Published in final edited form as:

J Biomech. 2011 May 17; 44(8): 1618–1620. doi:10.1016/j.jbiomech.2011.03.009.

Muscle Extracellular Matrix Applies a Transverse Stress on Fibers With Axial Strain

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Abstract

It is widely assumed that skeletal muscle contraction is isovolumic. This assumption has been verified at the single fiber and at the myofibril level. Model development and mechanical analyses often exploit this assumption when investigating skeletal muscle and evaluating muscle mechanical properties. This communication describes a method whereby individual muscle fibers and bundles of fibers, which include their constituent extracellular matrix (ECM), were tested to define the change in volume with axial strain. The results demonstrate that fibers are isovolumic, but bundles decrease in volume with strain. The loss of volume implicates a transverse force being applied to the fibers by the ECM. The nature and importance of this transverse force warrant further investigation.

Keywords

Muscle; Extracellular Matrix; Isovolumic

1. Introduction

Classic high resolution physiological and X-ray diffraction experiments in the 1960s demonstrated that muscle contraction is isovolumic at the muscle fiber (Huxley, 1969) and myofilament level (Elliott *et al.*, 1963). The isovolumic assumption is often exploited as a constraint in computing cross-sectional area changes with stretch in muscle mechanical models (Kyckelhahn *et al.*, 2003) even when using data derived from studies of the extracellular matrix (Trotter, 1991). However previous research has asserted that muscle volume decreases with strain in frog muscle (Baskin & Paolini, 1967).

To test the hypothesis of isovolumic properties in muscles subjected to passive deformation, we utilized a simple method of measuring the CSA of single muscle fibers and bundles while being stretched. Testing both single fibers and fiber bundles allows us to define the influence (if any) of the extracellular matrix (ECM) on isovolumic behaviour. If human muscle does not satisfy the isovolumic assumption, models may need to be refined. In

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Conflict of interest statement

The authors declare no conflicts of interest

addition, the structural basis for isovolumicity presents an intriguing topic of muscle physiology.

2. Methods

Ethical approval for this study conformed to the standards of the Declaration of Helsinki and was approved by the University of California, San Diego Institutional Review Board Human Research Protection Program. Biopsies were obtained during surgery that exposed the distal semitendinosus hamstring muscle of children. Biopsies were removed and placed directly into a glycerol relaxing solution prior to dissection and stored at -20°C . Biopsies were obtained from 4 patients with 3 fibers and 3 bundles tested from each biopsy.

For dissection of fiber or fiber bundle samples, muscles were removed from storage solution and transferred to a relaxing solution at pCa 8.0 and pH 7.1 consisting of (mM): imidazole (59.4), $\text{KCH}_3\text{O}_3\text{S}$ (86.0), $\text{Ca}(\text{KCH}_3\text{O}_3\text{S})_2$ (0.13), $\text{Mg}(\text{KCH}_3\text{O}_3\text{S})_2$ (10.8), K_3EGTA (5.5), KH_2PO_4 (1.0), Na_2ATP (5.1), and $50.0\ \mu\text{M}$ leupeptin. Single fiber segments (1.5–3 mm in length) were carefully dissected and mounted in a chamber in a custom apparatus. Fibers were secured using 10-0 monofilament nylon suture on one end to a force transducer (Model 405A, sensitivity 10 V/g, Aurora Scientific, Ontario, Canada) and on the other end to a titanium wire rigidly attached to a rotational bearing (Newport MT-RS; Irvine, CA). Segments displaying obvious abnormalities or discoloration were not used. Muscle images were captured using a Leica DFC 500 camera (Leica Camera Inc., Allendale, New Jersey) with two pictures taken—one to measure length and width (top view) and another to measure length and height (side view) using a 45° mirror, both at 50x magnification (Fig. 1)

The specimen (fiber or bundle) was brought to slack length, defined as passive tension just measurable above the noise level of the force transducer. Sample dimensions were measured optically with a cross-hair reticule mounted on the dissecting microscope and micromanipulator digital indicators mounted on an x-y stage. An image of the fiber was taken from both top and side views. The fiber was then loaded with strains of approximately 10% strain at 100 fiber lengths/sec. Each stretch was held for 3 minutes during which stress relaxation was allowed to occur and an image taken from both views, before a sequential stretch was made. Fibers were stretched in total to approximately 100% strain. Samples were discarded if any irregularities appeared along their length during testing, or if they were severed or slipped at either suture attachment point during the test. Muscle bundles consisted of approximately 20 fibers and their constitutive ECM.

After the experiment, images were analyzed with ImageJ (Version 1.43 from public domain NIH Image program developed at the U.S. National Institutes of Health and available on the Internet at <http://rsb.info.nih.gov/niH-image/>) to determine specimen height, width, and length. These dimensional data were used to calculate area, stress, strain, and volume. The slope of the relative volume to strain curve was used to investigate volume changes with strain (Fig. 2) and to calculate Poisson's ratio. Statistical significance was determined using the Student's T-test with a significance level (α) of $p < 0.05$.

3. Results

Stiffness values were within the range of those reported for human muscle fibers (26.4 ± 4.5 kPa SEM) and although bundles were more compliant (30.4 ± 5.7 kPa SEM) than those reported for other human muscles (Lieber *et al.*, 2003; Ward *et al.*, 2009), they are similar to recent data from human semitendinosus (Smith *et al.*, 2011, in press). There was no significant correlation between single fiber volume and strain ($r^2 = 0.238$), and the slope of the relative volume vs. strain curve was not significantly different from zero (0.070 ± 0.072

SEM; $p > 0.3$). In contrast to the results on single fibers, there was a highly significant negative correlation ($R^2 = 0.881$) between fiber bundle volume and strain, where axial strain always resulted in decreased volume and the slope of the relative volume vs. strain curve was negative and highly significantly different from 0 (-0.477 ± 0.075 SEM; $p < 0.0001$). The slope of the relative volume to strain data was significantly different between fibers and bundles ($p < 0.001$; Fig 3). These data demonstrate that, although human skeletal muscle fibers are isovolumic, bundles of fibers with their associated ECM are not.

A common material property measured when testing for a volume change is Poisson's ratio (ν)

$$\nu = -d\epsilon_d / d\epsilon_x$$

Where (ϵ_d) is the strain of the fiber diameter and (ϵ_x) is the strain length of the fiber. To approximate Poisson's ratio using the slope of the relative volume to strain curve (s) we use:

$$\nu = (1 - s) / 2$$

A Poisson's ratio of 0.5 indicates an isovolumic material, while a value greater than 0.5 indicates a material decreasing in volume with strain (impossible for isotropic materials). Here the Poisson's ratio of fibers was very close to 0.5 (0.465 ± 0.124 SD), yet the Poisson's ratio for bundles was much greater (0.738 ± 0.129 SD).

4. Discussion

The main result of this study is that, while single muscle fibers obey the isovolumic assumption, as has been previously demonstrated (Huxley, 1953; Elliott *et al.*, 1963; Elliott *et al.*, 1967), fiber bundles decrease volume with strain. The important distinction between fibers and bundles is that while fibers are single muscle cells, bundles contain single muscle cells and their associated ECM. Within normal muscle, the area fraction of ECM is approximately 5% (Lieber *et al.*, 2003). Given that the relative volume of bundles at 200% strain is approximately 60% of the volume at resting length, this decrease in volume cannot be accounted for simply from the ECM compartment, but the ECM must be applying transverse force to the fibers that decreases volume. As the primary ECM component of fiber bundles is the endomysium surrounding individual fibers, we suggest that endomysium applies transverse load to a fiber with strain. This study is limited by the resolution of our distance measurements, which led to a higher variability in fibers than bundles. A linear fit to the relative volume to strain curve was used, however this approximation would not be valid at very large strains, as it would require the volume to become negative at large strain. Using the slope of relative volume vs. strain curve allows us to average the effects over many points as opposed to measuring the change in volume only at one very large strain. It should be noted that the fibers in this experiment were permeabilized by glycerol and thus may be more compliant in the transverse direction than intact fibers. While this may be important for transverse strain, it would probably not have any impact on the transverse stress being applied to the fibers by the ECM.

Application of a transverse stresses to muscle fibers by the ECM during stretch represents an important aspect of muscle physiology. The results observed here are limited to a single human muscle and are not yet generalizable to all muscles or other species. Muscle is often assumed to be isovolumic for the purposes of modeling and analyzing data; however our data cast doubt on that assumption. This could be particularly important in muscle disease in which the ECM is altered and fibrotic (Serrano & Munoz-Canoves, 2010) or when muscle

fiber sizes are altered as in exercise-induced hypertrophy or sarcopenia. Altering the application of transverse stress could be critical to muscle adaptation and mechanotransduction. These transverse stresses warrant further investigation as to how they are modulated by ECM properties. It is clear that muscle is not always an isovolumic material.

Acknowledgments

We gratefully acknowledge the National Institutes of Health grants HD050837 and AR057393 and the Department of Veterans Affairs. We also thank Dr. Sam Ward for helpful discussions.

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Figure 1. Sample image of a bundle of fibers on the testing apparatus. (A) Bundle from both the top-view and the 45° mirror with the side-view. (B) Close-up of top-view. (C) Close-up of side-view. Red-lines represent the points where the bundle dimensions were measured.

Figure 2. Sample plot of relative volume vs. axial strain. The single fiber (open circles) shows little change in volume with strain, but the fiber bundle (filled circles) shows decreased volume with strain. Lines are linear best fits.

Figure 3.

Average change in volume with strain for single fibers and fiber bundles. The slope of the relative volume to strain for single fibers does not differ significantly from zero implying constant volume. The average slope of the bundles is negative indicating a loss of volume as bundles undergo strain. Error bar represent standard error of the mean.