Journal of Biomechanics 42 (2009) 1384–1389

Contents lists available at ScienceDirect

Journal of Biomechanics

journal homepage: www.elsevier.com/locate/jbiomech
www.JBiomech.com

2008 ASB Young Scientist Post-Doctoral Award

Passive mechanical properties of the lumbar multifidus muscle support its role as a stabilizer ☆

Samuel R. Ward [a,b,c], Akihito Tomiya [a], Gilad J. Regev [a], Bryan E. Thacker [c], Robert C. Benzl [a], Choll W. Kim [a], Richard L. Lieber [a,c,*]

[a] Department of Orthopaedic Surgery, University of California and Veterans Administration Medical Centers, San Diego, USA
[b] Department of Radiology, University of California and Veterans Administration Medical Centers, San Diego, USA
[c] Department of Bioengineering, University of California and Veterans Administration Medical Centers, San Diego, USA

ARTICLE INFO

Article history:
Accepted 25 September 2008

Keywords:
Lumbar multifidus
Muscle mechanics
Lumbar spine

ABSTRACT

The purpose of this study was to compare the passive mechanical properties and titin isoform sizes of the multifidus, longissimus, and iliocostalis muscles. Given our knowledge of each muscle's architecture and the multifidus' operating range, we hypothesized that multifidus would have higher elastic modulus with corresponding smaller titin isoforms compared to longissimus or iliocostalis muscles. Single-fiber and fiber-bundle material properties were derived from passive stress–strain tests of excised biopsies ($n = 47$). Titin isoform sizes were quantified via sodium dodecyl sulfate-vertical agarose gel electrophoresis (SDS-VAGE) analysis. We found that, at the single-fiber level, all muscles had similar material properties and titin isoform sizes. At the fiber-bundle level, however, we observed significantly increased stiffness (~45%) in multifidus compared to longissimus and iliocostalis muscles. These data demonstrate that each muscle may have a different scaling relationship between single-fiber and fiber-bundle levels, suggesting that the structures responsible for higher order passive mechanical properties may be muscle specific. Our results suggest that divergent passive material properties are observed at size scales larger than the single cell level, highlighting the importance of the extracellular matrix in these muscles. In addition to architectural data previously reported, these data further support the unique stabilizing function of the multifidus muscle. These data will provide key input variables for biomechanical modeling of normal and pathologic lumbar spine function and direct future work in biomechanical testing in these important muscles.

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1. Introduction

The posterior paraspinal muscles provide dynamic stability to the spinal column (Bogduk et al., 1992). Numerous studies have described the anatomical (Macintosh and Bogduk, 1986; Macintosh et al., 1993; Stokes and Gardner-Morse, 1999; Delp et al., 2001; Rosatelli et al., 2008) and histochemical (Rantanen et al., 1994) properties of these muscles with the goal of understanding normal and pathological spinal function. Similarly, imaging studies of the multifidus and other muscles have revealed pathologic changes associated with other spinal abnormalities such as chronic low back pain (Flicker et al., 1993; Parkkola et al., 1993), disk herniation (Zhao et al., 2000), scoliosis (Chan et al., 1999), and degenerative lumbar kyphosis (Kang et al., 2007). Paradoxically, surgery designed to treat these spinal disorders actually disrupt these muscles, and in turn, may lead to significant secondary structural and functional deficits (Skaf et al., 2005; Hazard, 2006; Sasaoka et al., 2006; Gille et al., 2007) which has been correlated with the development of post-surgical failed-back syndrome (Sihvonen et al., 1993; Turner et al., 2004; Taylor, 2006; Boswell et al., 2007).

The structure, function, and design of skeletal muscle has primarily been studied in the upper (Jacobson et al., 1992; Lieber et al., 1992) and lower extremities (Wickiewicz et al., 1983; Friederich and Brand, 1990), with very little data available for axial musculature. One universal finding in studies of extremity muscle is that skeletal muscle architecture, defined as the number and orientation of muscle fibers within a muscle, is the only accurate predictor of function (Powell et al., 1984; Lieber and Friden, 2001). Architectural properties of many spinal muscles were reported by Delp et al. (2001). However, it has been recently demonstrated that the multifidus muscle has a unique design amongst the

☆ IRB: Each author certifies that his or her institution has approved the protocol for this investigation and that all investigations were conducted in conformity with ethical principles of research.

* Corresponding author at: Department of Orthopaedic Surgery (9151), UC San Diego and VA Medical Center, 3350 La Jolla Village Drive, San Diego, CA 92161. Tel.: +858 552 8585x7016; fax: +858 552 4381.
E-mail address: rlieber@ucsd.edu (R.L. Lieber).

0021-9290/$ - see front matter Published by Elsevier Ltd.
doi:10.1016/j.jbiomech.2008.09.042

posterior spinal muscles; to stabilize the lumbar spine based on its very short fiber length, large physiological cross-sectional area, and specialized sarcomere length operating range (Ward et al., 2009).

In terms of design, the range over which sarcomeres operate and their passive mechanical properties are also functionally relevant. For example, it has been demonstrated that wrist flexors and extensors operate on opposite sides of the sarcomere length–tension curve, and have similar elastic moduli, which causes a precise mechanical balance between flexion and extension moments throughout the range of wrist motion (Lieber and Fridén, 1998). It has also been demonstrated that passive material properties of muscles do not simply scale between the single-fiber and fiber-bundle levels (Lieber et al., 2003). In the multifidus, it has been demonstrated that the muscle operates almost exclusively on the ascending limb of its length–tension curve even though the passive mechanical properties of its cells are similar to other extremity muscles (Ward et al., 2009). However, without understanding the single-fiber and fiber-bundle passive mechanical behaviors of each key lumbar spine muscle it is impossible to determine whether such properties are characteristic of all lumbar extensors or unique to the multifidus.

Previous research showed that passive mechanical properties of single muscle fibers vary between muscles (Prado et al., 2005) and with disease state (Fridén and Lieber, 2003). In these studies, the changes in single-fiber intrinsic mechanical properties were likely due to reorganization of intracellular load–bearing proteins. Since it is the major source of intracellular passive tension, a change in titin isoform would be the most likely cause of passive fiber elasticity modulation at the cellular level (Prado et al., 2005). Importantly, it has been shown that changes in passive tension are not limited to single muscle cells. For example, in spastic muscle fiber bundles, it has been suggested that extracellular matrix has important to passive mechanical load-bearing properties (Lieber et al., 2003). However, the passive mechanical properties and titin isoforms are virtually unknown for the human lumbar spinal musculature.

Therefore purposes of this study were: (1) to compare single-fiber and fiber-bundle passive mechanical properties of the multifidus, longissimus, and iliocostalis muscles, (2) to measure the titin isoform size in each muscle, and (3) to determine the relationship between titin size and passive single-fiber and fiber-bundle mechanical properties in each muscle. Given what is currently known about the architecture of each muscle, the operating range of the multifidus, and the passive mechanical properties of muscle, we hypothesized that multifidus would have larger elastic modulus and smaller titin isoforms compared to longissimus or iliocostalis muscles.

2. Materials and methods

2.1. Specimen and preparation

Under a University of California San Diego Human Subjects Protection Program approved protocol, muscle specimens were obtained from patients undergoing spinal surgery (Table 1). Using minimally invasive retractors placed in the intermuscular planes between the multifidus and longissimus, and the longissimus and iliocostalis muscles it was possible to identify each of these muscles. A small segment of the muscle was isolated by blunt dissection along natural fascicular planes. A specialized clamp was then slipped over the bundle with care to avoid undue manipulation or tension on the muscle. After harvest, the biopsy was immediately placed in relaxing solution composed of (in millimoles per liter): ethylene–glycol tetraacetic acid (EGTA), 7.5; potassium propionate, 170; magnesium acetate, 2; imidazole, 5; creatine phosphate, 10; adenosine triphosphate (ATP), 4; leupeptin, a protease inhibitor, 17 mg/ml; and E64 (a protease inhibitor) 4 mg/ml (Wood et al., 1975). This solution prevented depolarization across any site of disrupted membrane and proteolytic degradation, either of which can destroy the specimen. Single fibers or fiber bundles were either immediately dissected from the fresh biopsy or placed into a storage solution composed of relaxing solution mixed with 50% glycerol and stored at −20 °C. Samples stored in this manner have been shown to have stable mechanical properties for up to 3 months (Wood et al., 1975; Moss, 1979), but all fibers in this study were tested within 14 days.

Table 1
Patient demographics.

Muscle	*n*-size	Age	Gender		Biopsy level			
			Male	Female	L2–L3	L3–L4	L4–L5	L5–S1
Multifidus	23	57 ± 14[a]	11	12	1	2	5	15
Longissimus	7	69 ± 10	3	4	–	3	4	–
Iliocostalis	7	69 ± 10	3	4	–	3	4	–

Values are mean ± SD.
[a] Significant difference between multifidus and longissimus–iliocostalis.

2.2. Passive single-fiber and fiber-bundle mechanics

The single- and fiber-bundle testing protocol was designed to measure elastic material properties apart from any velocity dependent properties, as previously described (Fung, 1981; Fridén and Lieber, 2003; Lieber et al., 2003). Briefly, the dissected fiber or fiber-bundle segment was secured on either side to 125 μm titanium wires using 10-0 silk suture loops. One wire was secured to an ultrasensitive force transducer (Model 405A, sensitivity 10 V/g, Aurora Scientific, Ontario, Canada) and the other was secured to a micromanipulator. The sample was transilluminated by a 7 mW He–Ne laser to permit sarcomere length measurement by laser diffraction (Lieber et al., 1984). Resolution of this method is approximately 5 nm (Baskin et al., 1979). The system was calibrated with a 2.50 μm plastic blazed diffraction grating prior to experimentation (Diffraction Gratings, Inc., Nashville, TN). After calibration and mounting, samples were lengthened until force registered on a load cell that defined baseline load and slack sarcomere length. Baseline sample diameters were optically measured with a cross-hair reticule mounted on a dissecting microscope and micromanipulators on an x–y mobile stage. Force–displacement data were generated for each mounted sample in 250 μm increments after which stress-relaxation was permitted for 2 min and both sarcomere length and tension were again recorded. Segments were elongated through the theoretical limit of actin and myosin overlap in human muscle (Lieber et al., 1994). Force data were converted to stress by dividing force by the baseline cross-sectional area value and displacement was converted to strain subtracting sarcomere length from the baseline slack sarcomere length value and then dividing by the baseline slack sarcomere length value. The slope of the stress–strain curve between 2.0 and 4.25 μm was defined as the elastic modulus. Samples were discarded if they did not produce a clear diffraction pattern, if any irregularities appeared along their length, or if they were severed or slipped at either suture attachment point during the test.

2.3. Analysis of titin isoform

Single fibers were homogenized in sodium dodecyl sulfate-vertical agarose gel electrophoresis (SDS-VAGE) sample buffer and stored at −80 °C until analyzed by gel electrophoresis. SDS-VAGE sample buffer was comprised of 8 M urea, 2 M thiourea, 3% SDS w/v, 75 mM DTT, 0.03% bromophenol blue, and 0.05 M Tris–Cl, pH 6.8 (Warren et al., 2003). The molecular mass of titin in single fibers was determined using SDS-VAGE. This procedure has been described previously (Warren et al., 2003). An acrylamide plug was placed at the bottom of the gel to hold the agarose in place. The final composition of this plug was 12.8% acrylamide, 10% v/v glycerol, 0.5 M Tris–Cl, 2.34% N,N′-diallyltartardiamide, 0.028% ammonium persulfate, and 0.152% TEMED. The composition of the agarose gel was 1% w/v Sea Kem Gold agarose (Lonza, Basel, Switzerland), 30% v/v glycerol, 50 mM Tris–base, 0.384 M glycine, and 0.1% w/v SDS. This solution was poured over the acrylamide plug and kept warm to prevent premature solidification.

Titin standards were obtained from human cadaver soleus and rat cardiac muscle, which have known molecular weights of 3700 and 2992 kDa, respectively, based on sequence analysis of the 300 kb titin gene with a coding sequence contained within 363 exons (Labeit and Kolmerer, 1995; Freiburg et al., 2000). These tissues were also homogenized and stored at −80 °C until analysis. Before loading onto the gel, a titin standards "cocktail" was created by placing 4 μL human soleus standard and 8 μL rat cardiac standard into 98 μL sample buffer. Sample wells were then loaded with both biopsy and rat cardiac homogenate. Human soleus and rat cardiac titin homogenates were loaded into standard lanes. This facilitated titin quantification on each gel. Gels were run at 4 °C for 5 h at 15 mA constant current.

Agarose gels were fixed and stained according to the Silver Stain Plus (Bio-Rad, Hercules, CA) procedure except that gels were dried for approximately 20 h at 40 °C