The magnitude of the initial injury induced by stretches of maximally activated muscle fibres of mice and rats increases in old age

Susan V. Brooks and John A. Faulkner

Institute of Gerontology, Department of Physiology, and Bioengineering Program, University of Michigan, Ann Arbor, MI 48109-2007, USA

1. Our purpose was to compare the susceptibilities of muscles in animals of different ages to the injuries induced by stretching the contracting muscle. Single stretches provide an effective method for studying the factors that contribute to the initiation of contraction-induced injury. We hypothesized that, for maximally activated muscles in old compared with young or adult mice, the work input during a single stretch of any given strain is not different, but for a given work input the magnitude of the injury is greater.

2. The force deficit resulting from each single stretch was calculated as the decrease in the maximum isometric force expressed as a percentage of the maximum force prior to the stretch. Force deficits were compared 1 min after single stretches of *in situ* extensor digitorum longus (EDL) muscles of young, adult and old mice. In addition, measurements of force deficits immediately following single stretches of single permeabilized fibre segments from EDL muscles of young and old rats permitted investigation of the initial injury at the level of the contractile apparatus.

3. For maximally activated EDL muscles in young, adult and old mice, no differences were observed for the work input during stretches of any given strain. Furthermore, the relationships between the work and the resultant force deficit were not different for muscles in young and adult mice. In contrast, compared with the work–force deficit relationships for muscles in either young or adult mice, the relationship was significantly steeper for muscles in old mice. For single permeabilized fibres from muscles of old rats, the force deficits immediately after single stretches were greater than those observed for fibres from muscles of young rats. We conclude that the increased susceptibility of muscles in old animals to contraction-induced injury resides at least in part within the myofibrils.

Skeletal muscles can be injured by their own contractions (Fridén, Sjöström & Ekblom, 1983; Newham, Jones & Edwards, 1983*a*), and injury is most likely to occur when activated muscle fibres are stretched during pliometric contractions (McCully & Faulkner, 1985). The injury appears to be initiated by the mechanical disruption of the ultrastructure of specific sarcomeres (Fridén *et al.* 1983; Newham, McPhail, Mills & Edwards, 1983*b*; Wood, Morgan & Proske, 1993; Brooks, Zerba & Faulkner, 1995). The damage which occurs during the contractions can give rise to a delayed secondary injury characterized by an inflammatory response and ultimately the degeneration of severely damaged fibres (McCully & Faulkner, 1985; Jones, Newham, Round & Tolfree, 1986). The secondary injury, which is typically more severe than the initial injury, peaks between 1 and 5 days later (Newham *et al.* 1983*a*, *b*; McCully & Faulkner, 1985). While only direct demonstration of morphological damage to fibres confirms that an injury has occurred, the focal nature of the damage, widely dispersed both between fibres and along fibres, makes a quantitative morphometric analysis difficult. An indirect measure of the magnitude of an injury is provided by the force deficit, calculated as the decrease in the maximum isometric force expressed as a percentage of the maximum force prior to the stretch. Although the exact quantitative relationship between sarcomere disruption and the force deficit has not been determined, for a given contraction protocol, the force deficit gives the most reliable and reproducible measure of the amount of damage (Newham *et al.* 1983*b*; Brooks *et al.* 1995).

Morphological and functional evaluations made 3 days after a protocol of repeated pliometric contractions indicate that, compared with muscles in young or adult mice, muscles in old mice are more susceptible to contraction-induced injury (Zerba, Komorowski & Faulkner, 1990), but the more severe injury may be due to a greater susceptibility to the initial mechanical injury, or to a more severe secondary injury in response to a given initial injury, or both. Single stretches of

whole muscles and single permeabilized fibre segments provide an effective method for focusing on the factors that contribute to the initiation of contraction-induced injury (Brooks *et al.* 1995; Macpherson, Schork & Faulkner, 1996). Following single stretches of maximally activated extensor digitorum longus (EDL) muscles in young mice, the best predictor ($r^2 = 0{\cdot}76$) of the force deficit is the work done to stretch the muscle (Brooks *et al.* 1995).

Our purpose was to compare the susceptibility of muscles in animals of different ages to the initial mechanical injury induced by stretching a contracting muscle. The forces developed during stretches of maximally activated muscles from young and old mice are similar (Phillips, Bruce & Woledge, 1991), and 3 days after a protocol of repeated pliometric contractions, muscles of old mice demonstrate more severe injury than do muscles of young or adult mice (Zerba *et al.* 1990). Based on these observations, we hypothesized that, for maximally activated muscles of old compared with young or adult mice, the work done during stretches of any given strain is not different, but for a given work input the magnitude of the injury is greater. We compared the magnitude of the force deficits 1 min after single stretches of maximally activated *in situ* EDL muscles of young, adult, and old mice. In order to determine whether differences in the magnitude of the initial injury observed for whole muscles in mice of different ages occurred at the level of the contractile apparatus, we investigated the force deficits immediately following single stretches of single permeabilized fibre segments from EDL muscles of young and old rats.

Some results have been presented previously to the Biophysical Society (Brooks, Macpherson & Faulkner, 1994; Brooks & Faulkner, 1995).

METHODS

Adult (9–12 months) and old (25–28 months) C57BL/6 male mice and young (5–6 months) and old (27–34 months) F344 or F344 X Brown Norway male rats were obtained from specific pathogen free (SPF) colonies. Prior to experimentation, mice and rats were housed in an SPF barrier facility at the University of Michigan. All operations and protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (United States Public Health Service National Institutes of Health publication no. 85-23). For each experimental procedure, animals were anaesthetized with intraperitoneal injections of sodium pentobarbitone (80 mg kg^{-1} for young and adult animals, and 40 mg kg^{-1} for old animals) with supplemental doses (5–10 mg kg^{-1}) administered as needed to maintain a depth of anaesthesia that prevented responses to tactile stimuli.

In situ whole muscle experiments

Data were collected on fourteen adult and twenty-one old mice. All operative and experimental procedures have been described in detail previously (Brooks *et al.* 1995). Briefly, a small incision was made at the ankle of the anaesthetized mouse, a 5-0 nylon suture was tied around the distal tendon of the EDL muscle, and the tendon was cut distal to the suture. The mouse was placed on a platform maintained at 37 °C, and the experimental hindlimb was stabilized. The EDL tendon was attached to 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. The small regions of exposed muscle and tendon were bathed periodically with 0·9% NaCl solution at 37 °C. The muscle was activated through stimulation of the peroneal nerve with a pair of needle electrodes. The stimulation voltage and subsequently muscle length (L_o) were adjusted to be optimum for maximum isometric twitch force. Muscles were stimulated at increasing frequencies until the force reached a plateau. The force plateau was typically 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. Muscle fibre length (L_f) was estimated by multiplying L_o by the L_f/L_o ratio of 0·45

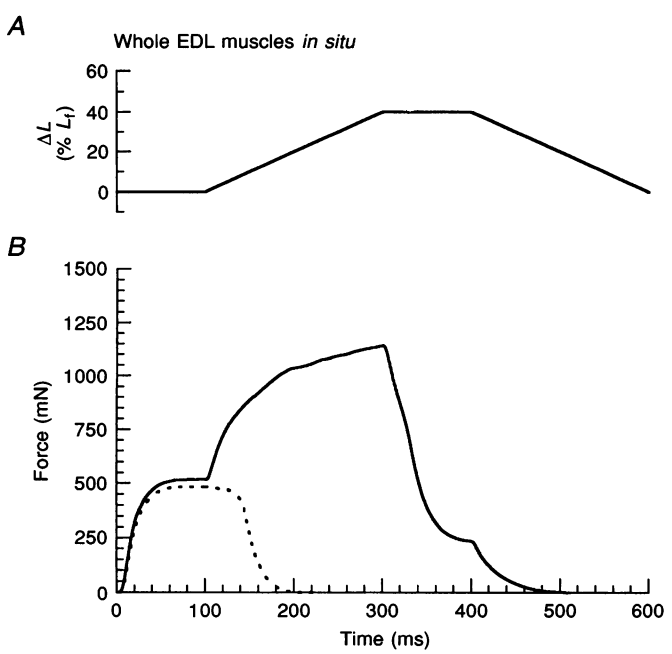

Figure 1. Representative experimental records of length and force during a single pliometric contraction of an *in situ* EDL muscle

A shows the imposed length change for a stretch of 40% strain at a velocity of 2 L_f s^{-1}. The magnitude of the length change is expressed as a percentage of muscle fibre length (L_f) where 0% corresponds to the optimum muscle length for force development (L_o). *B* shows force traces in millinewtons during the isometric and lengthening phases of a single pliometric contraction (continuous line), and the maximum force during an isometric contraction (dashed line) measured 1 min after the stretch. During the pliometric contraction, stimulation is terminated at the end of the lengthening ramp. The force deficit is calculated as the difference between the isometric forces developed before and 1 min after the stretch, expressed as a percentage of the force prior to the stretch. In this case, the force deficit = ((518 − 483)/518) × 100 = 6·8%.

(Brooks & Faulkner, 1988). The mean values for L_f were 5·78 ± 0·07 mm (± S.E.M.) and 5·85 ± 0·06 mm for muscles in adult and old mice, respectively.

Each muscle was exposed to a single stretch at 2 L_f s^{-1} with the muscle stimulated at the frequency that resulted in P_0 (Fig. 1). Single stretches were initiated from the plateau of the isometric contraction, and stimulation was terminated at the end of the lengthening ramp. All stretches were from L_0, and, following a 100 ms hold at the stretched length, muscles were returned to L_0 at the same velocity as occurred during lengthening. Single stretches were of 10, 20, 30, 40 or 50% strain relative to L_f. The fivefold variation in strain was designed to provide a wide range of work inputs for the detection of differences in the force deficit between muscles in mice of different ages. These length changes correspond to ~4 to ~20% of the EDL muscle length and are well within the physiological range for many muscles (Cutts, 1988).

The work input during the stretch was calculated for each muscle. The force deficit, observed 1 min after the stretch, provided the quantitative measure of the magnitude of the injury (Fig. 1). In all cases, the maximum isometric force prior to the stretch was not significantly different from P_0. Our contention that the force deficit observed at 1 min is a valid representation of the initial mechanical damage induced by a single stretch was confirmed previously by direct morphological evidence of damage to muscle fibres (Brooks *et al.* 1995). Furthermore, the morphological damage was comparable to that shown previously after repeated stretches of contracting muscles of humans (Fridén *et al.* 1983; Newham *et al.* 1983*b*).

After the final measurement of force *in situ*, EDL muscles were removed, and the anaesthetized mice were killed with an overdose of the anaesthetic. The tendons were trimmed from the muscle, which was then blotted dry and weighed to the nearest 0·01 mg. The mean wet mass of 12·22 ± 0·26 mg for twenty experimental muscles from adult mice was significantly greater than the mean mass of 10·98 ± 0·32 mg for twenty-three experimental muscles from old mice. Work input values determined from single stretches of maximally activated muscles were normalized by muscle wet mass. Total muscle fibre cross-sectional area (CSA) was calculated by dividing the muscle wet mass by the product of the L_f and the density of skeletal muscles, 1·06 mg mm^{-3}. P_0 was divided by the total muscle fibre CSA to obtain the specific P_0 (kN m^{-2}). Prior to the single stretch, the mean values for specific P_0 were 255·2 ± 4·9 and 209·8 ± 8·8 kN m^{-2} for muscles of adult and old mice, respectively. These values were similar to those reported previously for EDL muscles in adult and old mice (Brooks & Faulkner, 1988; Zerba *et al.* 1990).

Single permeabilized fibre experiments

The force deficits observed following single stretches of whole muscles could be the result of damage to force-generating or force-bearing components within the muscle fibres (Macpherson *et al.* 1996), damage to structures external to the muscle fibres (Warren, Hayes, Lowe, Prior & Armstrong, 1993*b*), disruption of the processes of excitation–contraction coupling (Warren, Lowe, Hayes, Karwoski, Prior & Armstrong, 1993*c*; Balnave & Allen, 1995), or some combination of these possibilities. To distinguish failure of excitation–contraction coupling or damage to extracellular structures from damage to intracellular myofibrillar components, a permeabilized single fibre preparation was used in which the contractile elements are activated directly by high-calcium solutions of standardized composition. Data were collected on fifty-one single permeabilized fibre segments from EDL muscles of two young and four old rats.

The procedures for preparing and studying single permeabilized fibre segments have been described in detail previously (Brooks & Faulkner, 1994). EDL muscles were removed and placed in cold mammalian Ringer solution (composition (mM): NaCl, 137; $NaHCO_3$, 24; glucose, 11; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 1; NaH_2PO_4, 1). Subsequently, anaesthetized rats were killed with an intravenous injection of 1 M KCl. Each muscle was dissected into small fibre bundles which were stored at −20 °C in skinning solution containing 125 mM potassium propionate, 5 mM EGTA, 2 mM ATP, 2 mM $MgCl_2$, 20 mM imidazole, and 50% (v/v) glycerol (Lynch, Duncan, Campbell & Williams, 1995). The solution was adjusted to pH 7 with KOH. For a given experiment, single fibre segments were pulled from the bundle with fine forceps and

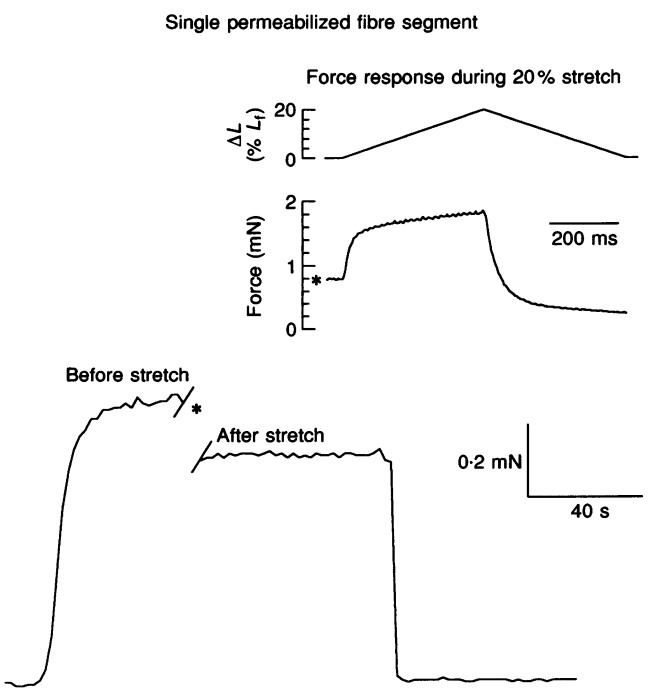

Figure 2. Experimental records of the length and force during a single stretch of a maximally activated single permeabilized fibre segment from a rat EDL muscle

The lower panel shows a representative trace for the isometric force (mN) developed when the fibre segment is immersed in maximal activating solution, pCa 4·5. The force trace is broken at the point during the contraction when a single stretch of 20% strain at 0·5 L_f s^{-1} was imposed. The imposed length change (upper trace; % L_f) and the force response (lower trace; mN) during the stretch are shown on a faster time scale in the upper panel. Note the force scale is also different in the upper panel. All stretches were initiated from the plateau of a maximal isometric contraction (*). In this case, the isometric forces before and after the stretch are 0·79 (*) and 0·63 mN, respectively, to give a force deficit of 20·3%.

transferred to a 15 °C bath containing a low-calcium relaxing solution comtaining 7 mM EGTA, 5·4 mM $MgCl_2$, 4·74 mM ATP, 14·5 mM creatine phosphate, 20 mM imidazole, 16 μM $CaCl_2$, and 79 mM KCl; to give a final ionic strength of 180 mM. Single fibre segments were mounted between a force transducer (Model 400; Cambridge Technology Inc.) and the lever arm of a servomotor (Model 300; Cambridge Technology Inc.). With the fibres immersed in relaxing solution, average sarcomere length was set to ~2·6 μm, and fibre segment length (L_f), width and depth were measured using a stereo-microscope (Model M3Z; Wild Heerbrugg Ltd, Heerbrugg, Switzerland), high-power objective, and a camera system (Models MPS 51 S, MPS45; Wild Heerbrugg Ltd). The mean L_f for the fifty-one fibres used in the study was 1·44 ± 0·04 mm. Single fibre P_o was determined by exposing the fibre segment to a maximal activating solution similar to the relaxing solution but with sufficient $CaCl_2$ to give a pCa of 4·5. Assuming an elliptical cross-section, the CSA of single fibres and specific P_o were calculated. Neither the mean CSA values of 3583 ± 275 and 3139 ± 199 μm^2 for fibres from muscles of young and old rats, respectively, nor the specific P_o values of 133·2 ± 7·0 and 129·2 ± 5·9 kN m^{-2} were significantly different, consistent with data on single permeabilized fibres from EDL muscles of adult and old mice (Brooks & Faulkner, 1994).

Throughout the experiment, fibre segments were cycled between an isometric contraction and short periods of isovelocity shortening at a velocity near V_{max} followed by a rapid restretch back to the initial fibre segment length while maximally activated. This cycling protocol allows the maintenance of a stable sarcomere striation pattern and a constant maximum isometric force for more than 10 min of continuous activation (Sweeney, Corteselli & Kushmerick, 1987). Stretches of fibre segments for the collection of experimental data were performed between cycles. From the plateau of a maximally activated isometric contraction at L_f, each fibre segment was exposed to a single stretch at 0·5 L_f s^{-1} (Fig. 2). For fibres from muscles of both young and old rats, stretches were of 5, 10 or 20% strain and fibres were returned to L_f at the same velocity as occurred during lengthening. With the fibre held at L_f, the force was monitored for approximately 30 s following the stretch before the fibre was relaxed by exposure to the pCa 9·0 relaxing solution (Fig. 2). The force deficit was calculated from the decrease in the maximum isometric force observed immediately after the stretch and was taken as an indirect estimate of the magnitude of the initial damage induced by the stretch to the myofibrillar structures within fibre segments (Macpherson *et al.* 1996). For all single permeabilized fibres used, the maximum isometric force before the stretch was not significantly different from P_o. In addition, relaxation after the stretch and subsequent reactivation after several minutes did not result in either an increase or a decrease in the force developed by the fibres.

Single stretches of passive muscle fibres

Both *in situ* whole muscles ($n = 25$) and *in vitro* single permeabilized fibre segments ($n = 16$) from adult and old animals were exposed to stretches without activation. Single stretches of passive whole muscles of adult and old mice were initiated from L_o, and were of 30, 50 or 60% strain relative to L_f at 2 L_f s^{-1}. Following a 100 ms hold at the stretched length, muscles were returned to L_o at the same velocity as occurred during lengthening. Similarly, stretches of passive single permeabilized fibres from EDL muscles of old rats were initiated from L_f, and were of 10, 20, 30 and 50% strain at 0·5 L_f s^{-1}. Force deficits resulting from single stretches of passive muscles and single permeabilized muscle fibres were calculated by comparing the P_o observed during a maximum isometric contraction ~1 min after the stretch and the P_o during a maximum isometric contraction prior to the stretch.

Statistical methods

From data associated with single stretches of maximally activated EDL muscles from adult and old mice, linear regressions were used to estimate the relationships between the work done to stretch the muscle and the resultant force deficit. Coefficients of determination for linear regression models are expressed as percentages ($r^2 \times 100$) in the Results section. A one-way analysis of variance (ANOVA) was used to determine whether differences existed between the muscles from adult and old mice and published data on EDL muscles of young mice (Brooks *et al.* 1995) for the mean work inputs at any of the strains used and for the work–force deficit relationships. For data from single permeabilized fibres from EDL muscles of young and old rats, descriptive variables and force deficit values are given as means ± 1 S.E.M. Differences between fibre segments from young and old rats were assessed by Student's two-tailed t test. For all statistical tests, the level of significance was set *a priori* at $P < 0·05$.

RESULTS

During single stretches of maximally activated EDL muscles in mice of all ages approximately 90% of the variability in the work input was explained by strain (Fig. 3). At any given strain, the peak and average specific forces (kN m^{-2}) developed during stretches were not different between the three age groups, resulting in no differences among the three age groups for the work input during single stretches (Fig. 3).

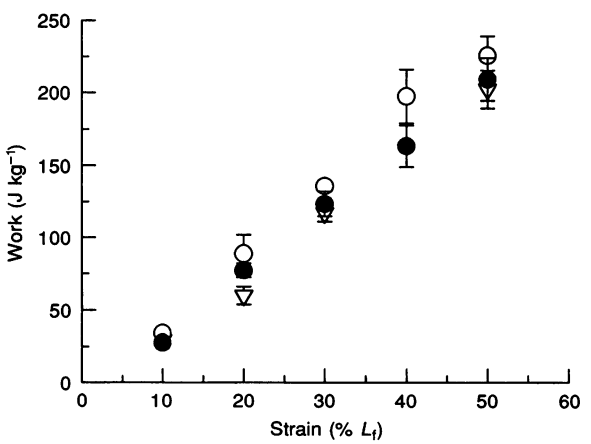

Figure 3. The relationship between the strain and the work input during single stretches of maximally activated muscles

Data are presented as the mean ± 1 S.E.M. for *in situ* EDL muscles in young (▽), adult (○) and old (●) mice. Strain is expressed as a percentage of optimum fibre length (L_f), and the work is normalized by muscle wet mass (J kg^{-1}). Data for young mice are reproduced from Brooks *et al.* 1995. Sample size is from 3 to 8 for each point.

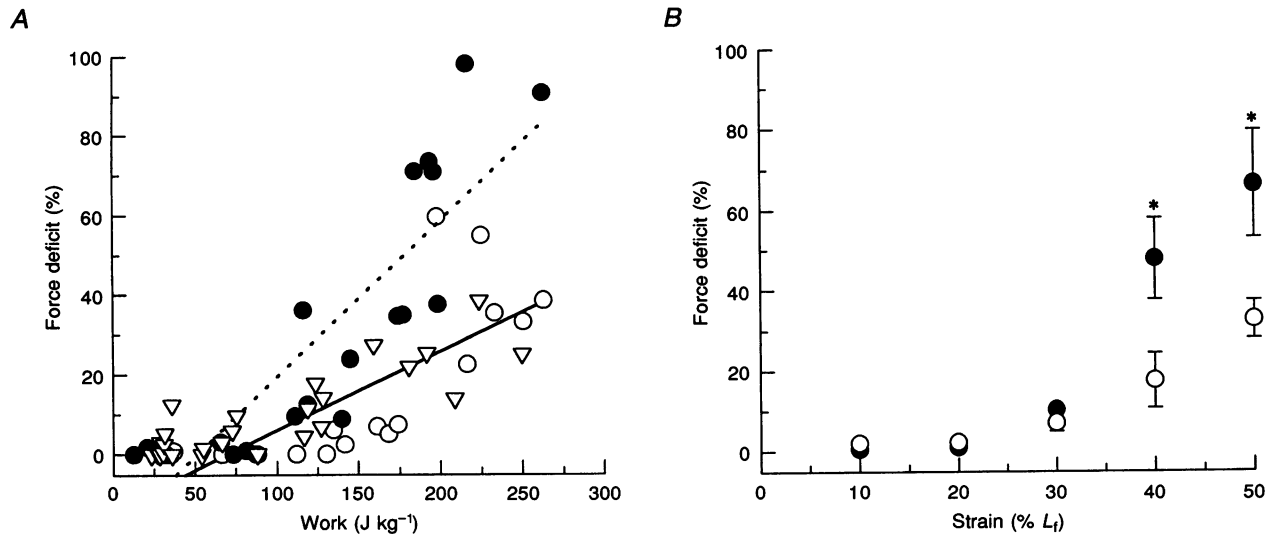

Figure 4. The force deficits following single stretches of maximally activated muscles

Data are presented for single stretches varying in magnitude but not velocity ($V = 2\ L_f\ s^{-1}$) for *in situ* EDL muscles of young (▽), adult (○) and old (●) mice in *A* and for pooled young and adult mice (○) and old mice (●) in *B*. The work input during the stretch is normalized by muscle wet mass (J kg^{-1}), strain is expressed as a percentage of optimum fibre length (L_f), and the force deficit observed 1 min after the stretch is expressed as a percentage of the isometric force developed just prior to the stretch. Each symbol in *A* indicates a data point from a single stretch. The coefficients of determination for the regression relationships for data from adult mice (continuous line) and old mice (dashed line) are 0·59 and 0·77, respectively. The slopes of the relationships, 0·20 for muscles in adult mice and 0·39 for muscles in old mice, are significantly different. Data for young mice ($r^2 = 0{\cdot}73$; slope = 0·13) are reproduced from Brooks *et al.* 1995. Data in *B* are presented as means ± 1 S.E.M. Sample size is from 3 to 12 for each point. * Significant difference ($P < 0{\cdot}05$) in the mean force deficits between the two groups.

The work input during single stretches of maximally activated muscles predicted the magnitude of the force deficit well for muscles in all age groups (Fig. 4*A*). Depending on the age group, the work explained between 59 and 78% of the variation in the force deficit. For muscles in young and adult mice, the relationships between the work input during a stretch and the resultant force deficit were not different (Fig. 4*A*). In contrast to the lack of difference between the relationships observed for young and adult mice, the work–force deficit relationship for muscles in old mice was significantly steeper than that of either of the other two age groups. The linear regression for data from muscles of adult and old mice are shown in Fig. 4*A* by the continuous and dashed line, respectively. The linear regression for the work–force deficit relationship for young mice has been presented previously (Brooks *et al.* 1995).

Although the coefficients of determination for the linear regressions are high (r^2, ~0·7), clearly single stretches that result in work inputs below some threshold value do not result in significant force deficits. The variability in both the work input and force deficit data precluded statistical determination of the threshold work values. Consequently, in addition to the comparisons of the work–force deficit

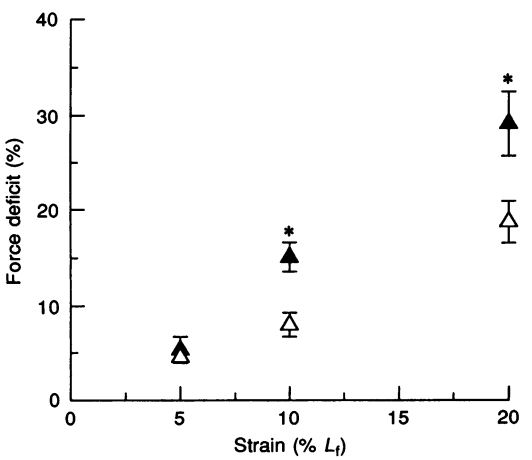

Figure 5. The force deficits observed immediately after single stretches of varying strains of maximally activated single permeabilized fibre segments

Data are presented as the mean ± 1 S.E.M. for single permeabilized fibre segments from EDL muscles of young (△) and old (▲) rats. 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 fibre segment prior to the stretch. Sample size is from 7 to 10 for each point. * Significant difference ($P < 0{\cdot}05$) in the mean force deficits between the two age groups.

relationships, the mean force deficits following single stretches at each strain were compared among the age groups. Because no differences existed between muscles in young and adult mice, these data were pooled. For both age groups, the force deficits following single stretches of 10 or 20% strain were not significantly different from zero (Fig. 4*B*). Consistent with the steeper work–force deficit relationship observed for muscles in old mice compared with young and adult mice, greater force deficits were observed for muscles in old compared with young and adult mice following single stretches of 40 and 50% strain as determined by Student's two-tailed *t* tests (Fig. 4*B*). A similar pattern was observed for maximally activated single permeabilized fibre segments from muscles of rats of different ages. The force deficits observed following 5% stretches were not different for fibres from old and young rats, but the mean force deficits following 10 and 20% stretches were 90 and 60% greater, respectively, for fibres from muscles of old compared with young rats (Fig. 5).

For passive whole muscles of adult and old mice, the production of significant force deficits required single stretches of sufficient strain to extend sarcomeres beyond thick and thin filament overlap, estimated to occur at a sarcomere strain of ~50% (Brooks *et al.* 1995). These data are consistent with data on *in situ* muscles in young mice (Brooks *et al.* 1995). Following single stretches of any given strain, no differences were observed among the three age groups for the mean force deficits of passive muscles. Similarly, following single stretches of 10, 20, 30 and 50% strain, the force deficits for passive single permeabilized fibres from EDL muscles of old rats were comparable with data reported for passive fibres from EDL muscles of adult rats (Macpherson *et al.* 1996).

DISCUSSION

Zerba *et al.* (1990) reported no difference in the force deficits of *in situ* EDL muscles of 3-month-old and 12-month-old mice 10 min and 3 days following seventy-five repeated pliometric contractions, and significantly greater force deficits at both time points for muscles in 27-month-old mice. Similarly, after single pliometric contractions we found no difference between the work–force deficit relationships for EDL muscles of young and adult mice but a steeper work–force deficit relationship for muscles in old mice. Both sets of data support the conclusion that, for the young and adult age groups, the susceptibility of muscle fibres to the damage associated with pliometric contractions is the same. In contrast, when conditions of force development, stretch, and number of contractions are sufficient to cause damage to muscle fibres, muscles in old animals will be injured more severely than muscles in young or adult animals. Furthermore, our data on the force deficits resulting from single stretches of maximally activated single permeabilized fibre segments from EDL muscles of young rats are in good agreement with the force deficits reported previously for the same types of fibres exposed to similar protocols (Macpherson *et al.* 1996). The greater force deficits that we observed for fibre segments from muscles of old rats compared with those from young rats support the conclusion that the basis for the increased susceptibility of muscles in old animals to the initial mechanical damage induced by stretch resides at least in part within the force-generating or force-transmitting structures of the myofibrils.

In determining the magnitude of injury, both the high forces developed during stretches (Fridén *et al.* 1983; Newham *et al.* 1983*a*; McCully & Faulkner, 1986; Warren, Hayes, Lowe & Armstrong, 1993*a*) and strains beyond optimum length for force development (Lieber & Fridén, 1993; Brooks *et al.* 1995) are of significance. This conclusion is supported by the coefficient of determination of greater than 0·70 between the work input during single pliometric contractions and the force deficit (Brooks *et al.* 1995; present study). Several investigators have proposed the working hypothesis that injury occurs when sarcomeres are stretched beyond thick and thin filament overlap (Newham *et al.* 1983*b*; Higuchi, Yoshioka & Maruyama, 1988; Wood *et al.* 1993). Individual sarcomeres might be pulled apart if local inhomogeneities exist in the strength of sarcomeres in series (Morgan, 1990). Consistent with the existence of weaker sarcomeres in series with stronger sarcomeres are the observations that, during maximal fixed-length contractions, sarcomeres at the ends of single intact fibres shorten while those in the middle region are stretched (Burton, Zagotta & Baskin, 1989), and sarcomeres at one end of single permeabilized fibre segments tend to shorten while those at the other end are stretched (Julian & Moss, 1980). Inequalities in sarcomere strength may arise from random variations in sarcomere lengths, in the number of cross-bridges attached at a given time, or in the inherent force-generating capacity of attached cross-bridges (Morgan, 1990). Lower metabolic rates of old animals (Mazzeo, Brooks & Horvath, 1984) reflect, at least in part, slower protein turnover in skeletal muscle (Richardson, 1981). Based on our observation that a given level of work input produces a greater force deficit for muscles of old compared with younger animals, we hypothesize that slower protein turnover results in a larger population of 'older' and weaker sarcomeres which are more likely to be stretched excessively and damaged.

The single permeabilized fibre preparation excludes any effects of single stretches on excitation–contraction coupling (Warren *et al.* 1993*c*), or on membrane or extracellular (Warren *et al.* 1993*b*) structures that transmit force. Consequently, we cannot rule out these effects as possible factors in the greater force deficits observed for the *in situ* whole muscles of old animals. Direct comparisons between data from experiments on single permeabilized fibres and those on whole muscles are difficult due to large differences in the work inputs required to induce comparable force deficits. In spite of these difficulties, the observation that the work input required to produce a given force deficit in permeabilized fibres is only one-third to one-fifth of that for

whole muscles indicates that membrane and extracellular force-transmitting structures do play a significant role in contraction-induced injury. Although connective tissue content (Alnaqeeb, Al Zaid & Goldspink, 1984) and the extent of collagen cross-linking (Palokangas, Kovanen, Duncan & Robins, 1992) increase in skeletal muscles with ageing, the lack of any difference among the age groups for the force deficits following single stretches of passive muscles *in situ* suggests that membrane and extracellular structures are not major factors in the increased susceptibility to injury of muscles in old animals. Finally, dramatic differences exist in the susceptibilities of different fibre types to contraction-induced injury (Jones *et al.* 1986; Macpherson *et al.* 1996), but because the percentages of type II fibres in EDL muscles of mice and rats of any age exceed 95% (Larsson & Edström, 1986; Florini & Ewton, 1989; Phillips, Wiseman, Woledge & Kushmerick, 1993), the samples of muscle fibre segments from both young and old rats probably consisted overwhelmingly of type II fibres. Consequently, we conclude that the greater susceptibility to contraction-induced injury observed for fibres in muscles of old animals was not the result of differences in fibre type.

Single contractions which result in work inputs of less than ~ 150 J kg^{-1} do not give rise to significant force deficits (Brooks *et al.* 1995; present study). In contrast, when loads are high, and sarcomere strains large, as may occur during accidental falls, the prevention of falls, or burst movements in sports events, severe injury to skeletal muscle fibres is highly probable (Brooks *et al.* 1995). The frequent occurrence of muscle injury under these circumstances supports the relevance of studying the injury induced by single contractions. Alternatively, the severe injuries observed following large numbers of small stretches (McCully & Faulkner, 1985; Zerba *et al.* 1990; Lieber & Fridén, 1993) demonstrate the importance of the number of contractions on the magnitude of injury. We conclude that activities involving either single or repeated contractions which are innocuous for muscles in young and adult animals may be injurious to muscles in old animals.

Alnaqeeb, M. A., Al Zaid, N. S. & Goldspink, G. (1984). Connective tissue changes and physical properties of developing and ageing skeletal muscle. *Journal of Anatomy* **139**, 677–689.

Balnave, C. D. & Allen, D. G. (1995). Intracellular calcium and force in single mouse muscle fibres following repeated contractions with stretch. *Journal of Physiology* **488**, 25–36.

Brooks, S. V. & Faulkner, J. A. (1988). Contractile properties of skeletal muscles from young, adult and aged mice. *Journal of Physiology* **404**, 71–82.

Brooks, S. V. & Faulkner, J. A. (1994). Isometric, shortening and lengthening contractions of muscle fiber segments from adult and old mice. *American Journal of Physiology* **267**, C507–513.

Brooks, S. V. & Faulkner, J. A. (1995). Injury to fibers following single stretches of extensor digitorum longus muscles of young, adult, and old mice. *Biophysical Journal* **68**, A75.

Brooks, S. V., Macpherson, P. C. D. & Faulkner, J. A. (1994). Contraction-induced injury to single permeabilized fibers from muscles of old rats. *Biophysical Journal* **66**, A305.

Brooks, S. V., Zerba, E. & Faulkner, J. A. (1995). Injury to muscle fibres after single stretches of passive and maximally stimulated muscles in mice. *Journal of Physiology* **488**, 459–469.

Burton, K., Zagotta, W. N. & Baskin, R. J. (1989). Sarcomere length behaviour along single frog muscle fibres at different lengths during isometric tetani. *Journal of Muscle Research and Cell Motility* **10**, 67–84.

Cutts, A. (1988). The range of sarcomere lengths in the muscles of the human lower limb. *Journal of Anatomy* **160**, 79–88.

Florini, J. R. & Ewton, D. Z. (1989). Skeletal muscle fiber types and myosin ATPase activity do not change with age or growth hormone administration. *Journals of Gerontology* **44**, B110–117.

Fridén, J., Sjöström, M. & Ekblom, B. (1983). Myofibrillar damage following intense eccentric exercise in man. *International Journal of Sports Medicine* **4**, 170–176.

Higuchi, H., Yoshioka, T. & Maruyama, K. (1988). Positioning of actin filaments and tension generation in skinned muscle fibres released after stretch beyond overlap of the actin and myosin filaments. *Journal of Muscle Research and Cell Motility* **9**, 491–498.

Jones, D. A., Newham, D. J., Round, J. M. & Tolfree, S. E. J. (1986). Experimental human muscle damage: morphological changes in relation to other indices of damage. *Journal of Physiology* **375**, 435–448.

Julian, F. J. & Moss, R. L. (1980). Sarcomere length–tension relations of frog skinned muscle fibres at lengths above the optimum. *Journal of Physiology* **304**, 529–539.

Larsson, L. & Edström, L. (1986). Effects of age on enzyme-histochemical fibre spectra and contractile properties of fast- and slow-twitch skeletal muscles in the rat. *Journal of the Neurological Sciences* **76**, 69–89.

Lieber, R. L. & Fridén, J. (1993). Muscle damage is not a function of muscle force but active muscle strain. *Journal of Applied Physiology* **74**, 520–526.

Lynch, G. S., Duncan, N. D., Campbell, S. P. & Williams, D. A. (1995). Endurance training effects on the contractile activation characteristics of single muscle fibres from the rat diaphragm. *Clinical and Experimental Pharmacology and Physiology* **22**, 430–437.

McCully, K. K. & Faulkner, J. A. (1985). Injury to skeletal muscle fibres of mice following lengthening contractions. *Journal of Applied Physiology* **59**, 119–126.

McCully, K. K. & Faulkner, J. A. (1986). Characteristics of lengthening contractions associated with injury to skeletal muscle fibres. *Journal of Applied Physiology* **61**, 293–299.

Macpherson, P. C. D., Schork, M. A. & Faulkner, J. A. (1996). Contraction-induced injury to single permeabilized muscle fibers from fast and slow muscles of the rat following single stretches. *American Journal of Physiology* **271**, (in the Press).

Mazzeo, R. S., Brooks, G. A. & Horvath, S. M. (1984). Effects of age on metabolic responses to endurance training in rats. *Journal of Applied Physiology* **57**, 1369–1374.

Morgan, D. L. (1990). New insights into the behavior of muscle during active lengthening. *Biophysical Journal* **57**, 209–221.

Newham, D. J., Jones, D. A. & Edwards, R. H. T. (1983*a*). Large delayed creatine kinase changes after stepping exercise. *Muscle and Nerve* **6**, 380–385.

NEWHAM, D. J., MCPHAIL, G., MILLS, K. R. & EDWARDS, R. H. T. (1983*b*). Ultrastructural changes after concentric and eccentric contractions of human muscle. *Journal of the Neurological Sciences* **61**, 109–122.

PALOKANGAS, H., KOVANEN, V., DUNCAN, A. & ROBINS, S. P. (1992). Age-related changes in the concentration of hydroxypyridinium crosslinks in functionally different skeletal muscles. *Matrix* **12**, 291–296.

PHILLIPS, S. K., BRUCE, S. A. & WOLEDGE, R. C. (1991). In mice, the muscle weakness due to age is absent during stretching. *Journal of Physiology* **437**, 63–70.

PHILLIPS, S. K., WISEMAN, R. W., WOLEDGE, R. C. & KUSHMERICK, M. J. (1993). Neither changes in phosphorus metabolite levels nor myosin isoforms can explain the weakness in aged mouse muscle. *Journal of Physiology* **463**, 157–167.

RICHARDSON, A. (1981). The relationship between aging and protein synthesis. In *Handbook of Biochemistry in Aging*, ed. FLORINI, J. R., pp. 79–101. CRC Press, Inc., Boca Raton, FL, USA.

SWEENEY, H. L., CORTESELLI, S. A. & KUSHMERICK, M. J. (1987). Measurements on permeabilized skeletal muscle fibers during continuous activation. *American Journal of Physiology* **252**, C575–580.

WARREN, G. L., HAYES, D. A., LOWE, D. A. & ARMSTRONG, R. B. (1993*a*). Mechanical factors in the initiation of eccentric contraction-induced injury in rat soleus muscle. *Journal of Physiology* **464**, 457–475.

WARREN, G. L., HAYES, D. A., LOWE, D. A., PRIOR, B. M. & ARMSTRONG, R. B. (1993*b*). Materials fatigue initiates eccentric contraction-induced injury in rat soleus muscle. *Journal of Physiology* **464**, 477–489.

WARREN, G. L., LOWE, D. A., HAYES, D. A., KARWOSKI, C. J., PRIOR, B. M. & ARMSTRONG, R. B. (1993*c*). Excitation failure in eccentric contraction-induced injury of mouse soleus muscle. *Journal of Physiology* **468**, 487–499.

WOOD, S. A., MORGAN, D. L. & PROSKE, U. (1993). Effects of repeated eccentric contractions on structure and mechanical properties of toad sartorius muscle. *American Journal of Physiology* **265**, C792–800.

ZERBA, E., KOMOROWSKI, T. E. & FAULKNER, J. A. (1990). Free radical injury to skeletal muscle of young, adult, and old mice. *American Journal of Physiology* **258**, C429–435.

Acknowledgements

The research was supported by a National Institute on Aging Grant, AG-06157.

Author's email address

S. V. Brooks: svbrooks@umich.edu

Received 25 March 1996; accepted 5 September 1996.