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# Effects of fiber type on force depression after active shortening in skeletal muscle

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ABSTRACT

The aim of this study was to investigate force depression in Type I and Type II muscle fibers. Experiments were performed using skinned fibers from rabbit soleus and psoas muscles. Force depression was quantified after active fiber shortening from an average sarcomere length (SL) of 3.2 μm to an average SL of 2.6 μm at an absolute speed of 0.115 fiber length/s and at a relative speed corresponding to 17% of the unloaded shortening velocity ($V_0$) in each type of fibers. Force decay and mechanical work during shortening were also compared between fiber types. After mechanical testing, each fiber was subjected to myosin heavy chain (MHC) analysis in order to confirm its type (Type I expressing MHC I, and Type II expressing MHC IId). Type II fibers showed greater steady-state force depression after active shortening at a speed of 0.115 fiber length/s than Type I fibers ($14.5 \pm 1.5\%$ versus $7.8 \pm 1.7\%$). Moreover, at this absolute shortening speed, Type I fibers showed a significantly greater rate of force decay during shortening and produced less mechanical work than Type II fibers. When active shortening was performed at the same relative speed (17% $V_0$), the difference in force depression between fiber types was abolished. These results suggest that no intrinsic differences were at the origin of the disparate force depressions observed in Type I and Type II fibers when actively shortened at the same absolute speed, but rather their distinct force–velocity relationships.



## 1. Introduction

The steady-state isometric force after active shortening of skeletal muscle is smaller than the purely isometric force at the same level of activation and the corresponding length. This phenomenon, referred to as force depression, was first described by Abbott and Aubert in 1952 and has been observed consistently in whole and isolated muscle preparations (Rassier and Herzog, 2004). The molecular mechanisms causing force depression remain unknown, but two primary mechanisms have been suggested. First, force depression has been associated with the development of sarcomere length non-uniformities upon active shortening (Morgan et al., 2000). According to this theory, force depression is caused by the development of large dispersions in sarcomere lengths during active shortening. Second, force depression has been associated with a stress-induced inhibition of cross bridges in the newly formed actin–myosin overlap zone following shortening owing to actin angular deformation (Marechal and Plaghki, 1979). According to this theory, active shortening causes a decrease in the probability of cross bridge attachment, thereby leading to a decrease in the proportion of force generating (strongly bound) cross bridges in the force depressed state at the same muscle length and overlap of thick and thin filaments compared to a purely isometric contraction.

Despite an abundance of information regarding the characteristics and mechanisms of force depression, there has been no investigation for comparing force depression among skeletal muscle fiber types. Fast (Type II) and slow (Type I) muscle fibers have distinctly different force–velocity properties (Bottinelli et al., 1991), and thus force and work for shortening at a given speed differs substantially between fiber types. It has been shown that the magnitude of force depression is directly related to force and work performed during shortening (Corr and Herzog, 2005; Dargeviciute et al., 2013; Leonard and Herzog, 2005; Minozzo and Rassier, 2013).

The aim of the present study was to investigate force depression in Type I and Type II muscle fibers. Based on the acknowledged differences in force–velocity properties and transient forces between fiber types (Bottinelli et al., 1991), we hypothesized that force depression following active shortening at a given absolute speed is greater in Type II than Type I fibers but would be similar between fiber types for similar relative speed of shortening.

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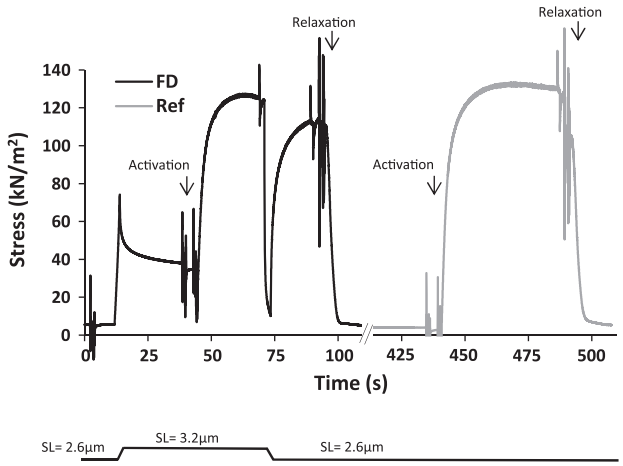


**Fig. 1.** Typical fiber response when passively stretched from an average SL of 2.6 μm to an average SL of 3.2 μm, activated, shortened to an average SL of 2.6 μm and then deactivated. The noise indicates the time when the fiber was transferred between solutions. The sudden change in stress indicates the stretch-release cycle performed to measure stiffness. The gray force trace indicates the stress produced by the reference isometric contraction performed at the average SL of 2.6 μm. (A stiffness test was also performed before active shortening but not used in the analysis).

## 2. Materials and methods

New Zealand white rabbits were euthanized according to a protocol approved by the University of Calgary's Animal Care and Ethics Committee. Strips of soleus and psoas muscles were skinned and fibers were isolated and mounted between a force transducer and a length controller as previously described (Joumaa and Herzog, 2013; Toursel et al., 2000). SLs were measured using optical diffraction of a He–Ne laser beam. Fiber cross sectional area and volume were calculated assuming the fiber has a cylindrical shape. Experiments were performed at 15 °C.

### 2.1. Maximal stress at an average SL of 2.6 μm

Soleus ($n=15$) and psoas ($n=22$) fibers were activated at an average SL of 2.6 μm in order to measure their maximal force at this length. Force was normalized to the fiber cross sectional area to obtain the maximal stress.

### 2.2. Active shortening contractions at a shortening speed of 0.115 fiber length/s

Soleus ($n=15$) and psoas ($n=15$) fibers were set at an average SL of 2.6 μm and then passively stretched to an average SL of 3.2 μm, held for 20 s and activated by changing the relaxing solution to a high calcium activating solution (Joumaa and Herzog, 2013). Fibers were then actively shortened to an average SL of 2.6 μm at a speed of 0.115 fiber length/s, held isometrically until steady-state isometric force was reached, and then deactivated (Fig. 1). After a rest period of 5 min, fibers were activated at an average SL of 2.6 μm in order to measure their purely isometric reference force.

### 2.3. Active shortening contractions at the same relative speed between Type I and Type II fibers

In order to perform active shortening at the same relative speed between Type I and Type II fibers, we measured the unloaded shortening velocity ($V_0$) for psoas ($n=5$) and soleus ($n=5$) fibers using the slack test method proposed by Edman (1979). Then we tested an additional group of psoas fibers in which we performed active shortening at the same relative speed (%$V_0$) as in soleus fibers. The unloaded shortening velocities were (mean ± SEM) 0.67 ± 0.09 fiber length/s and 1.77 ± 0.03 fiber length/s in soleus and psoas fibers, respectively. The initial active shortening experiments were performed at a shortening speed of 0.115 fiber length/s corresponding to a relative shortening speed of 17% of $V_0$ in Type I fibers. Thus, seven additional psoas fibers were actively shortened at a relative speed of 17% of their $V_0$ corresponding to 0.3 fiber length/s.

### 2.4. Stiffness measurements

Fiber stiffness was obtained using a quick stretch-release protocol of 0.2% fiber length (Ford et al., 1981; Joumaa et al., 2012; Mansson, 1989; Rassier and Herzog, 2005) at a speed of 1 fiber length/s. Stiffness was measured once the isometric steady-state had been reached after active shortening, and during the purely isometric reference contraction.

### 2.5. MHC content

The MHC content was determined in each fiber after mechanical testing using SDS-PAGE gel electrophoresis on 4.5% and 7.5% acrylamide stacking and separating gels respectively (Toursel et al., 2000). The gels were run in a Biorad Mini-Protean III unit at a constant voltage of 73 V for 40 h at 4 °C, stained with Coomassie Blue and scanned. Following electrophoresis, fibers were divided into two groups: Type I fibers expressing MHC I and Type II fibers expressing MHC IId.

### 2.6. Data analysis

#### 2.6.1. Force depression

Force depression was determined as the percent difference between the steady-state isometric force following active shortening and the purely isometric reference force at 2.6 μm average SL.

#### 2.6.2. Stiffness (instantaneous stiffness)

Stiffness was measured as the difference between the peak force reached after the quick stretch and the force immediately before the stretch divided by the amplitude of the stretch.

#### 2.6.3. Rate of force decline during shortening

Force during shortening is characterized by a steep initial phase and a slow final phase. The initial and final phases were fitted by two linear least-squares regression functions. The slope of the initial steep phase of shortening, $S1$, was taken as an estimate of the rate of force decline during shortening. The slope of the final slow phase of shortening, $S2$, was taken as an estimate of the behavior of the non-contractile (passive) element during shortening (Roots et al., 2007). $S1$ and $S2$ were normalized to the maximal isometric fiber force and compared between Type I and Type II fibers.

#### 2.6.4. Mechanical work

Mechanical work during shortening was calculated by trapezoidal numerical integration of the force–length curve during the shortening phase. To compare work performed between fibers, the mechanical work was normalized to fiber volume.

#### 2.6.5. Statistical analysis

All data reported are means ± SEM. The Student's $t$-test ($p < 0.05$) was used to compare data between Type I and Type II fibers.

## 3. Results

Fibers were analyzed for MHC composition (Fig. 2). Thirteen fibers isolated from the soleus muscle and tested for force depression expressed MHC I and were grouped as Type I fibers. The remaining two soleus fibers expressed MHC IIa and were excluded from the analysis. Three psoas fibers were excluded from analysis because they co-expressed MHCs IId/IIb. The remaining psoas fibers tested for force depression ($n=12$ shortened at a speed of 0.115 fiber length/s, $n=7$ shortened at a relative speed of 17% $V_0$) expressed MHC IId and were labeled Type II fibers and analyzed.

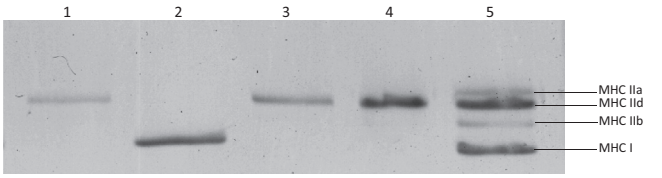


**Fig. 2.** Electrophoresis of MHCs. Lanes 1–3: fibers on which the mechanical measurements were performed. Lane 4: rabbit psoas muscle. Lane 5: rabbit muscles used as an electrophoretic marker. Fibers in lanes 1 and 3 expressed MHC IId and were identified as Type II. The fiber in lane 4 expressed MHC I and was identified as Type I.

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