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Short communication

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.

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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 and 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 behavior. If human muscle does not satisfy the isovolumic assumption, models may need to be refined. In 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), KCH_4O_3S (86.0), $Ca(KCH_4O_3S)_2$ (0.13), $Mg(KCH_4O_3S)_2$ (10.8), K_3EGTA (5.5), KH_2PO_4 (1.0), Na_2ATP (5.1), and 50.0 μM 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 405 A, 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 50× 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/s. Each stretch was held for 3 min 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

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