Contraction-induced injury to single muscle fibers: velocity of stretch does not influence the force deficit

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Lynch, Gordon S., and John A. Faulkner. Contraction-induced injury to single muscle fibers: velocity of stretch does not influence the force deficit. Am. J. Physiol. 275 (Cell Physiol. 44): C1548–C1554, 1998.—We tested the null hypothesis that the severity of injury to single muscle fibers following a single pliometric (lengthening) contraction is not dependent on the velocity of stretch. Each single permeabilized fiber obtained from extensor digitorum longus muscles of rats was maximally activated and then exposed to a single stretch of either 5, 10, or 20% strain [% of fiber length (Lf)] at a velocity of 0.5, 1.0, or 2.0 Lf/s. The force deficit, the difference between maximum tetanic isometric force (P o) before and after the stretch expressed as a percentage of the control value for P o before the stretch, provided an estimate of the magnitude of muscle injury. Despite a fourfold range from the lowest to the highest velocities, force deficits were not different among stretches of the same strain. At stretches of 20% strain, even an eightfold range of velocities produced no difference in the force deficit, although 40% of the fibers were torn apart at a velocity of 4 Lf/s. We conclude that, within the range of velocities tolerated by single permeabilized fibers, the severity of contraction-induced injury is not related to the velocity of stretch.

Skeletal muscle; skinned fibers; muscle damage; pliometric contraction

CONTRACTION-INDUCED INJURY to skeletal muscle fibers is a ubiquitous phenomenon most often associated with pliometric (lengthening) contractions (23). After single (6, 22) or repeated (18, 23, 34) pliometric contractions, a number of mechanical factors have been implicated in the initiation of the injury. These mechanical factors include the force developed (6, 22, 23, 34), the strain beyond optimum length for force development (5, 6, 18, 22), a combination of strain and average force, i.e., the work done to stretch the muscle fibers (5, 6, 22), the initial and final lengths of the muscle before and after stretch (14, 35), and the number of pliometric contractions performed (23). The damage to skeletal muscle immediately following a protocol of pliometric contractions appears to occur to small groups of sarcomeres dispersed throughout individual muscle fibers (6, 9, 12, 27, 28). The initial mechanical injury initiates a cascade of events that produces a more severe secondary, delayed-onset injury between 1 and 3 days after the initial injury (1, 9). The cascade includes an inflammatory response, free radical damage, phagocyte infiltration, and eventual phagocytosis of the cytoplasm in portions of the damaged fibers (9, 28).

Light or electron microscopy provides the only direct evidence of damage to muscle fibers, but microscopy does not provide a measure of the magnitude of the damage because of the focal nature of contraction-induced injury (9, 23, 24, 27, 28). After a protocol of pliometric contractions, the deficit in maximum isometric tetanic force (P o) provides potentially the best quantitative measure of the totality of the damage to skeletal muscle fibers (9). Unfortunately, repeated pliometric contractions cause both fatigue and injury, and each increases the force deficit. After a protocol of repeated pliometric contractions, the force deficit provides a valid measure of damage only after complete recovery from fatigue (9, 23, 24). For studies of the mechanical properties that contribute to contraction-induced injury, a single stretch is employed to obtain a force deficit that is free of fatigue (5, 6, 14, 21, 22).

One mechanical factor that has not been studied extensively but is potentially an influence on the magnitude of contraction-induced injury to skeletal muscle fibers is the velocity of stretch (24, 34). Most studies of contraction-induced injury have been conducted at muscle temperatures of 35–37°C, and the single velocity of stretch chosen has taken values that clustered between 0.5 and 2 fiber lengths/s (Lf/s). The studies have included in situ measurements of whole extensor digitorum longus (EDL) muscles of mice (6, 14, 23, 24), in vitro measurements of slow-twitch soleus muscles from rats (34), and in situ measurements of fast-twitch tibialis anterior muscles in rabbits (18). For fast- and slow-twitch muscles of mice, rats, and rabbits at 35°C, the velocities of stretch range from 4 to 30% of the maximum velocity of unloaded shortening (V o max). Single-stretch protocols of single permeabilized muscle fiber segments studied at 15°C have involved velocities similar to those used for whole muscles at 35°C (5, 21, 22), and experiments on single intact fibers have used velocities of 5 Lf/s with fibers at 22°C (2). With a Q 10 of 1.8 for V o max (30), the reduction in muscle temperature from 35 to 15°C produces an almost fourfold increase in the percentage of V o max attributable to a given velocity of stretch.

Two studies (23, 34) have investigated the force deficit induced in whole muscles by protocols of repeated pliometric contractions performed at different velocities of stretch, with each providing some evidence that the higher velocities produce a greater force deficit. Our purpose was to investigate the effect of the velocity of a single stretch on the magnitude of the contraction-induced injury observed for single permeabilized fiber segments from the fast-twitch EDL muscles of rats. We tested the null hypothesis that, as measured...
by the force deficit, the severity of the injury to maximally activated single muscle fibers following a single stretch is not dependent on the velocity of stretch.

METHODS

Experiments were performed on 145 single permeabilized fast-twitch fibers obtained from the EDL muscles of adult male Fischer 344 rats. The single permeabilized fiber preparation constitutes a valid preparation for the testing of our hypothesis because it has provided a valid preparation for testing hypotheses regarding the role of average force and strain in the induction of the injury in mice and rats with differences in age (5) and with differences in fiber type (22). A number of factors led to the selection of fast-twitch fibers for this study: 1) most of the data on contraction-induced injury have been collected on fast-twitch EDL muscles because investigations of the secondary damage and the subsequent recovery required an in situ muscle preparation and the EDL muscle was chosen for its accessibility (6, 9, 23, 24); 2) fast motor units are recruited selectively during stretches of activated muscles, and consequently fast-twitch fibers are most frequently exposed to pliometric contractions and are at the greatest risk of being injured (26); and 3) at different velocities of stretch, fast-twitch fibers provide a greater precision in the estimate of damage than slow-twitch fibers, because, after a single pliometric contraction of any given strain, the force deficit is fivefold greater for fast- than for slow-twitch fibers (22). The rats were anesthetized deeply with sodium pentobarbital. The EDL muscles were dissected tendon-to-tendon from both hindlimbs and then blotted dry on filter paper (Whatman no. 1). After the removal of the muscles, the rats were given an overdose of sodium pentobarbital and successful euthanasia was assured by opening the thoracic cavity.

Muscle fiber preparation. The excised muscles were tied to capillary tubes with silk suture, with the muscles at approximately resting length, placed in a skinning solution with a composition of (in mM) 125 potassium phosphate, 5 EGTA, 2 ATP, 2 MgCl₂, and 20 imidazole and 50% (vol/vol) glycerol (adjusted to pH 7.0 with 4 M KOH), and then stored at −20°C for up to 3 mo until required (20). For the measurement of single permeabilized fiber mechanics, the muscle was placed in a small petri dish with skinning solution. A bundle of permeabilized fibers was dissected from the muscle and pinned into the Sylgard base of the petri dish (Dow Corning, Midland, MI). Single fiber segments were plucked carefully from the muscle bundle, and small loops of 9-0 braided silk were tied to each end of an isolated fiber. This method of fiber attachment has been shown to reduce fiber end compliance significantly (16), to values even lower than the ~2–3% reported by one of us previously (21). The fiber was then transferred to a small bath filled with relaxing solution with a composition (in mM) of 7 EGTA, 20 imidazole, 5.4 MgCl₂, 14.5 creatine phosphate, 4.74 ATP, and 79 KCl, as well as 16 μM CaCl₂ (pCa 9.0). One end of the fiber segment was tied directly to a fixed post attached to a force transducer (model 400A, Cambridge Technology, Cambridge, MA), and the other end was tied directly to a post attached to the lever arm of a servomotor (model 300, Cambridge Technology). A Polaroid photomicrograph (×400) was taken, and the length of 100 sarcomeres was measured with an ocular graticule. Based on this measurement, the length of the fiber segment (L₁) was set to provide a sarcomere length of ~2.7 μm, which is within the optimal range for maximum force production of single permeabilized muscle fibers from the rat (32). Fiber width was measured using a stereomicroscope (Wild M3Z, Wild Heerbrugg) coupled with a high-power objective and camera system (models MPS 51 S and MPS45, Wild Heerbrugg). Fiber cross-sectional area (CSA) was estimated by assuming a circular fiber geometry, with a subsequent adjustment to an ellipse by multiplication by 1.7, based on previous measurements of width and depth of fibers (4, 5, 21, 22). All displacements of the servomotor lever arm and force sampling of single fibers were controlled by a microcomputer running ASYST software (Macmillan Software, New York, NY). Experiments were performed at 15°C.

Contractile activation and pliometric contraction protocol. Fibers were maximally activated by immersion in a bath containing activating solution with a composition (in mM) of 7 EGTA, 20 imidazole, 5.3 MgCl₂, 14.5 creatine phosphate, 4.81 ATP, 64 KCl, and 7 CaCl₂, (pCa 4.5). To maintain structural stability, fibers were cycled between an isometric contraction and short periods of isovelocity shortening (2 L₁/s), followed by a rapid return to initial L₁ (3, 21, 22). For the collection of experimental data, fibers were stretched between cycles. The P₀ was measured at L₁ immediately before each stretch. A single pliometric contraction protocol was employed to permit a direct comparison between the velocity of stretch and a force deficit free of fatigue (5, 6, 14, 21, 22). In each experiment, a fiber was maximally activated, and, when force had plateaued at P₀, the fiber was subjected to a single stretch of 5, 10, or 20% strain (%L₁). Each isovelocity stretch was performed at 0.5, 1.0, or 2.0 L₁/s (Table 1). The role of velocity at 20% strain was investigated further at velocities of 3.0 and 4.0 L₁/s. For type IIB single permeabilized fibers from the EDL muscles of rats at 15°C, the Vₘₐₓ is 2.4 ± 0.65 L₁/s (17). Consequently, our velocities of stretch of 0.5–4.0 L₁/s were from 21 to 167% of the Vₘₐₓ of the fibers studied at 15°C. Of the fibers subjected to the stretch at 4.0 L₁/s, 40% (6 of 15 fibers) tore apart during the pliometric contraction, compared with a failure rate of 5% or less for stretches at each of the slower velocities. Because the fibers that survived the 20% strain at 4L₁/s had P₀ values 37% greater than those of other fibers, a comparison was made with fibers that developed comparable P₀ values at 20% stretch at each of the other velocities (Table 2). Immediately after each single pliometric contraction, the fiber was still activated maximally, isometric force was recorded after a resting period of 8 s, and then the fiber was stretched at 4.0 L₁/s (17). Consequently, our force traces of single permeabilized fiber segments before, during, and after pliometric contractions at 0.5 and 4.0 L₁/s (Fig. 1) are comparable to those presented previously (5, 21, 22).

During a stretch, the highest force developed during the period of stretch was defined as the peak force (Pₚₖ). The average force (mN) developed during a stretch was calculated by integrating the area under the force curve during the period of the stretch and dividing by the elapsed time (22). The values for P₀, average force, and Pₚₖ were normalized (kN/m²) to the estimated CSA of each fiber. Because some single permeabilized fiber segments are damaged during removal from the bundles and attachment to the apparatus and subsequently generate abnormally low forces, experiments were performed only on fibers that developed forces of 100 kN/m² or greater (22). After each pliometric contraction, the work done to stretch a fiber was calculated from the product of the average force developed during the stretch and the displacement. Fiber mass was calculated from the product of L₁ and the fiber segment CSA, assuming a density of 1 mg/mm³. Values for work done (J/kg) and for the power absorbed (W/kg) during the stretch were normalized to the mass of the fiber (kg).

Statistics. The data on each experimental group of single permeabilized fiber segments are expressed as means ± SE.
In agreement with previous experiments on single stretches, the majority of the variation in the force deficit of maximally activated whole muscles (6) and single permeabilized fibers (22) was explained by the magnitude of strain imposed (see Table 1). Consequently, the data for each strain were compared separately. At each strain, the data were analyzed by a one-way ANOVA, with Bonferroni comparisons when significance was detected (P ≤ 0.05). To estimate the relationships among strain, velocity, $P_{\text{ss}}$, normalized work, normalized power, average force, and the magnitude of the force deficit, the data associated with single stretches of maximally activated single permeabilized muscle fibers were analyzed by multiple linear regression models. Initially, the predictive value of each variable alone was determined using a simple one-variable regression model. The relative importance of the independent variables for the force deficit was established using a stepwise regression analysis that determined the combination of variables that accounted for the largest portion of the variation in the force deficit. For this analysis, F tests were used to determine the significant regression relationships between the force deficit and the set of independent variables that reflects the contribution of the variable to the model. If independent level of significance of 0.10 was required for entry and inclusion in the model (6). The regression analyses were performed on the total sample of fibers (n = 125).

### RESULTS

**Effect of the stretch velocity on the force deficit.** The null hypothesis was supported, since, during single pliometric contractions at each of the three different strains, velocities of the stretch over a fourfold range of velocities had no effect on the force deficit (Fig. 2). At a 20% strain, no change was observed in the force deficit (Table 1), even with increases in the velocity of stretch of six- and eightfold (Table 2) and, as a consequence, a dramatic decrease in the duration of the stretch (Fig. 1). Furthermore, the velocity of stretch explained <1% of the variance in the magnitude of the force deficit and did not qualify for entry into the stepwise regression equation (Table 3).

Effect of stretch velocity on force, work input, and power absorption. For single stretches performed with the same strain, increasing the velocity had little effect on the average force or $P_{pk}$ generated during the stretch (Table 1). Therefore, the ratio of $P_{pk}$ to steady-state force was also unaffected by velocity. As a consequence, for stretches of the same strain, work input was not different among the three standard velocities or for 3.0 $L_s$/s with a 20% strain. In contrast, compared with the other four groups (Table 1), stretches at 4.0 $L_s$/s with a 20% strain produced a significant 32% increase in the average force and $P_{pk}$ and, as a result, a 35% increase in the work required to stretch the fiber. The increase in the average force and $P_{pk}$ of the 4.0 $L_s$/s velocity, 20% strain group appeared to be a function of the 37% increase in the $P_{ss}$ of this group compared with that of the other four groups (Table 1). Furthermore, the velocity of stretch explained <1% of the variance in the magnitude of the force deficit and did not qualify for entry into the stepwise regression equation (Table 3).

#### Table 1. Effects of velocity on some contractile parameters of single fibers during a single pliometric contraction at different strains

<table>
<thead>
<tr>
<th>Strain, %L_s</th>
<th>Velocity, $L_s$/s</th>
<th>No. of Fibers</th>
<th>$P_{ss}$, kN/m²</th>
<th>Average Force, kN/m²</th>
<th>$P_{pk}$, kN/m²</th>
<th>Force Deficit, %</th>
<th>Work, J/kg</th>
<th>Power, W/kg</th>
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</thead>
<tbody>
<tr>
<td>5</td>
<td>0.5</td>
<td>11</td>
<td>150 ± 14</td>
<td>173 ± 18</td>
<td>354 ± 35</td>
<td>3.4 ± 1.5</td>
<td>8.7 ± 0.9</td>
<td>87.2 ± 9.1</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>10</td>
<td>174 ± 15</td>
<td>192 ± 18</td>
<td>392 ± 35</td>
<td>2.4 ± 1.6</td>
<td>9.6 ± 0.9</td>
<td>191.7 ± 18.5</td>
</tr>
<tr>
<td>5</td>
<td>2.0</td>
<td>12</td>
<td>164 ± 15</td>
<td>181 ± 17</td>
<td>390 ± 34</td>
<td>3.8 ± 1.5</td>
<td>9.0 ± 0.9</td>
<td>361.7 ± 34.6</td>
</tr>
<tr>
<td>10</td>
<td>0.5</td>
<td>14</td>
<td>157 ± 12</td>
<td>191 ± 17</td>
<td>386 ± 30</td>
<td>7.2 ± 1.3</td>
<td>19.1 ± 1.7</td>
<td>95.4 ± 8.3</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>12</td>
<td>175 ± 8</td>
<td>212 ± 8</td>
<td>419 ± 13</td>
<td>9.9 ± 1.5</td>
<td>21.2 ± 0.8</td>
<td>218.8 ± 7.6</td>
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<tr>
<td>10</td>
<td>2.0</td>
<td>13</td>
<td>149 ± 11</td>
<td>203 ± 17</td>
<td>392 ± 27</td>
<td>7.4 ± 1.4</td>
<td>19.1 ± 1.4</td>
<td>383.1 ± 28.5</td>
</tr>
<tr>
<td>20</td>
<td>0.5</td>
<td>26</td>
<td>167 ± 10</td>
<td>227 ± 12</td>
<td>450 ± 21</td>
<td>15.0 ± 1.0</td>
<td>45.4 ± 2.4</td>
<td>123.5 ± 5.9</td>
</tr>
<tr>
<td>20</td>
<td>1.0</td>
<td>15</td>
<td>154 ± 10</td>
<td>227 ± 14</td>
<td>448 ± 27</td>
<td>18.0 ± 1.3</td>
<td>45.3 ± 2.0</td>
<td>228.8 ± 14.0</td>
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<tr>
<td>20</td>
<td>2.0</td>
<td>12</td>
<td>137 ± 14</td>
<td>207 ± 18</td>
<td>428 ± 31</td>
<td>14.6 ± 1.5</td>
<td>41.5 ± 3.6</td>
<td>414.9 ± 36.0</td>
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<tr>
<td>20</td>
<td>3.0</td>
<td>11</td>
<td>167 ± 12</td>
<td>242 ± 17</td>
<td>494 ± 33</td>
<td>20.1 ± 1.5</td>
<td>48.7 ± 3.5</td>
<td>727.7 ± 51.8</td>
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<tr>
<td>20</td>
<td>4.0</td>
<td>9</td>
<td>214 ± 15†</td>
<td>301 ± 20†</td>
<td>601 ± 36†</td>
<td>14.1 ± 1.7</td>
<td>61.2 ± 4.0†</td>
<td>1,224.0 ± 79.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Significant differences due to strain, with lines joining pairs of single or grouped data that are different (P ≤ 0.05). At each strain, the power absorbed at each velocity increased significantly with increasing velocity (P ≤ 0.05).†Value for 20% strain and 4.0 $L_s$/s is significantly greater than those for all other combinations of strain and velocity.

#### Table 2. Effects of velocity on some contractile parameters of single fibers during a single pliometric contraction of 20% strain

<table>
<thead>
<tr>
<th>Strain, %L_s</th>
<th>Velocity, $L_s$/s</th>
<th>No. of Fibers</th>
<th>$P_{ss}$, kN/m²</th>
<th>Average Force, kN/m²</th>
<th>$P_{pk}$, kN/m²</th>
<th>Force Deficit, %</th>
<th>Work, J/kg</th>
<th>Power, W/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>0.5</td>
<td>15</td>
<td>201 ± 8</td>
<td>517 ± 17</td>
<td>265 ± 9</td>
<td>14 ± 1.1</td>
<td>53 ± 1.9</td>
<td>132 ± 4.8</td>
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<tr>
<td>20</td>
<td>1.0</td>
<td>8</td>
<td>185 ± 6</td>
<td>513 ± 21</td>
<td>263 ± 10</td>
<td>15 ± 2.1</td>
<td>53 ± 2.1</td>
<td>264 ± 10.6</td>
</tr>
<tr>
<td>20</td>
<td>2.0</td>
<td>6</td>
<td>176 ± 8</td>
<td>505 ± 26</td>
<td>253 ± 14</td>
<td>12 ± 1.0</td>
<td>57 ± 2.3</td>
<td>508 ± 27.1</td>
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<tr>
<td>20</td>
<td>3.0</td>
<td>6</td>
<td>195 ± 9</td>
<td>575 ± 21</td>
<td>284 ± 12</td>
<td>19 ± 1.2</td>
<td>57 ± 2.3</td>
<td>852 ± 34.9</td>
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<tr>
<td>20</td>
<td>4.0</td>
<td>9</td>
<td>214 ± 15</td>
<td>601 ± 36</td>
<td>301 ± 20</td>
<td>14 ± 1.8</td>
<td>61 ± 4.0</td>
<td>1,224 ± 79.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. Effect of velocity on stretch on force deficit: no significant difference in force deficit among the 5 groups (F ratio = 1.92, P = 0.127). Effect of velocity on stretch on average force: no significant difference in average force among the 5 groups (F ratio = 1.49, P = 0.224).
each of the other groups. The increased $P_o$ of the 4.0-$L_f$/s velocity, 20% strain group likely arose from the weaker fibers being torn during the 4.0-$L_f$/s stretches. To test this hypothesis more rigorously, a subset of fibers was drawn from each of the other four velocity groups that had $P_o$ values within the range of those observed in the 4.0-$L_f$/s velocity, 20% strain group (Table 2). Although representing an eightfold range of velocities of stretch, groups equated as to the initial $P_o$ showed no differences for average force, $P_{pk}$, work, or, most importantly, force deficit. Last, increasing the magnitude of the strain increased the work required to stretch the fiber and the force deficit regardless of the velocity of stretch. Although work input increased as a function of strain, the power absorption did not. For stretches of the same strain, predictably, the power absorption increased linearly with increases in the velocity of the stretch (Table 1).

Prediction of the magnitude of the force deficit. With the inclusion of the total sample of fibers in the stepwise regression models, the coefficient of determination for magnitude of strain alone predicted 52% of the variance in the force deficit (Table 3). The strain was more effective in predicting the force deficit than any other variable or any combination of variables, including the work done to stretch the fiber (average force $\times$ displacement), which explained 33% of the variance (Table 1 and Fig. 2). The unusually low coefficients of correlation for average force and $P_{pk}$ result from the insignificant amount of variation in these two variables in the maximally activated single permeabilized fiber segments. The low coefficients of correlation for velocity and for power ($\text{force} \times \text{velocity}$) add further support for the null hypothesis that the force deficit is not dependent on velocity.

DISCUSSION

For maximally activated single permeabilized fiber segments, stretched at the velocities and through the
strains tested in this experiment, no relationship existed between the force deficit and the velocity of stretch. Velocities of 0.5–4.0 L/s extended the velocities of stretch beyond those normally used in studies of contraction-induced injury of single permeabilized fibers at 15°C (5, 21, 22) and even of those used for whole muscles at 35°C (6, 14, 18, 23, 24, 34). The only higher velocity of stretch, 5 L/s at 22°C, was used for single intact fibers (2). Under conditions of excessively high loading, even maximally activated muscle fibers may be stretched at velocities higher than their \( V_{\text{max}} \), but for most movements cocontractions occur such that for each agonist muscle shortening at a given velocity an antagonist muscle is being stretched at the same velocity (31). When velocities of stretch were compared as percentages of \( V_{\text{max}} \), the whole muscles during the various experiments at 35°C (6, 14, 18, 23, 24, 34) were stretched at between 4 and 30% of \( V_{\text{max}} \), whereas in the present study the single permeabilized fibers at 15°C were stretched at velocities ranging from 21 to 167% of \( V_{\text{max}} \). The observation that stretching maximally activated single permeabilized fibers at velocities almost twofold greater than the \( V_{\text{max}} \) had no effect on the force deficit adds even further support for the acceptance of the null hypothesis.

In contrast to the single permeabilized fibers being exposed to higher velocities of stretch relative to \( V_{\text{max}} \), the strains necessary to produce a given force deficit were lower for permeabilized fibers in vitro than for whole muscles in situ (6). For single strains initiated at resting length, a 5% strain was sufficient to produce a significant force deficit in single permeabilized fibers, whereas a 30% strain was required for whole muscles (5). Similarly, for the induction of an ~20% force deficit, a strain of 20% was required for single permeabilized fibers and a strain of 40% for whole EDL muscles (5). These force-deficit-strain relationships are comparable to other data on single permeabilized fibers from fast-twitch EDL muscles of mice (5) and rats (5, 22). The 50% lower strain required to produce an equivalent force deficit for single permeabilized fibers compared with that required for fibers in whole muscles is likely a function of the disruption of the integrity of the sarcolemma during the chemical “skinning” of the permeabilized fibers (7). Compared with intact fibers, permeabilized fibers, particularly fast-twitch permeabilized fibers, are known for their instability in sarcomere length (21), and consequently their increased susceptibility to injury is not surprising.

Considerable controversy has existed as to the mechanical event, or events, responsible for the injury to skeletal muscle fibers, with both the average force during the stretch (24) and the strain (18) proposed as key factors. The controversy was resolved by experiments on single stretches of maximally activated single permeabilized fibers (6, 22) and whole muscles (5, 14). Average force, strain (displacement), and the work done to stretch the fiber or muscle (average force \( \times \) displacement) were identified as the major mechanical factors in the generation of the initial injury to muscle fibers (5, 6, 14, 22). When initial length is held constant (14, 34) and activation is maximum (22), as in the present study, the force deficits were highly dependent on the magnitude of the strain. In the present study, the coefficient of determination of \( r^2 = 0.52 \) between force deficit and strain obtained for fibers stretched at the three different velocities is lower than the \( r^2 \) of ~0.8 reported for maximally activated whole EDL muscles (5) and single permeabilized fiber segments (22). The lower coefficient of determination for both strain and work input when data encompassing different velocities of stretch were included in the model is likely attributable to the increased variability of the force deficits introduced by the variety of the protocols. The high degree of dependence of the force deficit on strain was particularly evident due to the lack of any difference in average force either within groups at the same strain or among groups exposed to different strains.

In addition to the study of contraction-induced injury, stretches of maximally activated fibers and whole muscles have been utilized to investigate the reversal of chemical reactions (13), heterogeneity in sarcomere length (15), enhancement of mechanical performance (8), cross-bridge detachment and sarcomere “give” (11),

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**Table 3. Coefficients of determination and levels of significance for mechanical variables for one-variable regression models of force deficit following single stretches of maximally activated permeabilized muscle fibers at different velocities**

<table>
<thead>
<tr>
<th>Variable</th>
<th>( r^2 )</th>
<th>( p )</th>
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<tbody>
<tr>
<td>Strain</td>
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<td>0.000</td>
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<tr>
<td>Peak force</td>
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<td>0.334</td>
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<td>Average force</td>
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<td>Power</td>
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</tbody>
</table>
stiffness (10), and cross-bridge kinetics (19). For these diverse purposes, velocities of stretch > 0.5 L/s have been used frequently, but velocities of stretch > 3 L/s have been used rarely. For both whole muscles (11, 29) and single intact (19) and permeabilized (4) fibers, a number of investigators have noted that, at velocities of stretch > 0.5 L/s, the ratio of $P_{pk}/P_o$ increased steeply, reaching maximum values of 1.55–2.0. Apparently, there exist both a range of low velocities of stretch over which $P_{pk}/P_o$ displays a marked dependence on velocity and a range over which the ratio is independent of the velocity. When stretches are short and sarcomere lengths are homogeneous, the $P_{pk}/P_o$ approximates 2.0 (19). Our mean $P_{pk}/P_o$ of 2.8 reflects both the longer stretches and the greater heterogeneity in sarcomere lengths. For studies of contraction-induced injury with either single or repeated pliometric contraction protocols, the velocity of the stretch has been set consistently at between 0.5 and 2.0 L/s (4, 14, 18, 23, 34, 35), although Balnave and Allen (2) used a velocity of stretch of 5 L/s in a study of single intact fibers. A clear dependence of force deficit on average force is of relatively recent origin (22), but the assumption that velocities within this range will not play a significant role in the induction of the injury appears to be valid, since within this range the force is not dependent on velocity.

The lack of any difference in either the average force or $P_{pk}$ developed by single permeabilized fibers, despite an eightfold increase in the velocity of stretch with strains of 5–20%, is consistent with observations associated with the smaller stretches of single permeabilized fibers from muscles of mice (4), single intact muscle fibers from frogs (15, 19), and whole muscles from mice (29). To investigate the mechanism responsible for the plateau in force, Lombardi and Piazzesi (19) eliminated tendon compliance and nonuniformities in sarcomere lengths by establishing length clamp conditions. The measurements of stiffness, during the stretches of varying velocities and under conditions of steady-state force, indicated that the number of attached cross bridges was only 10–12% greater than the number measured during the development of $P_o$. The conclusion was that, during stretches of activated fibers above a force twofold greater than $P_o$, the increase in the negative strain on attached cross bridges increased greatly the rate constant for the detachment of the cross bridges (19). The increase in the rate constant for detachment results in the release of cross bridges with very small strains, < 1% of $L_o$, and then the reattachment of the cross bridges at a subsequent site on the actin filaments as the actin filaments are pulled past the cross bridges. The rate of attachment is unaffected and is sufficiently high to maintain the number of attached cross bridges slightly higher than that associated with $P_o$. Based on these experiments, the plateau of the average force and $P_{pk}$ during the stretch is attributed to the increase in the rate constant for the detachment of cross bridges strained negatively during the stretch (19).

In our experiments, the one exception of an increase in average force and $P_{pk}$ with an increase in velocity occurred during stretches of 20% strain at 4.0 L/s. These fibers represented an exceptional group of stronger fibers with a prestretch $P_o$ 40% greater than the value for fibers that tore. Fibers with comparable prestretch $P_o$ demonstrated equally high average force and $P_{pk}$ after stretches at lower velocities of stretch. The stronger fibers appear to have maintained more homogeneous sarcomere lengths during isometric contractions and then generated greater average force and $P_{pk}$ during resistance to the stretch (21). The results for stretches at 4.0 L/s through a 20% strain must be interpreted with caution, because of the high incidence of fiber breakage. This combination of velocity and strain is at or beyond the physiological limit for investigating pliometric contraction-induced injury with single permeabilized muscle fibers. In contrast, maximally activated single intact fibers from the flexor digitorum brevis muscle of the mouse can sustain repeated stretches of up to 50% strain at 5 L/s (2), suggesting that the sarcolemma contributes significantly to fiber integrity. Particularly during pliometric contractions, the sarcolemma is critical both for the transmission of force along the fiber (33) and the dispersion of the stress laterally.

Controversy exists as to the cellular components actually damaged by pliometric contractions (1, 9, 12, 25, 27). An early suggestion by Newman and her associates (27) was that “sarcomeres were pulled apart,” and subsequently Morgan (25) proposed that as a result of rapid elongation the weaker sarcomeres “popped”. During isometric contractions, laser diffraction studies of different regions along single permeabilized fibers have demonstrated that some regions of sarcomeres are frequently stretched onto the descending limb of the length-force relationship, whereas the majority of the regions of sarcomeres either shorten or remain at the same length (15, 21). The behavior of specific regions of a fiber to shorten, to hold length, or to be stretched is highly consistent during repeated isometric contractions (21). The conclusion is that the regions that shorten are intrinsically “stronger” and those that are stretched are intrinsically “weaker” (21, 25). The regions of a fiber that are stretched, particularly those stretched onto the descending limb of the length-force relationship, undergo further elongation when the fiber is stretched, and these regions also show the greatest amount of damage following a stretch (21). Half-sarcomeres with greater overlap of thick and thin filaments will have a greater probability of the cross bridges reattaching successfully during a stretch, just as they would under isometric conditions. Within the eightfold range of velocities of stretch, encompassing 21–167% of $V_{max}$ at 15°C (17), average force and $P_{pk}$ were maintained. Whether sarcomeres on the descending limb of the length-force curve are pulled beyond overlap of thick and thin filaments and injured is simply related to their combined active and passive force relative to the forces developed by the sarcomeres.
in series with them and the magnitude of the imposed strain.

This work was supported by National Institute on Aging Grant AG-06157. G. S. Lynch was supported by a C. J. Martin Research Fellowship from the National Health and Medical Research Council of Australia.

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Received 1 April 1998; accepted in final form 1 September 1998.

REFERENCES


