number of satellite cells expressing both N-CAM and the fetal antigen 1 proteins but that myogenin expression remained unchanged (Crameri *et al.* 2004*b*). In accordance with this, it was proposed that the satellite cells were being activated to re-enter the growth cycle but that they were not undergoing terminal differentiation (Crameri *et al.* 2004*b*). The present study confirms our previous hypothesis, by demonstrating an increase in expression of both N-CAM and Pax-7 in cells lying in the satellite cell position at 96 and 192 h postexercise. Pax-7 is a paired-box transcription factor that is critical for satellite cell biogenesis, in particular for satellite cell specification. It has been suggested that Pax-7 may be involved in maintaining proliferation and preventing precocious differentiation, while not exclusively promoting quiescence (Zammit *et al.* 2006). Pax-7 and myogenin expression have also been reported to be mutually exclusive during differentiation, with Pax-7 expression being upregulated in those satellite cells that escape differentiation and exit the cell cycle, while myogenin-expressing satellite cells undergo terminal differentiation (Olguin & Olwin, 2004; Fig. 13). In the leg that underwent voluntary exercise, we noted an increased expression at 96 and 192 h postexercise of N-CAM and Pax-7 in mononuclear cells lying in the satellite cell position. We did not note any significant increase in myogenin expression, suggesting that the satellite cells had entered the proliferative stage without progressing through to terminal differentiation. In contrast, the leg that was activated with electrical stimulation exhibited dramatic myofibre necrosis accompanied by a significant increase in mononuclear cells lying in the satellite cell position that expressed N-CAM and Pax-7 at 96 h postexercise, while all satellite cell markers (N-CAM, Pax-7 and myogenin) were increased 192 h postexercise. Of interest is the number of satellite cells at 192 h post-exercise following ES that expressed the different satellite cell markers: 22% expressed N-CAM; 7% Pax-7; and 14% expressed myogenin. These data are in keeping with previously published small animal studies showing that when muscle fibre necrosis has occurred, satellite cells are activated and a certain percentage will progress through to terminal differentiation while a second group will return to maintain the quiescent satellite cell pool. In this study, we show that while satellite cells can be activated after a single bout of intense exercise, terminal differentiation seldom occurs without the presence of myofibre necrosis.

Conclusions

In conclusion, the present study demonstrates that in the lower limbs of humans, muscle contractions derived from an artificial electrical impulse induce disruption of the myofibre proteins and result in terminal satellite cell differentiation following high-intensity eccentric contractions. The present intramuscular connective tissue responses add to the existing literature of a potential role of the ECM in some of the symptoms associated with the delayed onset of muscle soreness seen after physiological unaccustomed eccentric muscular exercise. Although acute physiological loading can activate satellite cells, only a marked disruption to the myofibre proteins, leading to inflammatory cell invasion within the myofibre, induced terminal differentiation within the affected myofibre in human skeletal musculature following high-intensity eccentric contractions in the lower limb. It is likely that the non-uniform activation of muscle that occurs with voluntary eccentric contractions protects against cytoskeletal damage, in contrast to electrically stimulated activity, and that this explains the conflicting findings between isolated animal models using electrical stimulation and human studies using voluntary exercise. The voluntary exercise therefore represents the physiological responses of skeletal muscle in the lower limb to high-intensity eccentric exercise, whereas electrical stimulation results in muscle fibre necrosis and subsequent regeneration of that tissue. It is yet to be determined whether other voluntary physiological models of eccentric exercise can induce the myofibre necrosis seen with electrical stimulation, particularly when using the upper body musculature.

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