ORIGINAL ARTICLE

The shift in muscle's length-tension relation after exercise attributed to increased series compliance

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Abstract Eccentric exercise can produce damage to muscle fibres. Here damage indicators are measured in the medial gastrocnemius muscle of the anaesthetised cat after eccentric contractions on the descending limb of the muscle's length-tension relation, compared with eccentric contractions on the ascending limb and concentric contractions on the descending limb. One damage indicator is a shift of the optimum length for peak active tension, in the direction of longer muscle lengths. The shift has been attributed to an increase in muscle compliance. It is a corollary of a current theory for the mechanism of the damage. With the intention of seeking further support for the theory, in these experiments we test the idea that other damage indicators, specifically the fall in twitch:tetanus ratio and in muscle force are due, in part, to such an increase in compliance. This was tested in an undamaged muscle by insertion of a compliant spring (0.19 mm N^{-1}) in series with the muscle. This led to a fall in tetanic tension by 17%, a shift in optimum length of 1.7 mm in the direction of longer muscle lengths and a fall in twitch tetanus ratio by 15%. The fall in tension is postulated to be due to development of non-uniform sarcomere lengths within muscle fibres. It is concluded that after a series of eccentric contractions of a muscle, the fall in force is the result of a number of interdependent

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factors, not all of which are a direct consequence of the damage process.

Introduction

Many of our everyday activities include eccentric contractions, where contracting muscles are forcibly lengthened. They are activities where we use our muscles as brakes to control and slow movements. Examples of eccentrically biased activities include skiing, horse riding and walking downhill. Other activities such as cycling and swimming do not have significant eccentric components. Eccentric exercise is distinct from other kinds of exercise in that someone who is unaccustomed to it becomes stiff and sore the day afterwards.

The reason for the stiffness and soreness is that eccentric contractions can lead to muscle damage. For a review of the subject, see Proske and Morgan (2001). What we mean by damage is the dysfunction of muscle directly attributable to the eccentric nature of the exercise, which takes a week or more to reverse. The fall in force immediately after the exercise, by itself, is an unreliable damage indicator since there will be some metabolic fatigue accompanying the exercise. Muscle can be fatigued without damaging it (Newham et al. 1983a, b; Morgan et al. 2004).

The cause of the damage is thought to be derived from the inherent instability of the descending limb of the length-tension relation for skeletal muscle (Gordon et al. 1966). This instability provides the basis for a

theory of non-uniform lengthening and disruption of sarcomeres stretched on the descending limb (Morgan 1990). An important prediction of the theory is that disrupted sarcomeres, lying in series with sarcomeres that are still functional, increase the muscle's series compliance, leading to a shift in optimum length for peak active tension in the direction of longer muscle lengths (Wood et al. 1993).

There is evidence from animal experiments in support of such a view. The shift in optimum length observed immediately after a series of eccentric contractions was seen to reverse over the next 5 h. Electron microscopic examination of muscle fixed immediately after the eccentric contractions showed many small areas of sarcomere disruption as well as a few larger areas. In muscle fixed 5 h after the contractions the small areas of disruption were no longer present and the number of larger areas was no greater than in muscles fixed immediately after the contractions. By implication, the reversal of the shift in optimum length was accompanied by many of the disrupted sarcomeres recovering their normal structure (Jones et al. 1997).

The shift in the length-tension relation has been previously used by us as a damage indicator (Whitehead et al. 2003; Morgan and Proske 2004). The most important factor determining the amount of damage from eccentric contractions is the length range over which the eccentric contractions are carried out (Talbot and Morgan 1998; Whitehead et al. 2003). Here we describe experiments designed to investigate further the length dependence of the damage, and we reassess the reliability of the various damage indicators.

The non-uniform lengthening of sarcomeres hypothesis is not universally accepted as the mechanism for the damage from eccentric exercise. In an alternative view, the first step in the series of events leading to damage is proposed to be an interference with the excitation–contraction (E–C) coupling system. This is believed to be responsible for the fall in force, changes in the muscle's force:frequency relation and the shift in optimum length (Warren et al. 2001). Here we have reassessed the fall in force and fall in twitch:tetanus ratio (force:frequency relation) as damage indicators. The hypothesis tested was that some of the changes in these measures were a consequence of the increase in muscle compliance and not due to the damage directly. This was tested by making measurements on an undamaged muscle after increasing compliance by inserting a spring between the muscle and its tendon. If our predictions were confirmed, they would add support to the proposal that the primary event in the damage process was disruption of sarcomeres, rather than interference with E–C coupling.

Methods

The experiments were carried out on four cats of both sexes with a weight range of 2.5–3.3 kg. The experiments were undertaken with approval from the Monash University Committee for Ethics in Animal Experimentation.

General anaesthesia was induced by an intraperitoneal injection of sodium pentobarbitone (40 mg kg^{-1}). The trachea was cannulated and end-tidal CO_2 concentration was monitored, to indicate the adequacy of ventilation. Rectal temperature was measured and core body temperature maintained at ∼38°C by means of a feedback-regulated heating blanket.

Depth of anaesthesia was kept adequate throughout the experiment with additional doses of anaesthetic (12 mg ml^{-1}) given when necessary through a cannula in the cephalic vein. The total supplementary dose given varied between animals and was approximately 80 mg over the 12 h period of the experiment. Depth of anaesthesia was assessed at intervals by testing reflex responses including the eye-blink, flexor withdrawal to a toe pinch and ear-flick. In addition the level of muscular tone was noted, as were the rate of respiration and end tidal CO_2 concentration. At the end of the experiments, animals were killed with an overdose of anaesthetic.

The animal was secured to a metal table with clamps on the lower jaw and thoracic vertebrae, steel pins in the pelvis and clamps on both ends of the tibia. A laminectomy was performed to expose ventral roots L7-S1, which were cut at their point of entry into the spinal cord. The left lower limb was dissected and the medial gastrocnemius muscle (MG) was separated from lateral gastrocnemius and soleus. The exposed muscle and spinal cord were covered by pools of paraffin oil in baths fashioned from skin flaps. The nerve to MG was located and dissected free from other nerves. All hind limb nerves, including nerves to hip muscles, were then cut, except for the nerve to MG.

Markers were placed on the MG tendon and the distal end of the tibia. The distance between the markers was measured with the ankle maximally flexed and the knee and hip in the position they would occupy later in the experiment. This defined the maximal physiological length of the muscle (L_{max}) and allowed muscle length to be expressed subsequently in relation to L_{max}.

The distal tendon of MG was identified and separated from the rest of the Achilles tendon, which was severed, leaving only the MG tendon attached to the calcaneum. The calcaneum was cut and a 2 mm diameter hole drilled through it. A threaded rod was passed through the hole and secured to the bone with a pair of

nuts and washers. At the other end, the rod was secured to an electromagnetic servomotor, which provided controlled muscle length changes. Interposed between the rod and servomotor were a strain gauge (Entran ELFS series, semiconductor load cell, 100 N range) to measure muscle tension.

In the experiments in which the effect of increasing compliance was tested on an undamaged muscle, a tension coil spring was inserted in series with the strain gauge. The spring was custom-made and compliance of the first spring constructed was 0.06 mm N^{-1}. When this was inserted it led to a shift of the muscle's optimum length, in the direction of longer lengths, of 0.9 mm. This was considered to be too small, given that the act of constructing the muscle's length-tension curve itself produced some, if small, shift in optimum length. Therefore a second, more compliant spring was constructed (0.19 mm N^{-1}). This produced larger shifts in optimum (1.3 mm, Fig. 3) and it was used in all subsequent experiments. When the additional compliance from the spring was not required, a metal bridge was placed across the spring to prevent it from extending. In this condition the system's compliance was 0.005 mm N^{-1}.

Experimental procedure

The tensions produced in MG by single-shock stimulation of the muscle nerve and of the collected ventral roots were measured at the start of each experiment, and the ventral roots were then divided into three parts, approximately equal in terms of the tension produced when stimulated separately. In any particular muscle, the twitch tension at optimal length (L_{opt}) differed by 10–16% between the piece generating the highest tension and the piece generating the lowest tension. Values of L_{opt} of each portion differed by between 0.9 and 2.0 mm.

For each part of the muscle the tetanus and twitch length-tension relations were measured over a length range between L_{max} and $L_{max}-20$ mm. To do that, muscle length was increased in computer-controlled steps of 2 mm amplitude and 30 s duration. At the end of each step, the muscle was stimulated via its ventral root supply, using a frequency ramp. This consisted of a train of stimulus pulses at linearly increasing frequency from 0 to 200 pulses per second (pps) over 1.5 s (Fig. 1). Peak active tension was determined as the difference between the peak tension during stimulation and the preceding passive tension at the same length. The reason for using a frequency ramp rather than a constant frequency tetanus was
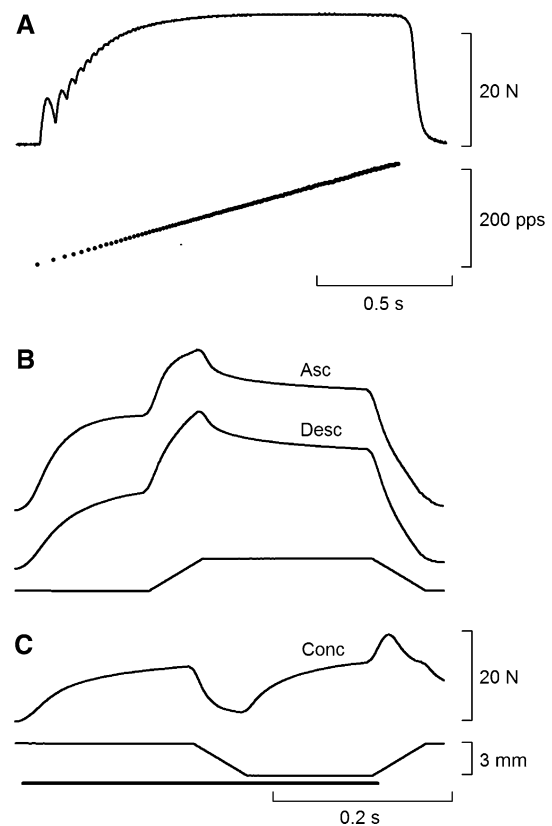

Fig. 1 Construction of length-tension curves. **a** The ventral root portion of the muscle nerve supply was stimulated with a frequency ramp. This consisted of a train of stimulus pulses of linearly increasing frequency from 0–200 pps (*lower trace*). The isometric tension recorded in the MG muscle is shown in the *upper trace*. Such trains were used in the construction of length-tension curves. Data from one experiment. **b** Example traces of length (*bottom*) and tension (*above*) during eccentric contractions. Tension traces are shown for contractions on the ascending limb (*Asc*) of the length-tension relation and for the descending limb (*Desc*). The precise length ranges are shown in Fig. 2. Isometric tensions (80 pps) before the stretches were not identical, although maximum isometric tensions (200 pps) were the same (Fig. 2 *upper*). **c** Example traces for length (*below*) and tension (*above*) during a concentric contraction. All of the data in the figure is from the same preparation

that it provided an immediate measure of twitch:tetanus ratio.

Values for peak active tension measured at each length were plotted against muscle length. A computer program provided a curve of best fit to the central data points, in the example of Fig. 2, 11 points. On other occasions fewer points were included in order to achieve a good fit in the central region of interest. A biquadratic equation was used to fit a curve to the data. The fitted curve provided values of L_{opt} and peak active tension.

During progress of the experiment, it became apparent that the act of constructing a length-tension curve, by itself, led to some fall in tension and a small shift in
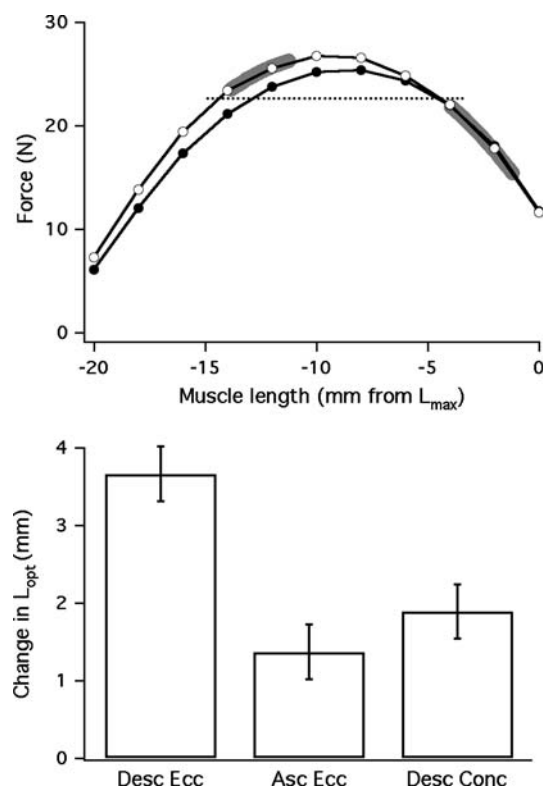
Fig. 2 Contractions on the ascending and descending limbs of the length-tension relation. *Upper panel* length-tension curves for two different portions of the MG muscle from the same preparation as the data shown in Fig. 1. One portion (*filled circles*) was subjected to 50 eccentric contractions carried out on the descending limb of the curve, over the range L_{max}−4 mm to L_{max}−1 mm (*shaded portion*). In this muscle L_{opt} was at L_{max}−7.9 mm at the start of the eccentric contractions. For the second portion (*open circles*) the eccentric contractions were carried out on the ascending limb, over the range L_{max}−14 mm to L_{max}−11 mm (*shaded portion*). The *dotted line* indicates that the isometric tension at the start of the eccentric contractions was about the same for the two sets of eccentric contractions. *Lower panel* change in optimum length (mean ± SEM) for peak active tension, given in mm, for five preparations after 50 eccentric contractions on the descending limb of the length-tension curve (*Desc Ecc*), three preparations after 50 eccentric contractions on the ascending limb of the curve (*Asc Ecc*) and three preparations for 50 concentric contractions on the descending limb (*Desc Conc*)

optimum length. In an attempt to achieve a stable baseline condition, a series of five consecutive length-tension curves was constructed and any falls in force and shift in optimum length were noted before the effects of inserting a spring or the eccentric contractions were measured.

The protocol for the eccentric contractions consisted of a series of 50 contractions at an interval of 30 s. Each contraction comprised a 0.4 s period of stimulation at 80 pps, during which the muscle was stretched by 3 mm at 50 mm s^{-1} from the initial length, the stretch starting 0.15 s after the start of the stimulation (Fig. 1). Eccentric contractions were carried out over two length

ranges, one lying on the ascending limb of the length-tension curve, the other on the descending limb (Fig. 2). Where contractions were done on the ascending limb, the length was adjusted to give an active tension at the start of the stretch which was about the same as the starting active tension on the descending limb. Total tension was, of course, not the same since there was more passive tension at the longer length. However passive stretches do not produce muscle damage (Lieber et al. 1991). For the descending limb the length range for the five muscles used in four experiments was L_{opt} + 4 mm to L_{opt} + 7 mm. For the three experiments on the ascending limb (three muscles) the starting length was between L_{opt}−4 mm and L_{opt}−7 mm (Fig. 2).

The concentric contractions on the descending limb, done in three experiments (three muscles), were a series of 50 contractions at an interval of 30 s. Each contraction consisted of stimulating the muscle for 0.4 s at 80 pps while shortening it from L_{opt} + 7 mm to L_{opt} + 4 mm at a velocity of 50 mm s^{-1} (Fig. 1).

Data collection and statistics

A PowerMac G3 computer with a NIDAQ card (National Instrument Corp., Austin TX, USA) running IgorPro (WaveMetrics, Lake Oswego OR. USA) and custom-written software were used to acquire, process and analyse all data, as well as being used to control stimulus timing and generate the waveforms controlling the muscle stretcher.

A one-way analysis of variance (ANOVA) was used to compare shifts in optimum after contractions on the descending limb of the length-tension relation compared with shifts on the ascending limb. A similar analysis compared the sizes of the force drops after the contractions at the different lengths. Where significance was indicated, a Scheffe post-hoc test was carried out. For all parameters measured, the mean and standard error of the mean (SEM) were calculated. Statistical significance was set at $P < 0.05$. The statistical program used was Data Desk (Ithaca, NY, USA)

Results

Length dependence of shift in optimum

The amount of damage produced by a series of eccentric contractions depends on the range of lengths over which the eccentric contractions are carried out (Whitehead et al. 2003). Here we have reassessed the

point by subjecting the MG muscle to eccentric contractions which were given over a length range that lay entirely on the ascending limb of the length-tension relation and this was compared in another preparation with contractions that lay entirely on the descending limb.

In a third preparation, instead of giving eccentric contractions on the descending limb, concentric contractions were used. The length ranges over which the contractions were carried out and the relationship between the remaining tension and shift in optimum length after the contractions are shown in Fig. 2.

The mean shift in optimum length after 50 eccentric contractions on the descending limb ($L_{opt} + 4$ mm to $L_{opt} + 7$ mm) was 3.7 (± 0.4 mm). This compared with a mean shift of 1.4 (± 0.4 mm) after 50 eccentric contractions on the ascending limb, beginning from a comparable starting tension. For the 50 concentric contractions on the descending limb, the mean shift was 1.9 (± 0.4 mm) (Fig. 2).

Statistical analysis carried out on the measured shifts in optimum length for the three groups of data, eccentric contractions on the descending limb, eccentric contractions on the ascending limb and concentric contractions on the descending limb showed significant differences between the groups (one-way ANOVA). Sheffe post-hoc tests showed a significant difference between the sizes of the shifts between eccentric contractions on the descending limb and eccentric contractions on the ascending limb ($P < 0.05$). Similarly there were significant differences between shifts after concentric contractions on the descending limb and eccentric contractions on the descending limb ($P < 0.05$). Shifts for eccentric contractions on the ascending limb were not significantly different from shifts after concentric contractions on the descending limb.

Inserting a spring

To test the idea that eccentric exercise leads to a shift in the optimum length of the muscle, as a result of an increase in series compliance, we have tried to model this in an undamaged muscle by insertion of a spring between the muscle tendon and tension transducer (see Methods).

During the process of inserting a spring, measuring the subsequent length-tension relation and then remeasuring the relation after removal of the spring, it became apparent that the act of stimulating the muscle to construct a length-tension curve itself produced some changes in active tension. It was therefore decided to construct a series of such curves in an attempt to achieve a stable baseline condition. In the
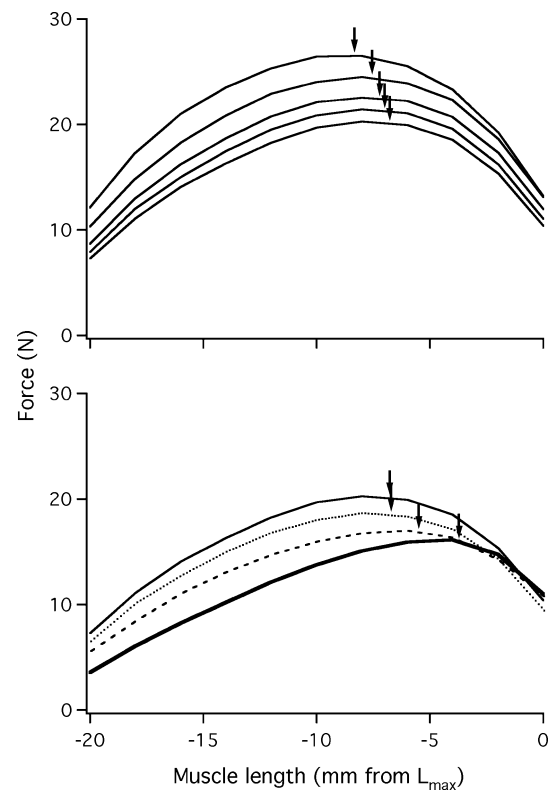

Fig. 3 Inserting a spring. *Upper panel* Five repeat measurements of the length-tension relation for a portion of MG from one experiment, showing successive drops in tension and changes in L_{opt}. The *arrows* indicate values of L_{opt} obtained from curve-fits. *Lower panel* continuing the sequence in the same experiment, the lowest of the five curves from the *upper panel* has been shown again (*upper*, *thin trace*), then a spring with a compliance of 0.19 mm N^{-1} was inserted in series with the muscle and the length-tension curve was retested (*dashed line*). After the spring was removed the curve was retested again (*dotted line*). Finally, 50 eccentric contractions were given over the range $L_{opt} + 4$ mm to $L_{opt} + 7$ mm and the length-tension relation was measured again (*bold line*)

example of Fig. 3, five successive length-tension plots led to a fall in peak tension of about 6N (24%). In addition, there was an accompanying small shift in optimum length of 1.5 mm in the direction of longer lengths.

After the effects of measuring the length-tension relation had been quantified, the experiment of inserting a spring between tendon and strain gauge was carried out using a spring with a compliance of 0.19 mm N^{-1}. Finally 50 eccentric contractions were carried out over the length range $L_{opt} + 4$ mm to $L_{opt} + 7$ mm (Fig. 3). Mean values for the falls in tension and changes in optimum length for eight muscle portions are shown in Fig. 4. By the second of the five repeated length-tension measurements, L_{opt} had increased by an average of 0.6 (± 0.2 mm SEM, $n = 8$), but by the fifth repeat the increase had declined to

only 0.2 ($\pm$ 0.3 mm) (Fig. 4). After the five repeats, the tension had fallen to 70 ($\pm$ 3%) of its initial value. With the spring in series the tension fell 17% further to 59 ($\pm$ 4%) of the initial value, accompanied by a further shift in L_{opt} of 1.7 ($\pm$ 0.3 mm) in the direction of longer lengths. This largely reversed to a mean optimum of 0.6 ($\pm$ 0.3 mm) longer than the initial value when the spring was removed. When the tension measured immediately before insertion of the spring was used as a reference value, inserting the spring led force to fall to 83% ($\pm$ 3) of its value and after removal of the spring it recovered to 93% ($\pm$ 1) of its value. Following the eccentric contractions carried out on the descending limb of the curve the tension fell 17% more to 55 ($\pm$ 1%) of the initial value, accompanied by a further shift in L_{opt} of 3.5 mm, making total shift since the start of the series 4.1 ($\pm$ 0.4 mm, Fig. 4, upper panel).

For the muscle pieces subjected to concentric contractions on the descending limb, the contractions were done first, and then the effect of the spring was tested. For the four experiments (four muscles) in which concentric contractions were used, they resulted in a mean shift of L_{opt} in the direction of longer lengths of 1.9 ($\pm$ 0.3 mm) and a reduction in tension to 85 ($\pm$ 5%) of the initial value (Fig. 2). The spring caused a further shift of 1.4 mm to a L_{opt} 3.3 ($\pm$ 0.5 mm) longer than the initial value and a further tension reduction of 16% to 71 ($\pm$ 11%) of the initial value (Fig. 4, lower panel). When the spring was removed again, L_{opt} moved back close to its pre-spring value. When expressed in terms of the tension immediately before inserting the spring, tension fell on its insertion to 82% ($\pm$ 8) of that value and on removal of the spring it measured 89% ($\pm$ 6) of the value.

Twitch:tetanus ratios

Our explanation for the damaging effects of eccentric contractions is based on mechanical events at the level of sarcomeres. In an alternative view, the initial step in the damage process is E–C uncoupling (Warren et al. 2001), see Introduction. Changes in E–C coupling manifest themselves as a change in the force:frequency relation (see, for example, Russ and Binder-Macleod 1999). A convenient measure of a change in force:frequency relation is the twitch:tetanus ratio. As E–C uncoupling proceeds, the twitch is expected to get smaller relative to tetanic tension and the ratio therefore falls. Here we propose an additional mechanism. The fall in twitch is, in part, the result of internal motion within fibres as a result of the increase in series compliance.
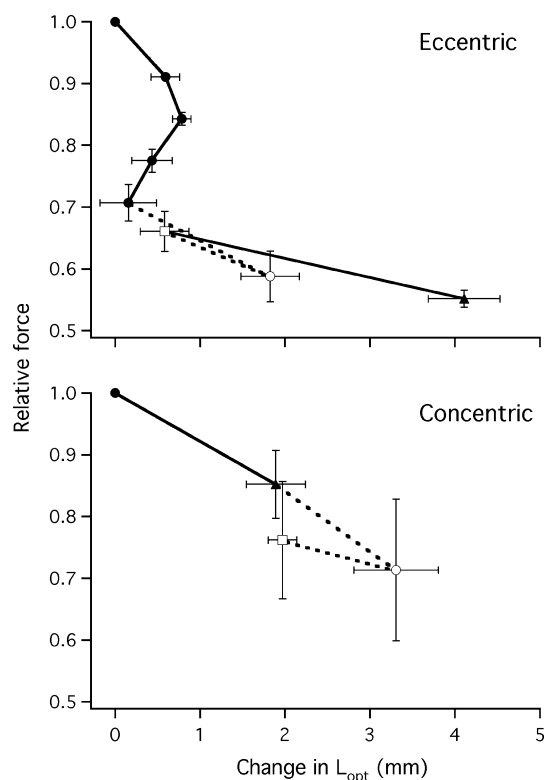
Fig. 4 Changes in optimum length and peak tension. *Upper panel* changes in optimum length (L_{opt}) and peak tension, expressed relative to the control value, during five repeat measures of the length-tension relation (*filled circles*, *bold continuous line*). Then a spring was placed in series with the muscle (*open circle*, *dashed line*), after removal of the spring (*open square*, *dashed line*) and after 50 eccentric contractions on the descending limb of the length-tension curve, also without the spring (*filled triangle*, *continuous line*). Values are means ($\pm$ SEM) from eight preparations. *Lower panel* changes in optimum length and peak tension after 50 concentric contractions on the descending limb (*solid line*, *filled symbols*), with a spring added in series with the muscle (*open circle*) and after removal of the spring (*open square*). Values are means ($\pm$ SEM) from four preparations

Since we had been using frequency ramps to measure length-tension curves, values for twitch:tetanus ratios were available for all of the above experiments. Example records of frequency ramps before and after concentric and eccentric contractions are shown in Fig. 5. Plots of twitch:tetanus ratio against muscle length indicated an increase in ratio up to a peak value which was typically reached at a length a little longer than the optimum length for peak active tension. At longer lengths twitch:tetanus ratio fell, much as did active tension (Fig. 5).

Values for twitch:tetanus ratio were measured at L_{opt} in four experiments. Average values are shown in Fig. 6, plotted against the tension remaining in the same sequences as in Fig. 4. The important observation made here was that twitch:tetanus ratio declined at a
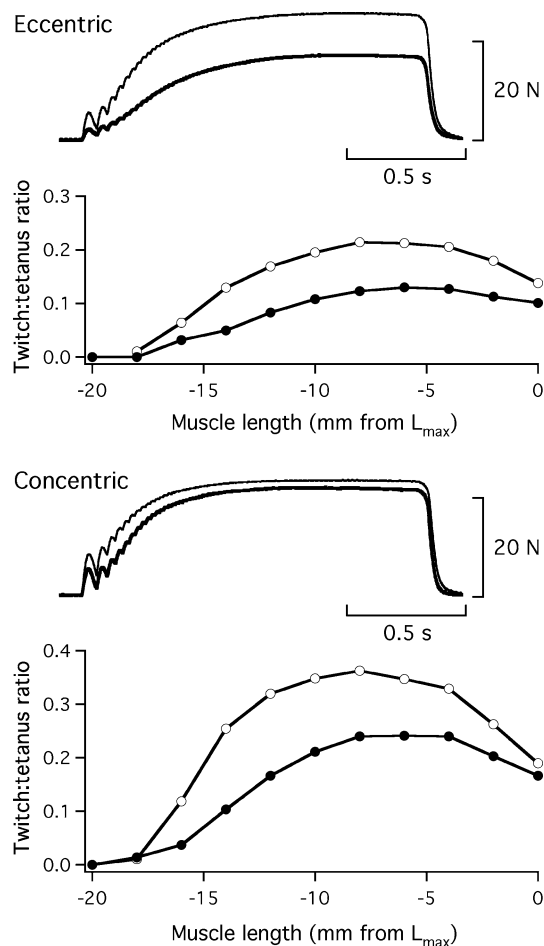
Fig. 5 The length dependence of twitch: tetanus ratios. *Upper panel* top traces, tension at optimum length in response to frequency ramp stimulation before (*thin trace*), and after (*bold trace*) 50 eccentric contractions carried out on the descending limb of the length-tension curve. *Below* are plotted twitch:tetanus ratios measured at each of a range of lengths, before the eccentric contractions (*open circles*) and after the eccentric contractions (*filled circles*). Data from one experiment. *Lower panel* tension measured at optimum length and length dependence of twitch:tetanus ratios, as shown in the upper panel, but for another MG portion from the same preparation, before and after 50 concentric contractions, carried out on the descending limb of the length-tension relation
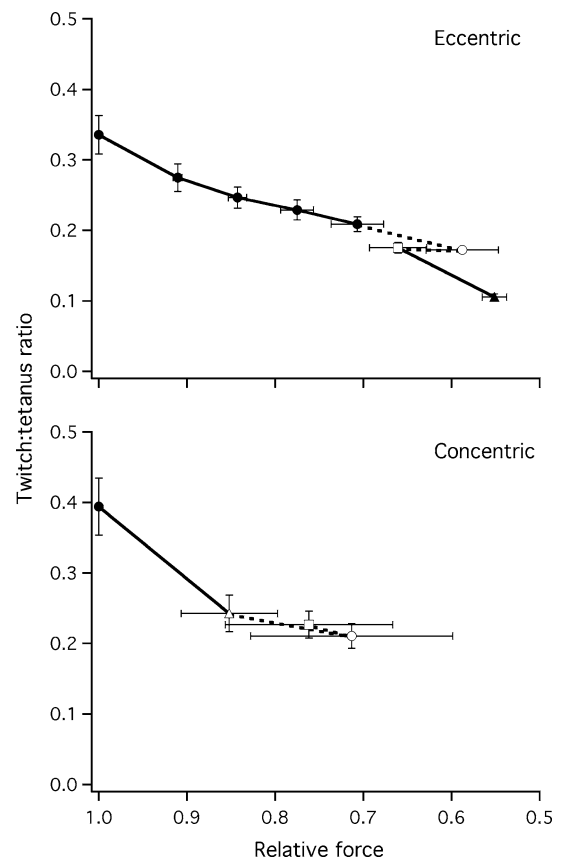
Fig. 6 Changes in twitch:tetanus ratio compared after eccentric and concentric contractions. *Upper panel* twitch:tetanus ratio measured at peak tension plotted against tension, expressed as a fraction of the control value. The *filled circles* (*continuous line*) are from five repeat measures of the length-tension relation, the *open circle* with a spring in series (*dashed line*), after removal of the spring (*open square, dashed line*) and after 50 eccentric contractions on the descending limb of the length-tension relation (*filled triangle, continuous line*). Pooled data from eight experiments. *Lower panel* similar plot from muscle pieces subjected to concentric contractions. Shows twitch:tetanus ratio at peak tension measured before (*filled circle*) and after (*open triangle*) the 50 concentric contractions on the descending limb, after insertion of a spring (*open circle, dashed line*) and after removal of the spring (*open square, dashed line*). Data from four preparations. Values in both panels are means ($\pm$ SEM) from pooled data for the same muscle portions as shown in Fig. 4

similar rate and by similar amounts regardless of whether the contractions were concentric or eccentric. In addition, increasing muscle compliance by inserting a spring lowered the twitch:tetanus ratio. So in the experiments involving eccentric contractions (Fig. 6) inserting the spring led to a fall of *t:T* ratio to 83% ($\pm$ 3) of the value before insertion. Removal of the spring left the ratio at 84% ($\pm$ 2). In the experiments using concentric contractions (Fig. 6), *t:T* ratio fell on inserting the spring to 83% ($\pm$ 8) of its pre-insertion value and it recovered to 89% ($\pm$ 6) on removal of the spring.

Discussion

The shift in length-tension relation

The central theme of this series of experiments is a study of the shift in length-tension relation of a muscle in the direction of longer muscle lengths after a series of eccentric contractions. As stated in the Introduction, our interpretation of the shift is that it is at least partly the result of an increase in series compliance of the muscle due to the presence of overstretched, disrupted

sarcomeres in myofibrils, in series with still functional sarcomeres. This interpretation is based on a theory of mechanical events triggered by the eccentric contractions and leading eventually to muscle damage and soreness (Morgan 1990).

It is unlikely that the shift in the length-tension curve is due to changes in non-muscle elements such as connective tissue or tendon. Isometric force levels for the eccentric contractions on the ascending limb of the length-tension curve (Fig. 2) were carefully adjusted to be similar to those on the descending limb, yet they effected very different sized shifts. Important additional evidence for a muscle origin of the mechanism of the shift is that the shift is progressive during repeated eccentric contractions, continuing to increment by similar amounts at a point where force levels have already fallen significantly (Wood et al. 1993). Finally, shifts in optimum measured after eccentric contractions carried out on fatigued muscle were not significantly different from shifts measured in unfatigued muscle (Morgan et al. 2004).

The available evidence supports the view that the fall in muscle force from fatigue, as distinct from damage, does not produce a shift in optimum length. In recent experiments we have shown that 200 concentric contractions of the MG muscle, arranged to cover a length range lying symmetrically about the optimum length, led to a 40% fall in force. Importantly this was not accompanied by a significant shift in optimum length (Morgan et al. 2004). We therefore believe that fatigue does not contribute to the shift in optimum. So in the experiment in which repeated length-tension curves were drawn (Fig. 3), peak tension falls as a result of fatigue from the series of contractions required to construct each curve. Of those, the contractions used to define the descending limb represent isometric contractions carried out at long muscle lengths. These are likely to produce some muscle damage (Clarkson et al. 1986) and therefore a shift in optimum.

The mechanism of the damage process associated with eccentric contractions derives from the instability of sarcomere lengths on the descending limb of the length-tension curve (Gordon et al. 1966). Active stretches carried out at long lengths, specifically at lengths beyond the optimum for peak active tension, are predicted to lead to sarcomere disruption. For a weak sarcomere, as it is lengthened it becomes ever weaker, leading to more rapid lengthening to beyond myofilament overlap until passive forces rise sufficiently to halt the movement. Then the next weakest sarcomere begins to lengthen (Morgan 1990). Some overstretched sarcomeres become disrupted on muscle

relaxation and are therefore no longer able to generate force.

We hypothesise that a point is reached where some myofibrils become sufficiently damaged by the eccentric contractions to no longer be able to contribute to the fibre's force output. The damage may be represented by a region of sarcomere disruption, the shearing of t-tubules (Takekura et al. 2001) damage to muscle membranes (Allen et al. 2005) or all of these, preventing the activation of some fibre segments. Any falls in force from incomplete activation should be recoverable with caffeine (Allen 2001).

Sarcomere instabilities are predicted not to be present at fibre lengths below the optimum so that damage levels are expected to be lower. That prediction was fulfilled, at least with respect to measurements of shift in optimum length. Eccentric contractions arranged to cover only the ascending limb, or only the descending limb and starting from similar levels of isometric tension produced more than a twofold difference in shift in optimum length (Fig. 2).

It was somewhat unexpected that eccentric contractions on the ascending limb of the curve produced a shift at all. Here a mean shift of 1.4 mm was observed. It implies that some, if small amounts of damage are produced over this length range. This was not predicted by consideration of sarcomere length-tension relations. During an active stretch on the ascending limb of the curve, if some sarcomeres were less able to resist the stretch because they had a shorter sarcomere length than others and were therefore weaker, they would be preferentially extended and in the process become stronger as they moved towards their optimum length. Therefore active stretches on the ascending limb lead to an inherently stable equilibrium, unlike the case for active stretches on the descending limb where the stretch further weakens the already weak sarcomeres, an unstable equilibrium.

Why then is there a shift in the optimum on the ascending limb? Some muscle damage must have occurred. Isometric contractions required to describe the descending limb of the test length-tension curve were likely to produce some damage (Fig. 4). In addition, it is known that motor units of MG have a wide range of length-tension relations (Brockett et al. 2002). Some of the fast-twitch units had optimum lengths 3 mm shorter than the whole muscle. Given that the active stretches on the ascending limb used in this study ended 2 mm short of the whole muscle optimum, for those fast units it would place a part of the stretch on the descending limb of their length-tension relation.

Some damage was also produced by the concentric contractions carried out on the descending limb since

here too there was a shift in optimum (Fig. 2). That was unexpected as well. Each concentric contraction consisted of an isometric contraction to get the muscle up to tension, followed by shortening. At the end of the shortening, the muscle was briefly isometric again before stimulation was stopped and it relaxed. It is known that isometric contractions at long lengths produce soreness and other indicators of damage (Clarkson et al. 1986). The most likely reason for the damage is that during the relaxation phase of the contraction, as tension falls, the tendon recoils, stretching the still partly active muscle. That represents an eccentric contraction. In other words, what were designed to be concentric contractions turned out, in practice, to contain an eccentric component. If the concentric contractions are carried out over a length range arranged to lie symmetrically about the optimum, there is no shift accompanying the fall in tension despite a 40% fall in isometric force (Morgan et al. 2004). Therefore there is no role for fatigue in the mechanism of the shift (c.f. Butterfield and Herzog 2005).

Inspection of the repeated length-tension curves for one preparation (Fig. 3) showed a progressive shift of optimum length in the direction of longer lengths. However pooling of the data from seven preparations showed that, on average, there was a small increase in optimum length from the first to the third curve followed by a small decrease during the last two (Fig. 4). Such a trend emphasises the dissociation between fatigue and shift. Given that the repeat measurements were accompanied by sizable falls in tension (30%), one explanation for this sequence of shifts relates to the fact that the largest, fatigable motor units in MG have the shortest optimum lengths (Brockett et al. 2002). These would become preferentially damaged, contributing to the initial shift in optimum in the direction of longer lengths. As they fatigued and their force output fell, their relative contribution to any shift would become less, leading to a small reversal of the whole-muscle shift (Fig. 4).

Inserting a spring

Our interpretation of the shift in optimum length after a series of eccentric contractions was that the muscle damage had increased the series compliance. The aim of this experiment was to try to model such a shift by inserting a spring in series with the tendon in an undamaged muscle, to see if it was able to reproduce some of the indicators of damage.

The average shift for all of the muscle portions where the spring was used ($n = 11$) was 1.6 (± 0.3 mm). An unexpected finding was that not only was there a shift in optimum length but also a fall in peak active tension with the spring inserted (mean of $17 \pm 3\%$). Insertion of a spring in an undamaged muscle to increase series compliance was expected to reduce the twitch tension and therefore the twitch:tetanus ratio (Fig. 6). The reason is that during a twitch there would not be sufficient time to fully stretch the extra series compliance and therefore twitch tension would fall. However the extra compliance was not expected to reduce tetanic tension. During tetanic stimulation there is sufficient time for the series compliance to become fully stretched, up to the tension of the tension generators.

One explanation for the lower tetanic tension is that the additional internal motion within the muscle produced by the extra compliance, leads some muscle fibres to undergo non-uniform length changes. The stronger, end-portions of these fibres would take up more of the shortening than segments in the middle. That is, instead of all sarcomeres being at optimum length some would be on the ascending limb of their force:length curve, others would be on the descending limb. The result would be an overall reduction in tension output. It is known that shortening contractions can lead to the development of sarcomere non-uniformities and a consequent fall in force (Julian and Morgan 1979; Morgan et al. 2000). This is quite different from the postulated non-uniform distribution of length changes during eccentric contractions. Could such internal motion lead to damage? The substantial recovery of tension after removal of the spring suggests not. The remaining tension deficit would have arisen from fatigue as a result of construction of the length-tension curve used to determine the shift.

Previous measurements of tendon compliance for cat MG gave a value of 0.06 mm N^{-1} (Walmsley and Proske 1981). This is a similar value to that for soleus (Proske and Morgan 1984). It meant that our stiffer spring was of about the same stiffness as the tendon. Therefore the spring doubled the total compliance and that led to a small shift in optimum (0.9 mm). Inserting the more compliant spring (0.19 mm N^{-1}) effectively quadrupled the compliance and it led to a larger shift in optimum (mean 1.8 mm). Since the shifts observed after the eccentric contractions were nearly twice as large again (mean 3.4 mm), the damage must have led to an overall increase in compliance of about eightfold.

If our interpretation is correct and an increase in series compliance can lead to a fall in tension in an undamaged muscle, it raises the possibility that at least part of the fall in tension seen after a series of eccentric contractions is due to the compliance change, not the damage or fatigue. This conclusion led us to re-examine

some of our earlier human data. In the experiments of Jones et al. (1997), subjects carried out a period of eccentric exercise in ankle extensor muscles by walking backwards on a forwards-moving treadmill. This led to a fall in torque as well as a shift in optimum angle for peak torque. Plotting both the fall in torque and the shift in optimum angle on the same time scale (Fig. 7) showed that one was a mirror image of the other. When a large shift was present, torque was low and when the shift began to reverse, it was accompanied by some recovery of torque. The conclusion from all of this is that we believe we have identified a new factor, an increase in muscle compliance, which is able to contribute to the fall in tension after a series of eccentric contractions.

To conclude, there are at least three mechanisms available to account for the fall in force after a series of eccentric contractions. These can be distinguished, to some extent, by their differences in time-course. The first is fatigue, that is, metabolic fatigue associated with repeated activations of the muscle. This would be expected to have fully recovered by 2 h after the exercise (Jones et al. 1997). The second factor is the shift in optimum. This is associated with sarcomere disruption, some of which is reversible, some irreversible (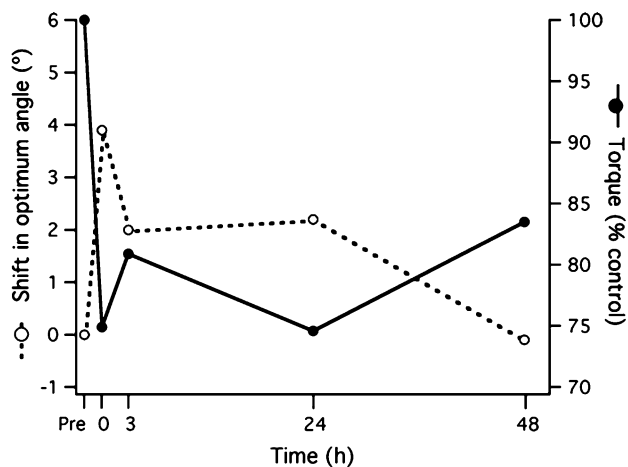Talbot and Morgan 1996; Jones et al. 1997). The shift, representing an increase in series compliance and probably associated with some, not damage-related fall in force, reverses by 2 days post-exercise (Jones et al. 1997). This is the result of recovery of normal function in some areas of disruption. Where the disruption is sufficiently extensive, over time, these myofibrils, which were initially able to generate some force, would stop working altogether and they would therefore no longer contribute to the shift in optimum. There is evidence of a delayed fall in force after eccentric exercise (Jones et al. 1989; Faulkner et al. 1993; MacIntyre et al. 1995). Finally, immediately after the eccentric contractions some parts of the muscle, myofibrils or even whole fibres, would have been damaged so severely to stop functioning straight away. Their full recovery would be expected to take a week or more (Jones et al. 1997; Brockett et al. 2001; Bowers et al. 2004).

The twitch:tetanus ratio

The main purpose of making twitch:tetanus ratio measurements was that they might provide some comment on the origin of E–C coupling changes seen after a series of eccentric contractions. Our explanation for the initial steps in the damage process from eccentric contractions is based on a series of mechanical events (Morgan 1990). In an alternative hypothesis (Warren et al. 2001), 75% of the loss in tension was attributed to E–C uncoupling. We have discussed the relative merits of the two hypotheses previously (Proske and Morgan 2001) and simply provide these additional observations to help further distinguish between them.

A well-known consequence of eccentric contractions is low-frequency depression. This has been used to support the case for E–C coupling dysfunction as the primary event in the damage process (Morgan and Allen 1999). The twitch:tetanus ratio is one measure of low frequency depression. For another measure, see Parikh et al. (2004). The main observation made here is that there is a progressive decline in twitch:tetanus ratio, regardless of whether the contractions are eccentric or concentric (Fig. 6). It suggests that a major factor determining the twitch:tetanus ratio is fatigue (see also Parikh et al. 2004). In addition, the ratio fell in an undamaged muscle when series compliance was increased by inserting a spring. It implies that any increase in compliance is also likely to reduce the ratio, independently of the damage, although the effect of increased compliance was small.

Another observation was that twitch:tetanus ratios were length-dependent and they peaked at lengths a little longer than the optimum length for peak tension (Close 1972). There was no substantial change in

Fig. 7 Changes in peak torque and optimum angle for peak torque in human ankle extensor muscles after eccentric exercise. Optimum angle (*open circles*, *dashed line*) and peak torque (*filled circles*, *continuous line*) before and at various times after a period of eccentric exercise of human ankle extensor muscles. The 2 h exercise required subjects to walk backwards, using a toe-to-heel action, on an inclined, forwards moving treadmill. Values for optimum angle and peak torque were obtained from torque-angle curves constructed using paired pulse stimulation of the tibial nerve. Measurements were made before the exercise (pre), immediately afterwards (0), at 3, 24 and 48 h. Shift in optimum angle was expressed relative to the pre-exercise value. Pre-exercise torque was assigned 100% and post-exercise values were expressed relative to that. Data redrawn from (Jones et al. 1997)

length dependence of the twitch:tetanus ratio after either eccentric or concentric contractions (Fig. 5).

One possible explanation for the shift in optimum length of the muscle after a series of eccentric contractions is that it has resulted from E-C uncoupling (for a discussion, see Proske and Morgan 2001). Here we have demonstrated, in the absence of damage, both a shift in optimum length and a change in twitch:tetanus ratio, as a result of the increase in compliance. The observations emphasise that some of the commonly measured indicators of damage from eccentric exercise can interact with one another. So damage may increase series compliance and this will affect the other measures, including force drop, low frequency depression and shift in optimum. It leads to the conclusion that no one measurement of muscle mechanical properties after eccentric exercise is likely to provide a direct measure of the amount of damage present.

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