Injury to muscle fibres after single stretches of passive and maximally stimulated muscles in mice

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1. Our purpose was to investigate the initial mechanisms responsible for contraction-induced injury. Most studies of mechanisms of contraction-induced injury have been based on observations made either shortly after many repeated contractions at the peak of fatigue, or days after, at the peak of delayed onset injury. As a result, conclusions based on these studies are complicated by interactions of mechanical and biochemical events, as well as the passage of time. We studied the initial mechanical events associated with contraction-induced injury immediately following single stretches of whole skeletal muscles of mice *in situ*.

2. We tested the hypothesis that immediately following a single stretch, the severity of contraction-induced injury is a function of both strain and average force. Consequently, the work done to stretch the muscle would be the best predictor of the magnitude of injury. Extensor digitorum longus muscles were adjusted to optimum length for force (L_o). Passive (not stimulated) and maximally activated muscles were exposed to single stretches of 10, 20, 30, 50 or 60 % strain, relative to muscle fibre length (L_f), at a rate of $2\ L_f\ \mathrm{s}^{-1}$.

3. The magnitude of injury was represented by the force deficit 1 min after the stretch expressed as a percentage of the maximum force prior to the stretch. The occurrence of injury was confirmed directly by electron microscopic analysis of the ultrastructure of muscle fibres that were fixed immediately following single stretches.

4. For active muscles, a single stretch of only 30 % strain produced a significant force deficit, whereas for passive muscles, a larger strain was required. Stretches of greater than 50 % strain resulted in greater force deficits for passive than for maximally activated muscles. For either condition, the work done to stretch the muscle was the best predictor of the magnitude of injury, accounting for 76 % of the variability in the force deficit for maximally activated muscles, and 85 % for passive muscles.

During everyday activities, contracting muscles are commonly stretched by antagonistic muscles or by external loads. Whole muscles exposed to repeated stretches while contracting show significant deficits in maximum force (McCully & Faulkner, 1985, 1986; Lieber & Fridén, 1993) as well as histological (McCully & Faulkner, 1985; Jones, Newham, Round & Tolfree, 1986; Ogilvie, Armstrong, Baird & Bottoms, 1988) and ultrastructural (Fridén, Sjöström & Ekblom, 1983; Newham, McPhail, Mills & Edwards, 1983) evidence of damage. Although demonstration of morphological damage to fibres is vital to confirm directly that an injury has occurred (McCully & Faulkner, 1985, 1986), the widely dispersed nature of the damage makes a quantitative assessment of damage to fibre morphology difficult (Ogilvie *et al.* 1988). Because of the limitations involved in a quantitative evaluation of the morphological damage, the deficit in the development of maximum isometric tetanic force is the most valid measure of the totality of the damage (Newham, McPhail, Jones & Edwards, 1983;

McCully & Faulkner, 1985; Faulkner, Jones & Round, 1989; Brooks & Faulkner, 1990; Zerba, Komorowski & Faulkner, 1990; Warren, Hayes, Lowe & Armstrong, 1993).

The time courses of the decrease in force and the damage to the morphology of fibres in the days following a protocol of injurious contractions indicate an initial injury followed by a cascade of events leading to a secondary injury (Fridén *et al.* 1983; Newham *et al.* 1983*b*; Brooks & Faulkner, 1990; Zerba *et al.* 1990). In the present study, we chose to focus on the direct association of the initial injury with specific mechanical events within the contracting muscle. High forces (Newham *et al.* 1983*a*; Fridén *et al.* 1983; McCully & Faulkner, 1985) and strain beyond optimum length for force development (Newham, Jones, Ghosh & Aurora, 1988; Faulkner *et al.* 1989) have been implicated as the mechanical factors responsible for the initiation of injury since these factors distinguish damaging lengthening contractions from those during which muscles remain at a fixed length or shorten with no damage. Studies of the

relative importance of force (McCully & Faulkner, 1986; Warren et al. 1993) and strain (Lieber & Fridén, 1993) in the induction of an injury by lengthening contractions have produced disparate conclusions. The inconsistency of the findings of these studies may be the result of differences in experimental models and protocols, in particular, the fibre type compositions of the muscles studied, the use of both in vitro and in situ preparations, the time at which the evaluation of injury was made, and the number and frequency of repeated contractions (McCully & Faulkner, 1986; Lieber & Fridén, 1993; Warren et al. 1993).

Immediately following a number of repeated contractions, the decrease in force reflects fatigue as well as injury (McCully & Faulkner, 1985; Faulkner et al. 1989; Lieber & Fridén, 1993). To focus on the mechanical factors that contribute to the initiation of injury induced by stretching muscles, fatigue must be minimized or eliminated. Warren et al. (1993) minimized fatigue in an in vitro muscle preparation by limiting the number of contractions to only five and by providing 4 min rest periods between contractions. Our approach was to develop a protocol that induced contraction-induced injury following a single stretch. Our purpose was to analyse the injury 1 min after single stretches of passive and maximally activated in situ extensor digitorum longus (EDL) muscles of mice. We tested the hypothesis that, following single stretches of passive or maximally activated muscles, the severity of the injury is a function of both the strain beyond optimum length for force development and the average force developed during the stretch, and the product of the two, the work done to stretch the muscle, provides the best prediction of the force deficit. A brief report of these results was presented to The Physiological Society (Zerba & Faulkner, 1993).

METHODS

Data were collected on young (2–6 months) male mice obtained from a specific pathogen-free mouse colony. Prior to experimentation, the mice were housed in a barrier-protected animal facility at the University of Michigan. All operations and protocols were conducted in accordance with the guidelines for the care and use of laboratory animals published by the United States Public Health Service, National Institutes of Health publication no. 85-23. For each experimental procedure, mice were anaesthetized with intraperitoneal injections of sodium pentobarbitone (80 mg kg^{-1}). Supplemental doses were administered as needed to maintain an adequate depth of anaesthesia. Whole muscle mechanics were measured in situ.

Measurements of whole muscle mechanics in situ

A small incision was made at the ankle, and the distal tendon of the EDL muscle was exposed. A nylon suture (5-0) tie was tied tightly around the tendon immediately adjacent to the distal end of the muscle fibres. The tendon was cut distal to the suture, folded back onto itself, and once again, the suture was tied tightly around it. This method was effective for preventing the tendon from slipping during stretches. Great care was taken to stabilize the

hindlimb of the mouse. The mouse was placed on a platform which was maintained at 37 °C. The entire medial surface of the experimental limb was supported by the platform and the knee was placed within a Plexiglass clamp. The medial and lateral surfaces of the knee were secured in the clamp between two sharpened screws, and the foot was affixed to the surface of the platform. The tendon of the EDL muscle was attached to the tip of the lever arm of a servomotor (Model 305, Cambridge Technology Inc., Watertown, MA, USA) which controlled the length of the muscle and measured the force developed by the muscle. The servomotor was controlled by a computer to move the lever at a constant velocity through a given displacement. The computer was also used for acquisition and storage of data. The stabilization of the limb and the attachment to the transducer provided a system of low compliance, estimated to be less than 3 μm g^{-1}.

The small regions of exposed muscle and tendon were kept moist throughout the experiment by bathing them periodically with 37 °C isotonic saline solution. The muscle was activated through stimulation of the peroneal nerve with a pair of needle electrodes inserted adjacent and parallel to the nerve. The stimulation voltage and subsequently muscle length were adjusted for maximum isometric twitch force (P_t). The optimal muscle length (L_o) for P_t is also the optimum for the development of tetanic force (Brooks & Faulkner, 1988). A frequency–force curve was produced by stimulating muscles at increasing frequencies from 10 to 350 Hz until the force reached a plateau. The maximum isometric tetanic force was achieved at ~250 Hz and was defined as P_o. With the muscle at L_o, muscle length was measured with calipers, based on well-defined anatomical landmarks determined previously by extensive dissections of mice of the same strain. Muscle fibre length (L_f) was determined by multiplying the muscle length at L_o by the L_f/L_o ratio of 0·44 (McCully & Faulkner, 1985). For the fifty-two EDL muscles used in the study, the mean L_f was 5·87 ± 0·36 mm (S.D.).

P_o was measured immediately before a single stretch and again 1 min afterward. After the final in situ force measurement, EDL muscles were removed from the mice, and the mice were killed with an overdose of the anaesthetic. The tendons were trimmed from the muscle, which was blotted dry and weighed to the nearest 0·01 mg. The mean wet mass of fifty-two experimental muscles was 11·54 ± 2·52 mg (S.D.). Total muscle fibre cross-sectional area (CSA) was calculated by dividing the wet mass of the muscle by the product of the L_f and the density of skeletal muscles, 1·06 mg mm^{-3}. The P_o was divided by the total muscle fibre CSA to obtain the specific P_o (kN m^{-2}). Prior to the single stretch of passive or active muscles, the mean value for specific P_o was 220 ± 29 kN m^{-2} (S.D.), $n = 52$. The wet mass, P_o, and specific P_o of control EDL muscles were similar to values reported by us previously (Brooks & Faulkner, 1988, 1990; Zerba et al. 1990).

Protocol for single stretches

Each muscle was exposed to a single stretch in situ with the muscle either passive (not stimulated), or maximally activated (Fig. 1). Maximally actived muscles were stimulated at the frequency which resulted in P_o. Single stretches of active muscles were initiated from the plateau of an isometric contraction (Fig. 1). Stimulation of active muscles was terminated at the end of the lengthening ramp. Both passive and active muscles were returned to L_f at the same velocity as occurred during lengthening. A lengthening velocity of 2 L_f s^{-1} was used for all stretches of both active and passive muscles. This velocity of lengthening

corresponds to ~10% of the maximum velocity of unloaded shortening (V_{max}) of the EDL muscle of the mouse adjusted from a temperature of 25 °C (Brooks & Faulkner, 1988) to 35 °C by a Q_{10} of 1·8 (Ranatunga, 1984).

Muscle strain was varied systematically. With repeated contractions, a stretch to 10% of L_f beyond L_o was sufficient to injure EDL muscle fibres in mice (McCully & Faulkner, 1985; Zerba et al. 1990). Unstimulated EDL muscles of mice that were exposed to protocols of 225 stretches of less than 20% strain showed no morphological evidence of injury and no force deficit either immediately following the repeated stretches or at 3 days (McCully & Faulkner, 1985). Consequently, in an attempt to induce an injury with a single stretch, stretches were initiated at L_f and were of 10, 20, 30, 50 or 60% strain relative to L_f for active muscles and limited to strains of greater than 20% for passive muscles. For pennate muscles that extend across two joints, such as the gastrocnemius muscle, 60% strain of muscle fibres is within the physiological range of motion. Although fibres in EDL muscles *in vivo* are typically exposed to strains of no greater than 30–35% (Ashton-Miller, He, Kadhiresan, McCubbrey & Faulkner, 1992), the longer stretches were included as a model of other muscles, as well as to make comparisons between passive and active muscles.

Forces produced by passive and maximally activated muscles during stretches were not controlled, but allowed to vary freely and measured. The peak force achieved during a contraction was measured directly from the force trace, and the average force produced was calculated by integrating the force–time curve during a single stretch and dividing the value by the duration of the ramp stretch. The contribution of average force to the magnitude of injury was examined, in addition to the peak force, since the average force is more representative of the force generating capacity of the muscle during the entire period of the stretch and the average force is related to the total work done to stretch the muscle. Work was calculated for each muscle and was normalized by the wet mass (joules kg^{-1}).

Very high peak forces were achieved during stretches of both passive and maximally activated muscles (Fig. 1). Even stretches of only 30% strain resulted in peak forces for passive muscles close to the level of P_o, and peak forces were often several fold higher than P_o for larger stretches (Fig. 2). If a muscle is held at long length, force decays rapidly resulting in steady state forces that are much lower than the peak forces observed during stretches. To determine if the stretches utilized in the present study resulted in unusually high steady state forces, the relationship between
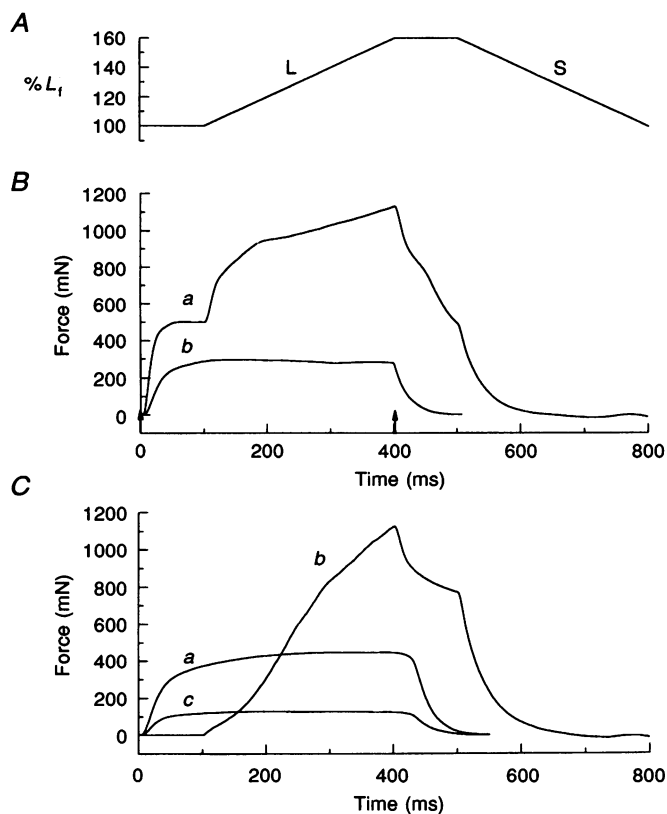

Figure 1. Representative recordings of length and force traces during single stretches of 60% strain at a velocity of 2 fibre lengths s^{-1}

A, position of the lever during the lengthening (L) and shortening (S) phase of the contraction. The displacement of the lever is expressed as a percentage of fibre length where 100% indicates the optimum fibre length for force development (L_f). *B*, force traces during the isometric and lengthening phase of a single contraction (*a*), and the maximum force during an isometric contraction (*b*) measured 1 min after lengthening. The small arrows above the time axis indicate the onset and termination of electrical stimulation. *C*, force traces during isometric contractions before (*a*) and 1 min after (*c*) the 60% stretch (*b*) of a passive muscle.

muscle length and the steady-state passive and active forces was determined in a separate group of EDL muscles, over the full range of muscle lengths used in the single stretch experiments. The muscle length was set initially at L_0. In order to obtain the steady state length–tension characteristic, the muscle was held at each length for ~3 min before the resting tension was noted. Following the measurement of resting tension, the muscle was maximally activated and the active tension was recorded. This procedure was repeated as the muscle was shortened, returned to L_0, and finally lengthened in increments separated by ~3 min. These data are shown in Fig. 2 along with the peak force data from the single stretches. The data indicate that although the experimental stretches imposed resulted in brief exposures to very high peak forces, the muscles were not near their limits of extensibility.

Evaluation of contraction-induced injury

Following a single stretch of either a passive or an active muscle, a 1 min rest period was selected as a time when fatigue would not be a factor, and the force deficit would be due exclusively to injury. The observation that no force deficit is evident 1 min following isometric contractions of similar duration to the lengthening contractions used in the present study supports our contention that fatigue did not influence the measurement of P_0 at 1 min. The force deficit, calculated as the decrease in P_0 observed 1 min after the stretch expressed as a percentage of P_0 prior to the stretch, provided the quantitative measure of the magnitude of the injury (Fig. 1).

For our single stretch protocol to be a valid model of contraction-induced injury, confirmation was required that the presence of a force deficit was accompanied by direct morphological evidence of damage to muscle fibres, and that the damage induced by a single longer stretch was comparable to that shown previously with repeated shorter stretches of contracting muscles. Light microscopic evidence of cellular damage is not observed until several days after the initial injury (McCully & Faulkner, 1985). Consequently, two control EDL muscles and seven experimental muscles were examined by electron microscopy (EM). Muscles were removed from the mice and placed in 0·1 M cacodylate-buffered Karnovsky's fixative solution (3% glutaraldahyde and 3% formaldahyde) within 3 min after a single stretch. Each muscle was divided into three parts, the belly region and two end regions. Each region was subsequently divided into two portions for cross-

and longitudinal sections and fixed for 4 h at 4 °C, pH 7·4. The muscles were then washed overnight in rinsing cacodylate buffer, post-fixed in a buffered solution of 1% osmium tetroxide, and dehydrated through a graded ethanol series. Each specimen was then propylene oxide embedded in epoxy resin and polymerized for 3 days at 45 °C and 1 day at 60 °C. Semi-thin sections (1 μm) were cut on a Sorvall MT5000 ultramicrotome (DuPont, Newton, CT, USA) and stained with 1% Toluidine Blue for light microscopic evaluation. Ultra-thin sections were obtained by cutting the specimens with a diamond knife on the same ultramicrotome and post-staining in 1% uranyl acetate and lead citrate. The sections were examined with a Philips CM-10 transmission electron microscope (Philips Electronic Instruments Co., Mahwah, NJ, USA) operating at 60 kV.

The EMs of longitudinal sections of control EDL muscles of mice showed no irregularity in fibre structure (Figs. 3A and B), whereas those of EDL muscles that presented significant force deficits exhibited similar ultrastructural abnormalities to those previously been reported to accompany contraction-induced muscle damage (Fridén et al. 1983; Newham et al. 1983b). Examples of the morphological damage observed following single stretches of 60% strain of passive and maximally activated muscles are shown in Figs 3 and 4, respectively. The total number of sarcomeres that exist in a muscle or a single fibre does not permit a complete or even representative quantitative analysis of ultrastructural damage at the level of single sarcomeres. In spite of this limitation, our observations of the ultrastructure of sarcomeres (Figs 3 and 4) indicated that the force deficits induced by single stretches were accompanied by mechanical damage to muscle fibres, and, furthermore, the mechanical injury was qualitatively similar to that observed following protocols of repeated stretches of contracting whole muscles of human beings (Friden et al. 1983; Newham et al. 1983b).

Statistical analyses

From data associated with single stretches of passive and maximally activated muscles, multiple linear regression models were used to estimate the relationships of strain, peak and average forces, and work with the magnitude of force deficit. Initially, the predictive value of each variable alone was determined using a simple one-variable regression model. The relative importance of the independent variables on the force deficit was established using a stepwise regression analysis which determined the combination
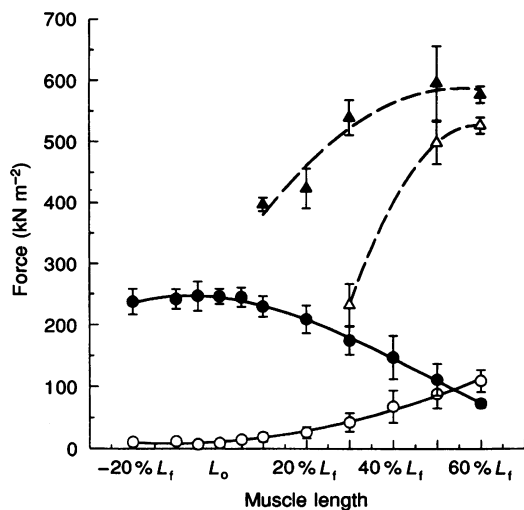

Figure 2. The relationship between final muscle length and passive or maximally activated force

The circles show the relationship between muscle length and passive (O) and maximally activated (●) force recorded ~3 min after placing the muscle at each given length. Sample size is from 4 to 6 for each point. The data given by the triangles are the peak forces achieved during single stretches at 2 L_f s^{-1} of passive (△) and maximally activated (▲) muscle starting at L_0 and ending at the length at which the data point is plotted. Sample size is from 6 to 8 for each point. Data are presented as means ± S.E.M.

of variables that accounted for the largest portion of the variation in the force deficit. For this analysis, *F* tests were used to determine significant regression relationships between the force deficit and the set of independent variables (Neter, Wasserman & Kuter, 1985) which reflect the variable's contribution to the model if included. A level of significance of 0·10 was required both for entry into the model and inclusion in the model. The coefficients of determination and partial coefficients of determination are expressed as percentages in the results section of the text ($r^2 \times 100$).

RESULTS

For passive muscles, single stretches of 50% strain or greater were necessary to produce a significant force deficit (Fig. 5, inset). When maximally activated muscles were exposed to single stretches, force deficits resulted from stretches of 30% strain or greater (Fig. 5). Despite the smaller strains necessary to produce injury in an active compared with a passive muscle, following stretches of
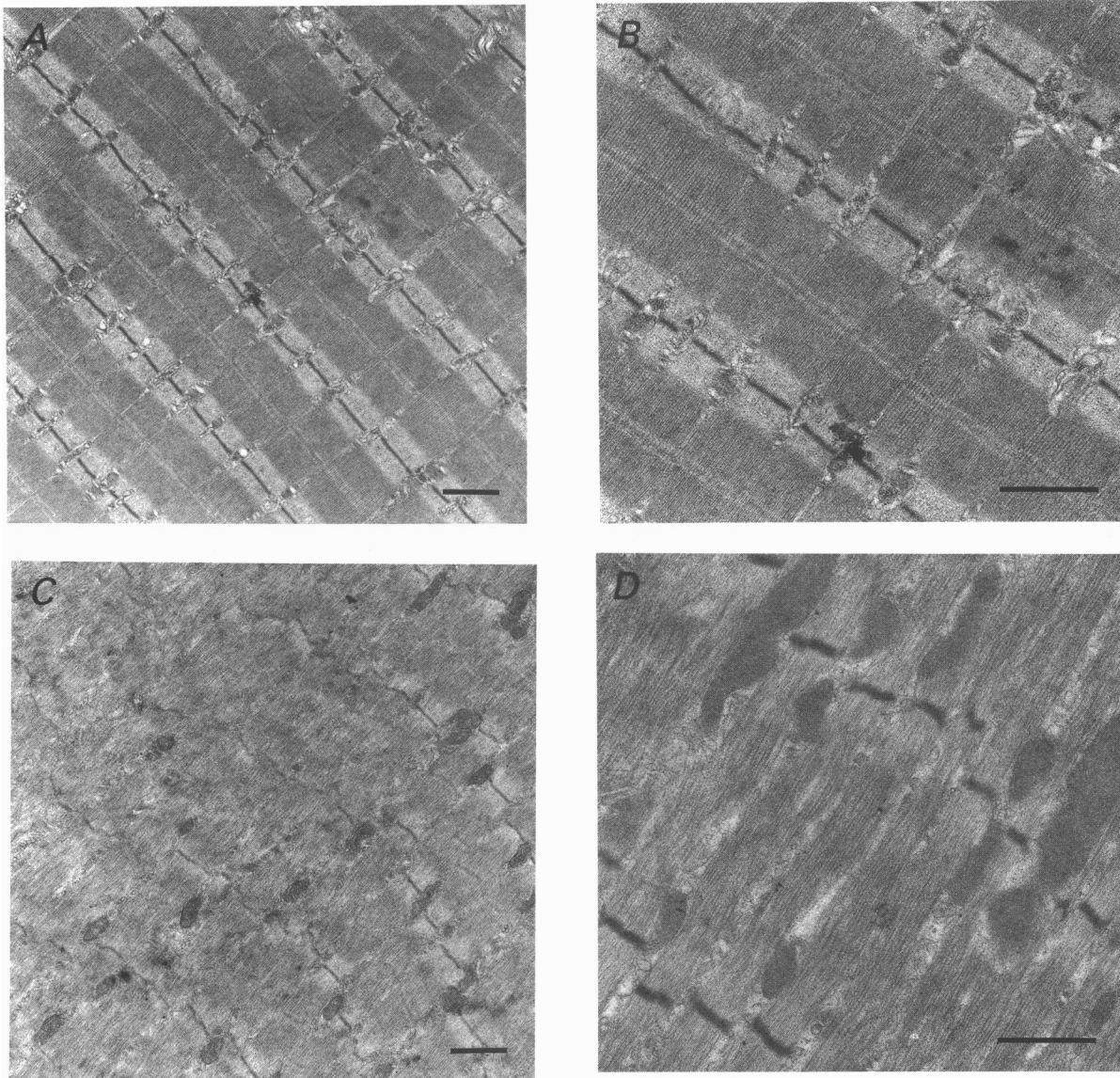

Figure 3. Longitudinal sections of a control extensor digitorum longus (EDL) muscle (*A* and *B*) and of an EDL muscle exposed to a 60% stretch while unstimulated (*C* and *D*)

Note that in *A* and *B*, the fibre shows normal striation patterns, whereas the injured fibres in *C* and *D* show a wide variety of severe, widely dispersed ultrastructural abnormalities. The ultrastructural damage observed includes disorganisation of myofilaments, misalignment of adjacent sarcomeres, and distortion or absence of Z-lines (*C*). In addition, many areas of overstretched sarcomeres and widening of Z-lines were observed following large stretches of passive muscles (*D*). For *A* and *C*, the magnification is × 17 820, and for *B* and *D*, the magnification is × 31 050. All scale bars represent 1 μm.
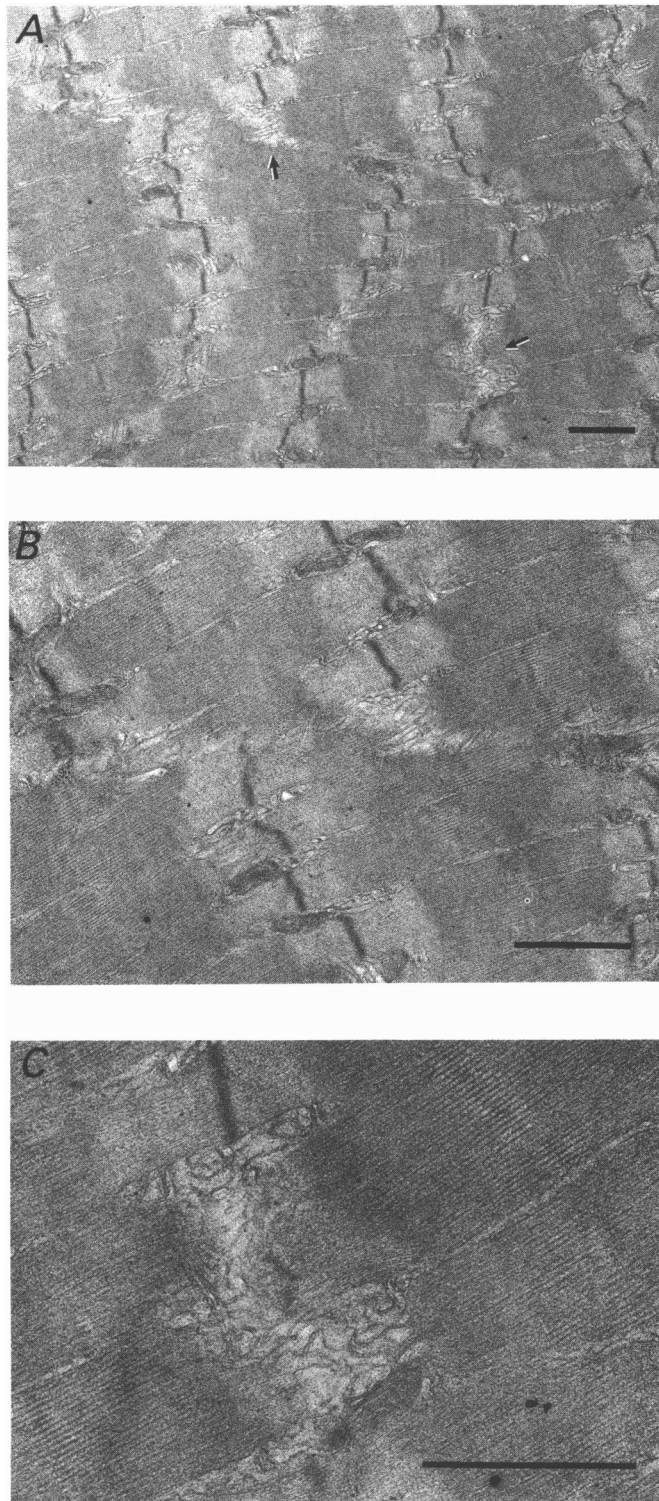
Figure 4. Longitudinal section of a fibre immediately after a single 60 % stretch of a maximally activated EDL muscle

The injured fibre shows focal abnormalities, with misaligned and overstretched sarcomeres, disruption or absence of Z-band material, thin filaments displaced from thick filaments, and disrupted striation patterns. Sarcomeres in series range from severely damaged to those with normal characteristics. *B* and *C* show the regions in *A* that are indicated with arrows at higher levels of magnification. For *A*, the magnification is × 17 820; for *B*, × 31 050; and for *C*, × 56 700. All scale bars represent 1 μm.

Table 1. Coefficients of determination for each one-variable model of the force deficit following single stretches of passive and maximally activated muscles

	Passive	Maximally activated
Strain	0·73 (0·0001)	0·69 (0·0001)
Peak force	0·57 (0·0001)	0·31 (0·0008)
Average force	0·75 (0·0001)	0·50 (0·0001)
Work	0·85 (0·0001)	0·76 (0·0001)

Levels of significance are given in parentheses.

60% strain, significantly smaller force deficits were observed for active than for passive muscles (Fig. 5). The force deficit of 93 ± 4% produced by single 60% stretches of passive muscles implies that these muscles may have been strained to failure resulting in complete abolition of the ability to generate force. To determine whether this was the case, four of the seven passive muscles that were exposed to 60% stretches were monitored during a period of recovery. At 10 min intervals following the stretch, the muscles were activated maximally and P_o was measured. Each of the four muscles showed improvements in force development over time, recovering from a mean force deficit of ~90% at 1 min to a mean force deficit of ~58% within 30 min. The partial recovery of force by muscles exposed to very long stretches provides further support for our conclusion that, in spite of the high forces achieved during the stretches, the muscles were not stretched beyond their range of extensibility.

Table 1 gives the results of the one-variable regression models. When interactions among variables were ignored, each had a significant relationship with the force deficit following a single stretch of either a passive or a maximally activated muscle. Apart from the peak force of maximally activated muscles, each of the other variables could explain more than 50% of the variation in the force deficit. Of all of the variables, the work done to stretch the muscle was the

best predictor of the magnitude of injury, explaining 85% and 76% of the variation in the force deficit for passive and maximally activated muscles, respectively (Fig. 6).

As with the one-variable models, the stepwise regression models indicated that work was the dominant factor in explaining the force deficit for either passive or maximally activated muscles (Table 2). In spite of the fact that work was the best predictor of the magnitude of the force deficit for both passive and maximally activated muscles, the more important component of work was different for each condition. Following single stretches of passive muscles, if work was not included as an independent regresser in the stepwise analysis, average force explained the majority of the variation in the force deficit (Table 2). The importance of average force is illustrated by the relationship between average force and force deficit for passive muscles which indicates that the high variablity in the force deficit observed following stretches of 50% strain (Fig. 5, inset) is related to the average force developed during the stretch (Fig. 7). With activation, the average forces developed during stretches increased dramatically compared with the passive condition due the presence of attached cross-bridges. In fact, the distribution of average forces generated during stretches of passive muscles did not overlap with the average forces generated by active muscles (Fig. 7). Furthermore, only strain made a significant contribution to

Figure 5. The relationship between the strain and the force deficits observed 1 min after single stretches of maximally activated muscles (●)

Strain is expressed as a percentage of optimum fibre length (L_f), and the force deficit is expressed as a percentage of the maximum force developed by the muscle prior to the stretch. Data are presented as means ± s.e.m. Sample size is from 6 to 8 for each point. The inset shows the relationship between strain and the force deficits observed 1 min after single stretches of passive muscles (○). Data presented in the inset are for individual muscles. Sample size is 20 for passive muscles.
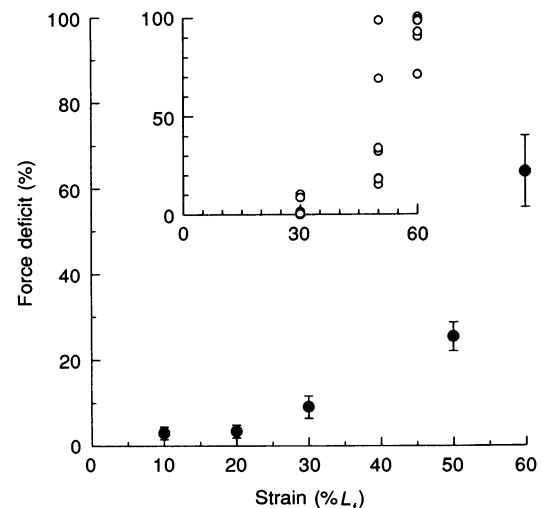
Table 2. Results of the stepwise regression analyses of the force deficit following single
stretches of passive and maximally activated muscles

	Strain	Peak force	Average force	Work	Model r^2	P
Passive						
Work as a regresser	—	0·05	—	0·85	0·90	0·0001
Work not included	0·06	0·09	0·75		0·90	0·0001
Maximally activated						
Work as a regresser	0·06	0·03	—	0·76	0·85	0·0001
Work not included	0·69	—	—		0·69	0·0001

Partial coefficients of determination are shown for each variable for the analyses that included work as an
independent regresser and for the analyses that did not include an explicit work term in the model.
Dashes indicate that the contribution of a variable to the variance in the force deficit did not reach a level
of significance necessary for inclusion in the stepwise regression analysis. The coefficients of determination
and the levels of significance for the models are also given.

the force deficit for maximally activated muscles when work
was not included as an independent regresser in the
stepwise analysis (Table 2).

DISCUSSION

For both passive and maximally activated muscles, the
work done during a single stretch provided the best
prediction of the resultant force deficit. Despite the
strength of work as a predictor of damage, the relationship
between the force deficit and work was quite different for
the two conditions of activation. For work inputs that
produced force deficits, a given amount of work produced
greater force deficits after stretches of passive than of active
muscles. The difference between passive and active muscles
in the relationship between force deficit and work arose
from the conditions under which injuries occurred with
respect to the components of work, average force and
strain. Passive muscles were injured by large stretches, but
at forces often less than P_o, whereas maximally activated
muscles were injured by relatively small stretches, but at
average forces between two- and threefold greater than P_o.

When work was excluded as an independent variable, for
passive muscles, average force predicted the force deficit
best, whereas for active muscles, strain was the best
predictor. The force generated by stretching a passive
muscle is totally dependent on strain and is borne largely
by parallel elastic structures, such as the thick filament
anchoring protein, titin (Horowits, 1991; Wang, McCarter,
Wright, Beverly & Ramirez-Mitchell, 1993). For a given
sarcomere strain, the parallel elastic component bears the
same load in the presence or absence of attached cross-
bridges. Consequently, the same magnitude of sarcomere
strain would injure a given sarcomere in a passive and an
active muscle. For active muscles, the high average forces
appeared to influence the distribution of muscle strain
among different sarcomeres in series along the length of
myofibrils. Higher average forces developed by active
compared with passive muscles increased the likelihood of
large strains and subsequent injury of some isolated
sarcomeres during stretches of the muscle fibres of
relatively small magnitudes. In contrast, during large
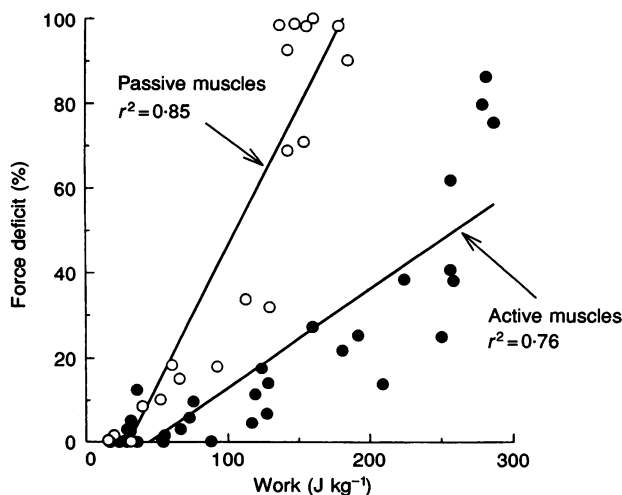stretches, the presence of actively cycling cross-bridges

Figure 6. The force deficit observed 1 min after single
stretches of passive (O) and maximally activated (●)
muscles in relation to the work done to lengthen the
muscle

Data are for single stretches varying in magnitude but not
velocity ($v = 2\ L_f\ \mathrm{s}^{-1}$). Each symbol indicates a data point
from a single stretch. The r^2 values represent the coefficients
of determination for the regression relationships. The slopes
of the relationships, 0·23 for active muscles and 0·66 for
passive muscles, are significantly different. Sample size is 20
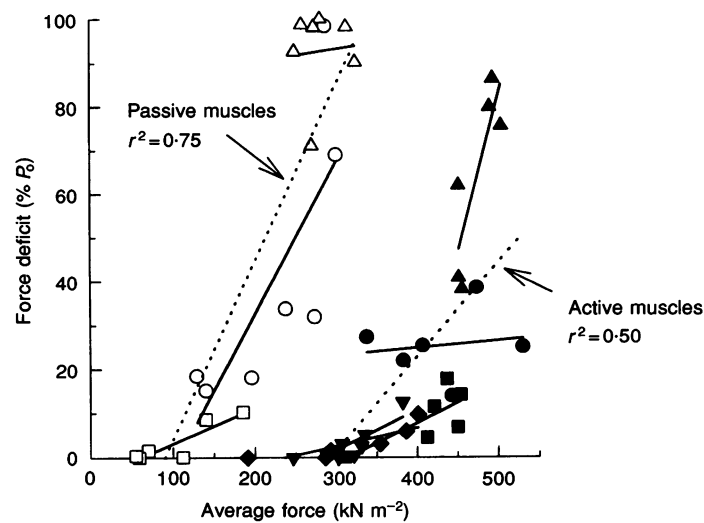for passive muscles and 32 for active muscles.

Figure 7. The force deficit observed 1 min after single stretches of passive (open symbols) and maximally activated (filled symbols) muscles in relation to the average force generated during the stretch

Data are for single stretches varying in magnitude, indicated by the shape of the symbol (10 % strain, diamond; 20 %, inverse triangle; 30 %, square; 50 %, circle; 60 %, triangle). Each symbol indicates a data point from a single stretch. Continuous lines show the regression relationships for data from stretches of a single strain. Dashed lines show the regression relationships for all data from each condition of activation. The r^2 values represent the coefficients of determination for the overall regression relationships. Sample size is 20 for passive muscles and 32 for active muscles.

appeared to protect some sarcomeres from experiencing severe strains and being injured.

A recent study by Warren *et al.* (1993), using protocols of five stretches of activated rat soleus muscles, each contraction separated by 4 min, concluded that the peak force during the stretch was the most important factor in the initiation of contraction-induced injury, while neither strain nor work contributed significantly to the variance in the force deficit. Many of the stretches used by Warren and his colleagues did not lengthen the muscle beyond L_0, and their most injurious protocol stretched muscle fibres to < 30% L_f beyond L_0 resulting in force deficits of only 14%. In addition, the slow fibre types of the soleus muscles of rats may be less susceptible to contraction-induced muscle damage than the fast fibre types in EDL muscles (Jones *et al.* 1986; Lieber, Woodburn & Fridén, 1991). The relatively modest stretches used and the minimal severity of the resulting injury may account for the lack of a significant contribution of strain to the magnitude of the force deficit in the study of Warren *et al.*

In contrast to our finding that a single stretch of a maximally activated muscle must be of at least 30% strain relative to L_f to produce a force deficit, protocols of repeated stretches to ~10% beyond L_f produced severe muscle injury (McCully & Faulkner, 1985, 1986; Brooks & Faulkner, 1990; Zerba *et al.* 1990; Lieber & Fridén, 1993). A protocol of 225 repeated stretches to 10% L_f beyond L_0 resulted in a 65% force deficit and a 20% loss in the

number of fibres in the total muscle cross-section at 3 days (Brooks & Faulkner, 1990), and 900 repeated stretches of 12·5% strain caused a 40% force deficit 1 h after completion of the 30 min contraction protocol (Lieber & Fridén, 1993). The large force deficits and severe morphological damage observed in these previous studies attest to the influence of the number of stretches on the magnitude of injury, as well as the importance of the cumulative effects of the initial mechanical injury and the resultant cascade of chemical and metabolic factors (McCully & Faulkner, 1985, 1986; Brooks & Faulkner, 1990; Zerba *et al.* 1990; Lieber & Fridén, 1993). Through the use of single stretches and the evaluation of the force deficit within 1 min of the stretch, the present study separated the original mechanical disruption from any metabolic events that may be initiated by the mechanical damage.

Several investigators have suggested that muscle fibres are injured when individual sarcomeres are stretched excessively and the actin and myosin filaments are pulled apart (Newham *et al.* 1983 *b*; Higuchi, Yoshioka & Maruyama, 1988; Wood, Morgan & Proske, 1993). At L_f, EDL muscles of mice have an average sarcomere length of 2·52 μm (Brooks & Faulkner, 1994). Assuming thick and thin filament lengths of 1·6 and 1·02 μm, respectively, no overlap of thick and thin filaments would exist at a sarcomere length of 3·64 μm, corresponding to a sarcomere strain of ~50%. In the present study, the first sign of significant injury to passive muscles occurred following

single stretches of 50% strain, and stretches of 60% strain resulted in nearly complete elimination of force production. We propose that, despite our the lack of knowledge of the actual sarcomere strains, these observations are consistent with the hypothesis that passive fibres are injured when single sarcomeres are stretched beyond thick and thin filament overlap. Once sarcomeres are stretched beyond overlap of thick and thin filaments, no differences would exist between stretched sarcomeres in active and passive fibres. Consequently, the mechanism of the injury to individual sarcomeres and the dependence of the magnitude of the injury on the magnitude of the stretch beyond overlap of thick and thin filaments would not be different.

Passive single fibres that were stretched such that sarcomeres were lengthened beyond the point of thick and thin filament overlap, and then returned to L_f and fixed for electron microscopy, contained thin filaments that did not re-enter the thick filament array (Higuchi *et al.* 1988; Brown & Hill, 1991). EMs showed that a buckling of the thin filaments had occurred, and a force deficit was observed (Higuchi *et al.* 1988). These observations also provide support for the working hypothesis that injury occurs when sarcomeres are stretched beyond thick and thin filament overlap (Higuchi *et al.* 1988; Brown & Hill, 1991). The observation that single (present study) or repeated stretches of both passive single fibres (Higuchi *et al.* 1988) and whole muscles (McCully & Faulkner, 1985) through strains insufficient to result in lengthening beyond overlap did not give rise to force deficits provides further indirect support for this hypothesis.

During stretches of passive (Higuchi *et al.* 1988) and active (Julian & Morgan, 1979; Lombardi & Piazzesi, 1990; Brown & Hill, 1991) single muscle fibres, heterogeneity in sarcomere length has been reported. In addition, the degree of heterogeneity in sarcomere length increases with increasing fibre length and is increased further by activation (Julian, Sollins & Moss, 1978; Julian & Morgan, 1979; Burton, Zagotta & Baskin, 1989; Horowitz & Pollack, 1993). In modeling active muscle fibres, Morgan (1990) predicted that, due to random variations in the strengths of individual sarcomeres, stretches take place non-uniformly by the rapid, uncontrolled lengthening beyond thick and thin filament overlap of relatively few sarcomeres, while stronger sarcomeres are able to resist lengthening and may be protected from severe strains, even during large stretches. Although no evidence exists for sudden transitions to extreme elongation of sarcomeres, our observation of significant force deficits attributable to focal damage of small groups of sarcomeres following short stretches of maximally activated fibres is consistent with the development of non-uniformities in sarcomere length.

In conclusion, the strong relationship between the work done to lengthen the muscle and the magnitude of damage has implications for the circumstances under which injury may occur during the activities of daily living. The majority of contractions involve low loads with the muscle experiencing small stretches (Goslow, Seeherman, Taylor, McCutchin & Heglund, 1981), and most exposures to repetitive contractions are of short duration. Under both circumstances, contraction-induced injury is an unlikely event. In contrast, high loads and large stretches may occur during accidental falls, or during the single burst movements characteristic of sports events and of sudden escape behaviours. When loads are high, and sarcomere strains large, severe focal injury to skeletal muscle myofibrils is probable. The partial recovery of force following large stretches of passive muscles indicates that some aspects of the disruption of the thick and thin filament array may be immediately reversible, but damage may also involve cytoskeletal proteins (Horowits, 1991; Wang *et al.* 1993) and muscle membranes (McNeil & Khakee, 1992). Physical activities involving highly repetitive contractions of relatively small stretches may also result in severe injury (Fridén *et al.* 1983; Newham *et al.* 1983*a,b*; McCully & Faulkner, 1985; Jones *et al.* 1986; Ogilvie *et al.* 1988; Faulkner *et al.* 1989; Brooks & Faulkner, 1990; Zerba *et al.* 1990; Lieber & Fridén, 1993). We conclude that contraction-induced injury occurs under a wide variety of circumstances, but in each case is initiated by similar mechanisms.

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