# Myosin heavy chain co-expression result in the heightened susceptibility following a standardized eccentric contraction

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## ABSTRACT

BACKGROUND: From our previous work, we found the reductions in Ca2+-activated force, a marker of fiber damage, were 3-fold greater for fibers co-expressing type IIa and IIx MHC compared to fibers expressing type I or type IIa MHC when fiber specific force was equal. PURPOSE: The goal of present study was to test two hypotheses, derived from our previous work, that the heightened susceptibility of type IIa/IIx fibers result from the influence of IIx MHC (Hypothesis 1) or the co-expression of mixture MHC (Hypothesis 2). METHODS: We utilized soleus and extensor digital longus muscle from C57BL/6 mice to address the full range of mammalian limb muscle MHC. Ca2+-activated force of single skinned muscle fibers was evaluated before and after a standardized eccentric contraction (25% of fiber length 50% of maximal shortening velocity). RESULTS: Multiple linear regression indicated that the independent variables pre-eccentric specific force and a mixture of MHC isoform accounted for over 90% of the variability in post-eccentric force. Regardless of fiber type, fiber producing greater pre-force experienced greater post-eccentric force deficits. When pre-eccentric force was held constant, fiber expressing a mixture MHC showed significantly greater force deficit than fibers expressing a single MHC isoform. CONCLUSION: In summary, we found that fibers containing the mixture of MHC isoforms are more susceptible to eccentric contraction than fibers expressing a single MHC isoform. In other words, the heightened susceptibility of polymorphic fibers is due to the mixture of MHC isoform expression. However, the faster MHC, such as type IIx and/or IIb MHC isoform were not associated with the susceptibility to eccentric contraction.

## Introduction

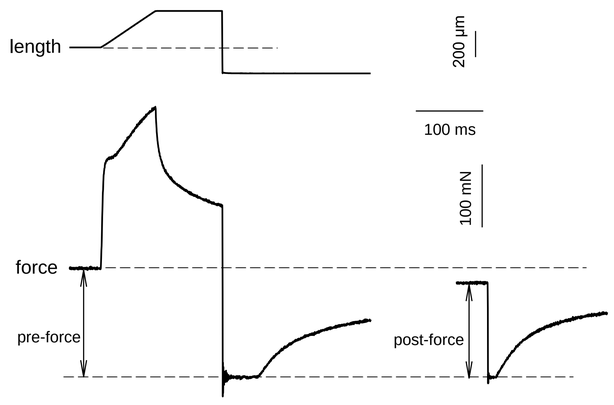
Skeletal muscles produce their greatest force during active stretch. This mode of contractile activity has important clinical relevance because when performed at high intensity or in excess, these so-called lengthening, eccentric, or pliometric contractions induce prolonged muscle weakness, delayed onset muscle soreness, and inflammation.

Morgan (Biophys J 57: 209-221, 1990) has proposed a model in which active stretch lengthens a subset of sarcomeres onto the descending limb of their length-tension relationship causing them to be rapidly over-extended beyond myofilament overlap. The structural perturbations produced by these over-extended, or “popped”, sarcomeres may then be transmitted radically and longitudinally within the fiber, disrupting other cellular structures and leading to impaired muscle function. In this model, sarcomere heterogeneity is the key initiating step in eccentric muscle damage

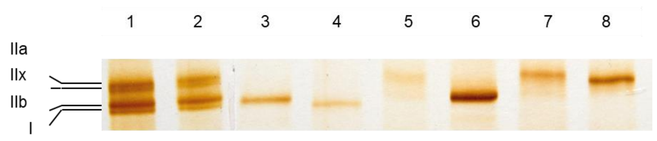
While this “popping sarcomere” hypothesis is supported by a number of observations, it doesn’t address why or how sarcomere length heterogeneity arises. It is known that some cells are more susceptible to lengthening contractions than other cells but the characteristics of susceptible cells have never been examined in detail. Identification of susceptible cells may provide important clues for understanding why sarcomere length heterogeneity arises during lengthening contractions.

## Methods

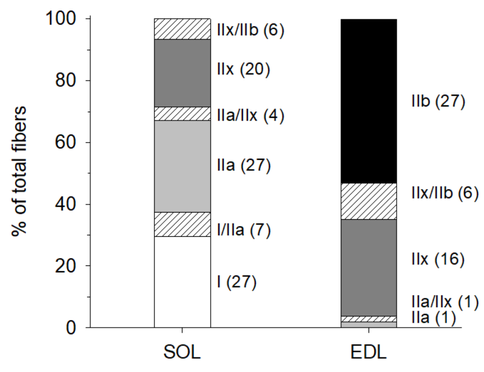
**Study Protocol**: Chemically skinned fiber bundles were prepared from soleus (SOL) and extensor digitorum longus (EDL) muscles of C57BL/6 mice. Unloaded shortening velocity (Vo) was determined using a slack test (pCa 4.5, 15°C). For the eccentric treatment, fibers were activated, allowed to reach their peak pre-treatment force, and subjected to a single lengthening contraction of standardized magnitude (0.25 fiber length) and velocity (0.50 Vo). The fiber was returned to its original length and Ca2+-activated several times to establish post-treatment force.




**Fiber MHC isoform content:** Following the physiological measurements, SDS-PAGE was used to identify fiber myosin heavy chain (MHC) isoform content. Below is a representative silver stained polyacrylamide gel where a MHC standard was run in Lanes 1 and 2 and single fiber segments in lanes 3-8.




We collected data on 91 soleus (SOL) and 51 extensor digitorum longus (EDL) fibers. The MHC isoform content of these fibers is illustrated at the right. Solid shaded areas represent the proportion of MHC ***monomorphic*** fiber segments, i.e. those expressing a single MHC isoform. Hatched areas represent the proportion of MHC ***polymorphic*** fiber segments, i.e. single segments co-expressing two different MHC isoforms. The numbers in parenthesis represent the absolute number of fibers. Ten of these fibers were eliminated from analysis (see next panel).




## Results

**1. Fiber breakage during eccentric treatment**

Ten fibers either completely or partially broke during the eccentric treatment. These fibers were not included in subsequent statistical analysis.

Breakage per MHC isoform:

| | |
|---|---|
| 3 expressed type I MHC | 11% of all type I fibers |
| 1 expressed type IIa MHC | 4% of all type IIa fibers |
| 2 expressed type IIx MHC | 10% of all type IIx fibers |
| 1 expressed type IIx/IIb MHC | 17% of all type IIa/IIx fibers |
| 3 expressed type IIb MHC | 11% of all type IIb fibers |

Breakage per MHC co-expression:

| | |
|---|---|
| 9 monomorphic fibers | 8% of all monomorphic fibers |
| 1 polymorphic fiber | 4% of all polymorphic fibers |

Because there was no evidence that fiber breakage was related to a specific fiber MHC isoform or to MHC co-expression, fiber breakage does not bias any conclusions drawn from our data.

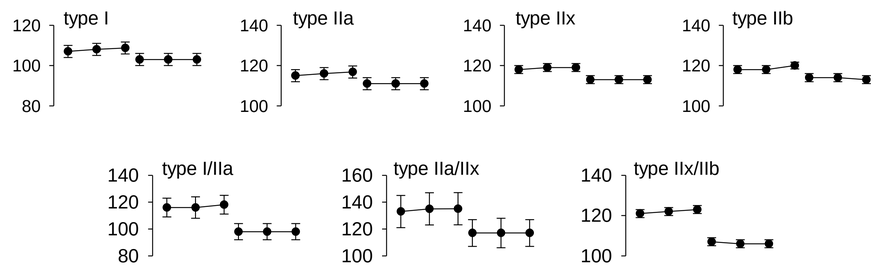
**2. Pre-treatment characteristics**

Fibers were all studied at similar sarcomere length. As expected, unloaded shortening velocity (Vo, normalized to fiber length, FL) varied with myosin heavy chain (MHC) isoform content. Fiber compliance, calculated as the displacement axis intercept of the slack test (normalized to fiber length) averaged slightly over 3% for all fibers with no differences between groups.

| MHC | n | SL, µm | $V_o$, FL/s | compliance |
|---|---|---|---|---|
| I | 24 | 2.61 ± 0.001 | 1.88 ± 0.09 | 2.9 ± 0.3 |
| I/IIa | 7 | 2.60 ± 0.001 | 3.61 ± 0.36 | 4.4 ± 1.1 |
| IIa | 27 | 2.61 ± 0.001 | 3.70 ± 0.22 | 3.1 ± 0.3 |
| IIa/IIx | 5 | 2.60 ± 0.004 | 3.58 ± 0.27 | 3.5 ± 0.8 |
| IIx | 34 | 2.60 ± 0.002 | 5.44 ± 0.21 | 3.2 ± 0.2 |
| IIx/IIb | 11 | 2.60 ± 0.002 | 5.95 ± 0.46 | 2.8 ± 0.4 |
| IIb | 24 | 2.60 ± 0.002 | 6.12 ± 0.17 | 3.2 ± 0.3 |

**3. Stability of force measurements**

To ensure that force changes after the eccentric treatment were not due to run-down of the preparation, we examined peak $Ca^{2+}$-activated force attained on the three activations immediately preceding the lengthening contraction (the last two activations of the slack test and the fixed-end contraction leading into the lengthening ramp) and the three activations immediately following the lengthening contraction. As can be seen below, force was stable before and after the lengthening contraction for all fiber types (all values in $kN/m^2$).




**4. Force responses to lengthening**

Pre-treatment force was defined as the peak force attained by the fiber during the fixed-end portion of the eccentric contraction treatment.

Post-treatment force was defined as the maximal force attained over a minimum of 3 post-eccentric activations.

In subsequent analyses, we used the relationship between pre- and post-eccentric $Ca^{2+}$activated force as an index of damage to the myofilament lattice.

| MHC | pre-force $kN/m^2$ | eccentric contraction: peak ecc force $kN/m^2$ | eccentric contraction: peak ecc / pre ecc force | eccentric contraction: work done J/liter | pre to post force change: $kN/m^2$ | pre to post force change: % of pre |
|---|---|---|---|---|---|---|
| I | 109 ± 3 | 219 ± 6 | 2.02 ± 0.04 | 48.5 ± 1.5 | -5.1 ± 0.4 | -4.8 ± 0.5 |
| I/IIa | 118 ± 7 | 248 ± 14 | 2.10 ± 0.07 | 54.7 ± 4.0 | -18.6 ± 1.4 | -15.5 ± 1.2 |
| IIa | 117 ± 3 | 253 ± 6 | 2.17 ± 0.04 | 55.8 ± 1.5 | -5.3 ± 0.4 | -4.5 ± 0.5 |
| IIa/IIx | 136 ± 11 | 296 ± 14 | 2.21 ± 0.09 | 62.0 ± 5.1 | -18.1 ± 2.3 | -13.4 ± 1.5 |
| IIx | 119 ± 2 | 281 ± 5 | 2.38 ± 0.04 | 61.1 ± 1.4 | -5.1 ± 0.4 | -4.5 ± 0.5 |
| IIx/IIb | 124 ± 2 | 281 ± 7 | 2.27 ± 0.05 | 61.4 ± 1.9 | -18.0 ± 1.2 | -14.4 ± 0.9 |
| IIb | 120 ± 2 | 292 ± 4 | 2.43 ± 0.03 | 62.2 ± 1.1 | -5.9 ± 2.5 | -4.7 ± 0.7 |

## Conclusion

Our data do not support the idea that faster fibers are more sensitive to eccentric contractions than slow fibers, at least at the level of the myofilament lattice.

Instead, multiple linear regression revealed that the post-eccentric $Ca^{2+}$-activated force loss could be described by a model consisting of a *strain dependent component* and a *MHC co-expression* dependent *component*.

*Strain component*: For every 10 $kN/m^2$ increase in pre-eccentric specific force, the force reduction following eccentric contraction increased 0.6 $kN/m^2$. This component appears to be an innate property of fibers as it was *independent* of fiber MHC isoform expression.

*MHC co-expression component*: The model revealed that eccentric-induced damage to the myofilament lattice is *exacerbated in fibers that co-express multiple MHC isoforms*. For a monomorphic and polymorphic fiber of identical pre-eccentric specific force, our model predicts that the polymorphic fiber would show a 14 $kN/m^2$ greater force reduction when subjected to a single standardized eccentric contraction in comparison to the monomorphic fiber.

We conclude that MHC co-expression, rather than the expression of a specific MHC isoform per se, predisposes a fiber to eccentric-induced damage at the level of the myofilament lattice.