Severity of contraction-induced injury is affected by velocity only during stretches of large strain

SUSAN V. BROOKS AND JOHN A. FAULKNER
Departments of Physiology and Biomedical Engineering, Institute of Gerontology, University of Michigan, Ann Arbor, Michigan 48109-2007

Received 23 August 2000; accepted in final form 26 March 2001

Brooks, Susan V., and John A. Faulkner. Severity of contraction-induced injury is affected by velocity only during stretches of large strain. J Appl Physiol 91: 661–666, 2001.—Our purpose was to investigate the effect of velocity of stretch on contraction-induced injury to whole skeletal muscles. Single stretches provide an effective method for studying factors that initiate contraction-induced injury. We tested the null hypothesis that the severity of injury is not dependent on the velocity of the stretch. From the plateau of maximum isometric contractions, extensor digitorum longus muscles of mice were administered single stretches in situ of 30–50% strain relative to muscle fiber length ($L_o$) at rates of 1–16 $L_o$/s. The magnitude of injury was represented by the isometric force deficit 1–10 min after the stretch. Although the null hypothesis was not supported because the force deficit was affected by velocity ($r^2 = 0.09$), the effect was relatively weak and was not significant except at the largest strain. Velocity had no effect on peak or average force or work input, factors established to have significant relationships with the force deficit. Velocity may play a minor role in contraction-induced injury, but its importance is negligible relative to that of strain.

SKELETAL MUSCLES IN VIVO ROUTINELY function not only as stabilizers and motors during the performance of fixed length and shortening contractions, respectively, but also as shock absorbers when muscles are stretched during lengthening contractions. Muscles exposed to single or repeated lengthening contractions show significant deficits in maximum force (3, 14, 18, 22, 32), histological (15, 16, 19, 24), and ultrastructural (3, 14, 23, 29, 32) evidence of damage and shifts to longer optimum lengths for isometric force (14, 28, 32). The damage to skeletal muscle immediately after a protocol of lengthening contractions appears to occur to small groups of sarcomeres widely dispersed throughout individual muscle fibers (21) and among fibers (24), making quantitative morphometric analysis difficult. Although the exact quantitative relationship between the number of disrupted sarcomeres and the decrease in force has not been determined, for a given contraction protocol the deficit in force generation gives the most reliable and reproducible measure of the amount of damage (3, 11, 14, 23).

Single stretches of both whole muscles (3) and single muscle fibers (20, 21) provide an effective method for studying the direct association of the initial injury with specific mechanical events within contracting muscles. After a single lengthening contraction, the strongest predictors of the magnitude of the force deficit are strain beyond optimum length ($L_o$) for force development and the work done during a stretch (3, 21). Two studies of isolated whole muscles in vitro have produced conflicting results regarding the effect of the velocity of stretch on the force deficit. Using protocols of five lengthening contractions of rat soleus muscles, Warren et al. (31) reported that a small percentage of the variation in the force deficit was explained by velocity, but velocities of stretch were restricted to $\sim 8$ to $\sim 25 \%$ of the $V_{\text{max}}$. Furthermore, the most injurious protocol produced a force deficit of only $\sim 14 \%$. In contrast, after 5–60 lengthening contractions of toad sartorius muscles, Talbot and Morgan (28) found no relationship of force deficit with velocity, but only two velocities, $\sim 50$ and $\sim 67 \%$ of $V_{\text{max}}$, were studied. A study using single permeabilized fibers also found no effect of velocity of stretch on the force deficit immediately after single lengthening contractions (20), but the authors cautioned against the generality of the conclusion based on a high incidence of fiber breakage during stretches at the highest velocity. Our purpose was to analyze the magnitude of the damage to in situ extensor digitorum longus (EDL) muscles of mice after single maximum lengthening contractions over a wide range of velocities. We tested the null hypothesis that after single stretches of maximally activated muscles the force deficit is not dependent on the velocity of stretch.

METHODS

Data were collected on 51 specific-pathogen-free, male, CD-1 mice of mean body mass 31.9 $\pm$ 3.9 (SD) g. Before 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 Guide for...
the Care and Use of Laboratory Animals [DHHS Publication No. 85-23 (NIH), Revised 1985, Office of Science and Health Reports, Bethesda, MD 20892]. For each experimental procedure, mice were anesthetized with intraperitoneal injections of pentobarbital sodium (80 mg/kg). Supplemental doses were administered as needed to maintain an adequate depth of anesthesia.

Measurements of whole muscle mechanics in situ. Whole muscle mechanics were measured in situ as described previously (3). A small incision was made at the ankle, and the distal tendon of the EDL muscle was exposed. Nylon suture (5-0) was tied tightly around the tendon immediately adjacent to the distal end of the muscle fibers. The tendon was cut distal to the suture, folded back, and the suture was once again tied tightly around it. This method prevented the tendon from slipping during stretches. The mouse was placed on a platform maintained at 37°C. The experimental limb tendon from slipping during stretches. The mouse was placed on a platform maintained at 37°C. The experimental limb was stabilized by securing the knee within a clamp between two sharpened screws and taping the foot to the surface of the platform. The tendon of the EDL muscle was attached to the tip of the lever arm of a servomotor (range of 5 N; model 305, Cambridge Technology, Watertown, MA) that controlled the length of the muscle and measured the force developed by the muscle. The stabilization of the limb and the attachment to the transducer provided a system of low compliance, estimated to be <3 μm/g.

Throughout the experiment, the small region of exposed muscle and tendon was kept moist by periodic applications of 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 isotonic twitch force. The muscle was stimulated at increasing frequencies until a maximum force (P0) was reached, typically at ~250 Hz. With the muscle at L0, muscle length was measured with calipers, on the basis of well-defined anatomic landmarks determined previously by extensive dissections of CD-1 mice. Muscle fiber length (Lf) was determined by multiplying the muscle length at L0 by the L0/Lf ratio of 0.44 (3). For the 84 EDL muscles included in the study, the mean Lf was 5.78 ± 0.24 (SD) mm.

P0 was measured immediately before a single stretch and again beginning 1 min after the stretch and at 1-min intervals until the force stabilized (Fig. 1). A stable force was defined as a smaller than 1% variation in force from one minute to the next. After the final in situ force measurement, EDL muscles were removed from the mice, and the mice were killed with an overdose of the anesthetic. The tendons were trimmed, and the muscle was blotted dry and weighed. The mean wet mass of 84 experimental muscles was 10.4 ± 1.7 (SD) mg. Total muscle fiber cross-sectional area was calculated by dividing the wet mass of the muscle by the product of the Lf and the density of skeletal muscles, 1.06 mg/mm3. The P0 was divided by total muscle fiber cross-sectional area to obtain the specific P0 (kN/m2). Before the single stretch, the mean value for specific P0 was 230 ± 25 (SD) kN/m2 (n = 84). The wet mass, P0, and specific P0 of control EDL muscles were similar to values reported by our laboratory previously (3).

Protocol for single stretches. Each muscle was exposed to a single stretch in situ with the muscle stimulated at the frequency that resulted in P0. Stretches were initiated from the plateau of an isometric contraction at L0. Stimulation was terminated at the end of the lengthening ramp, and muscles were returned to L0 at the same velocity as occurred during lengthening. Both the magnitude and velocity of the stretches were varied systematically. For EDL muscles, a single stretch with a length change of 30% strain (%L0) was required to produce a significant force deficit (3). Although fibers in EDL muscles in vivo are typically exposed to strains of no greater than ~35% (1), for pennate muscles that extend across two joints, such as the gastrocnemius muscle, a 60% strain of muscle fibers is within the physiological range of motion. Consequently, stretches of 30, 40, and 50% strain were used in the present study, with the 50% stretches included as a model of other muscles.

Muscules were stretched at a velocity of 1, 2, 4, 8, or 16 L0/s. On the basis of muscle temperatures measured in the present study of 31 ± 1°C (SD) and a temperature coefficient of 1.6 for Vmax of EDL muscles of mice between 25 and 35°C (9), we calculated a Vmax of ~14 L0/s for in situ mouse EDL muscles. Consequently, the velocities used corresponded to a range from less than 10% of Vmax to slightly greater than 100% Vmax. This range of velocities was designed to encompass the range expected for muscles in vivo, particularly during accidental falls or during the rapid decelerations characteristic of movements in vigorous sports events.

During stretches, force was not controlled but varied freely, and forces produced were measured. The peak force achieved during a contraction was measured directly, and average force was determined by integrating the force-time curve during the lengthening ramp and dividing the value by the duration of the ramp stretch. Both the work done to stretch the muscle and power absorbed during the stretch were calculated for each muscle and normalized by muscle wet mass. High peak forces, >1 N, were achieved briefly during some stretches (see Fig. 1 of Ref. 3), but previous studies indicate that muscles are not near their limits of extensibility during stretches of the magnitudes used in the present study (3, 17).

Evaluation of contraction-induced injury. The magnitude of the injury resulting from a single stretch was assessed by the deficit in the ability to generate isometric force. In a previous study that used one relatively low velocity of stretch (3), force remained stable during many minutes subsequent to an initial measurement made at 1 min. In the present study, force measurements were made at 1-min intervals.
RESULTS

Previous studies of maximally activated in situ EDL muscles in mice using a single velocity of stretch (3) showed that 90% of the variability in the work done to stretch a muscle was explained by the magnitude of the stretch. Despite a 16-fold range of velocities used in the present study, 70% of the variation in work input was still explained by strain (Fig. 2). Under circumstances of a single velocity of stretch, strain and work input were equally strong predictors of the magnitude of injury (3). In the one-variable regression models in the present study (Table 1), strain and work also provided the strongest predictions of the force deficit with either variable giving a coefficient of determination of ~30%. Although strain and work were the best predictors of the force deficit after single stretches of maximally activated muscles, the velocity of stretch and the power absorbed during a stretch each also had a significant effect on the force deficit (Table 1). Although the relationships were significant, the coefficients of determination for velocity and power were relatively poor with 9% of the variation in the force deficit explained by velocity and 12% by power. The similarity in the relationships of the force deficit with either velocity or power absorbed is explained by the observation that, across all strains used in the present study, velocity explained 94% of the variation in the power absorbed during the stretch (Fig. 2).

For the relationship between velocity and force deficit with the inclusion of the total sample of muscles, the low coefficient of determination was due in large part to a statistically significant \((P = 0.01)\) interaction between velocity and strain. The interaction resulted from the effect on force deficit of varying velocity being

\[ \text{Work} = r^2 = 0.70, P < 0.001 \]

\[ \text{Power} = r^2 = 0.94, P < 0.001 \]

\[ \text{Strain} = r^2 = 0.01, P = 0.37 \]

\[ \text{Velocity} = r^2 = 0.0005, P = 0.84 \]
Fig. 3. Force deficits after single stretches of maximally activated muscles. Values are presented for single stretches varying in both strain and velocity of in situ extensor digitorum longus muscles of mice. Each symbol indicates a data point from a single stretch, and vertical bars show means ± SE. Sample size is 5–8 for each bar. Strain is expressed as %L
, and velocity is expressed in L/s. Force deficits were calculated from the stable force values 1–10 min after the stretch and are expressed as a percentage of the isometric force just before the stretch. Within a given strain, the shading of the bar indicates a significant (P < 0.05) differences in the mean force deficit between velocities. For stretches of 30% strain, the mean force deficits were not different for any velocity. At 50% strain, mean force deficits were not different for 1, 2, and 4 L/s, or for 8 and 16 L/s, but mean force deficits after stretches at 8 or 16 L/s were greater than those for 1, 2, or 4 L/s. Lowercase letters below the bars indicate differences in the mean force deficit between strains for a given velocity. For example, for stretches at 1 L/s, mean force deficits were not different for 30% (a) and 40% (a, b) strain or for 40% (a, b) and 50% (b) strain but were different for 30% (a) and 50% (b) strain.

Table 1. Effect of variables on force deficit

<table>
<thead>
<tr>
<th>Variable</th>
<th>One-Variable Models</th>
<th>Stepwise Multiple-Regression Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All data (n = 84)</td>
<td>30% Strain (n = 27)</td>
</tr>
<tr>
<td>Strain</td>
<td>0.35</td>
<td>0.19</td>
</tr>
<tr>
<td>Velocity</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>Peak force</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Average force</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Work</td>
<td>0.26</td>
<td>NS</td>
</tr>
<tr>
<td>Power</td>
<td>0.12</td>
<td>NS</td>
</tr>
<tr>
<td>Model r²</td>
<td>0.26</td>
<td>0.09</td>
</tr>
</tbody>
</table>

Values are coefficients of determination and partial coefficients of determination for one-variable and stepwise multiple-regression models, respectively, of the force deficit after single stretches of maximally activated in situ extensor digitorum longus muscles of mice. n, No. of muscles. For velocity and power, one-variable models were determined with data from stretches of different magnitudes (30, 40, or 50% strain) analyzed separately as well as for the entire data set (all data). The coefficient of determination of the multiple regression model, including strain and velocity, is also given. NS, contribution of the variable to the variance in the force deficit did not reach a level of significance (P < 0.05) necessary for inclusion in the model.

highly dependent on the strain (Fig. 3) with increasing proportions of the force deficit explained by velocity as the size of the stretch increased. The interaction between velocity and strain is illustrated in Table 1, which shows the relationships of force deficit with velocity and power when data were grouped by strain. For stretches of 30% strain, the force deficit was unrelated to velocity or power. In contrast, as the size of the stretch was increased to 40 and subsequently 50% strain, 19 and 31%, respectively, of the variation in the force deficit was explained by velocity (Table 1). The complexity of the interaction of strain and velocity was emphasized further by the observation that, over the five different velocities, stretches of 50% strain resulted in force deficits that spanned the entire range from ~0 to ~100% (Fig. 3).

As with the one-variable models, the stepwise multiple-regression analysis indicated that strain was by far the dominant factor in explaining the force deficit after single stretches of maximally activated muscles. The stepwise regression model demonstrated that the combination of strain and velocity was more effective in predicting the force deficit than strain alone (Table 1) and that velocity added little to the predictive value of the regression model. The linear combination of strain and velocity in the multiple-regression model explained 44% of the variation in force deficit, with partial coefficients of determination of 35% for strain and only 9% for velocity.

Unlike previous studies in which peak force and average force each had a significant effect on the force deficit after a single stretch at a single velocity (3, 24), when a wide range of velocities was used, neither peak nor average force was a significant predictor of the force deficit (Table 1). In addition, at any given strain or when all strains were analyzed together, velocity had no effect on the peak force (Fig. 4), average force (Fig. 4), or work done (Fig. 2) during the stretch.

**DISCUSSION**

On the basis of the significant relationship between force deficit and the velocity of stretch, we rejected our
hypothesis that the magnitude of damage after a single lengthening contraction would be independent of velocity. Despite our overall rejection of the hypothesis, the relationship between force deficit and velocity was weak, particularly when compared with that of force deficit and strain. The superiority of strain as a predictor of force deficit is consistent with previous studies of whole muscles (3, 18, 28) and single permeabilized fibers (20, 21). Furthermore, even the weak relationship between force deficit and velocity was restricted to combinations of high velocities and large strains. In addition, the considerable variability in force deficits after single stretches at high velocities and large strains raises questions as to the physiological relevance of the result. In the group exposed to a 50% strain at 8 \( L_f/s \), force deficits were almost 100% for two muscles and <40% for others. The large and variable force deficits are reminiscent of those observed previously after large strains of passive muscles (3) or stretches that approached the threshold of complete mechanical failure (11). The magnitudes of strain in the present study were less than half the breaking strain of mouse EDL muscles (17), but the velocities were much higher than those studied in association with failure properties of muscles. The combined high velocity-high strain conditions in the present study may have placed some fibers near their threshold for tears (30).

In the present study, the range of velocities, from \( \sim 7 \) to \( \sim 1/144 \% V_{max} \), was of greater magnitude than in any previous study of contraction-induced injury. Many contractions of muscles associated with vigorous sports events, such as jumping, sprinting, kicking, and throwing, result in stretches of activated muscles at velocities within this range (5, 12). The velocities were also comparable to those required of human muscles in vivo to arrest a fall (K. M. DeGoede and J. A. Ashton-Miller, unpublished observations; 4). During the rapid phase of the arrest of a mild fall, the rotation of the elbow joint occurs at \( \sim 1,000/s \) over at least 10° (DeGoede and Ashton-Miller, unpublished observations; 4). The corresponding stretch of individual fibers in the triceps brachii muscle occurs at a velocity of 3 \( L_f/s \) (6, 25). By three independent methods, \( V_{max} \) for human triceps brachii muscles was estimated to be \( \sim 4 L_f/s \). The three estimates were based on (1) twitch contraction times of human triceps brachii muscles (10) and the relationship between the reciprocal of the contraction time and \( V_{max} \) (7), (2) \( V_{max} \) measured on single permeabilized fibers from human triceps muscles (10) corrected to 37°C, and (3) the force-velocity relationships of bundles of human fibers of varying fiber type composition (8) and the composition of human triceps brachii muscle (10). On the basis of a \( V_{max} \) of 4 \( L_f/s \), triceps brachii muscles were stretched at \( \sim 75\% V_{max} \) during the mild falls studied by DeGoede and Ashton-Miller (unpublished observations) and Dietz et al. (4). During unexpected falls, much higher velocities over larger arm deflections are certain. The potential for damaging stretches during falls is amplified by high levels of activation during the fall arrest (DeGoede and Ashton-Miller, unpublished observations; 4) and the high load involved with absorbing essentially the entire body weight by the triceps brachii muscle.

Previous investigations of the effect of velocity on contraction-induced injury have involved protocols of repeated lengthening contractions of whole muscles in vitro (28, 31) and in situ (22) and single stretches of single permeabilized fibers (20). The single-stretch protocols have significant advantages. The absence of fatigue permits investigations of the impact of velocity of stretch on the initial injury (20), and the added complication of the effect of the number of contractions on the magnitude of the damage is avoided. Using two velocities of stretch of similar magnitudes, \( \sim 50 \) and \( \sim 67\% V_{max} \), Talbot and Morgan (28) reported that, for toad sartorius muscles, force deficit was not affected by velocity. After stretches of single permeabilized fibers with a range of velocities similar to that of the present study and strains of 5–20%, Lynch and Faulkner (20) found no effect of velocity on force deficit. The results of these two studies (20, 28) are consistent with the observation of McCully and Faulkner (22) that 3 days after an in situ protocol of repeated lengthening contractions, the contraction-induced injury was not related to the velocity of stretch. Contrary to the reports of no effect of velocity on force deficit (20, 22, 28), Warren et al. (31) reported that force deficit was dependent on velocity for whole muscles in vitro after stretches with maximum strains of \( \sim 30\% \) and maximum velocities of \( \sim 25\% V_{max} \). Despite the similarity of the conclusion of the present study with that of Warren et al., comparisons of our findings with any of these previous experiments (20, 22, 28, 31) are confounded by the use of different preparations, different numbers of contractions, different fiber types, and different species.

The restriction to stretches of small strains by Lynch and Faulkner (20) was necessary because of a high incidence of fiber breakage during larger strains. Fiber breakage appeared to result from the disruption of the membrane and supporting structures during the process of permeabilization (20). In the permeabilized-fiber preparation, the disruption of the membrane excludes any effects on the force deficit of excitation-contraction coupling (2) or damage to membrane and extracellular structures that normally transmit forces (26, 27). Consequently, these factors arise as potentially responsible for any effects of velocity on the force deficit observed for whole muscles in situ but not for single permeabilized fibers. Impairments in excitation after stretches of activated muscles are maintained for at least 60 min (2) and may remain for several days (13). In contrast, the rapid partial recovery of force observed in the present study after some stretches at high velocity suggests that the effect of velocity to increase the force deficit was not due to further disruption of excitation-contraction coupling.

Despite the dominance of strain and work as predictors of the force deficit in the present, as well as previous, studies (3, 18, 20, 21, 28), only \( \sim 30\% \) of the variation in the force deficit in the present study was
explained by either of these variables. The coefficient of determination of $-30\%$ is drastically different from that of $-70\%$ reported in our laboratory's previous studies. The key difference appears to be that our laboratory's previous studies included only a single velocity of stretch (3). Similarly, for maximally activated single permeabilized fibers, Lynch and Faulkner (20) reported a coefficient of determination of only $52\%$ between strain and force deficit for single stretches at three different velocities compared with a coefficient of determination of $-80\%$ for fibers stretched at a single velocity (21). As proposed by Lynch and Faulkner (20), the lower coefficients of determination for both strain and work in the present study compared with those reported previously (3) are likely explained by an increased variability in the force deficits introduced by the 16-fold range in velocity. The effect in the present study of velocity on force deficit, albeit small, is independent of any effect of velocity, overall or at any given strain, on the peak force, the average force, or the work done during the stretch. Peak force (22, 31), average force (3, 21), and work (3, 20, 21) are all factors reported previously to play significant roles in the magnitude of the force deficit.

This research was supported by National Institute on Aging Grant AG-06157.

REFERENCES


