Journal of Biomechanics 47 (2014) 1506–1509

Contents lists available at ScienceDirect

Journal of Biomechanics

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

Short communication

Post-mortem timing of skeletal muscle biochemical and mechanical degradation

Lori J. Tuttle [a], Marianna Alperin [b], Richard L. Lieber [c,*]

[a] Doctor of Physical Therapy Program, Exercise and Nutritional Sciences, San Diego State University, San Diego, CA, USA
[b] Department of Reproductive Medicine, Division of Female Pelvic Medicine and Reconstructive Surgery, University of California San Diego, San Diego, CA, USA
[c] Departments of Orthopaedic Surgery and Bioengineering, University of California San Diego and VA San Diego Healthcare System, San Diego, CA, USA

ARTICLE INFO

Article history:
Accepted 13 February 2014

Keywords:
Muscle
Titin
Collagen
Myosin heavy chain

ABSTRACT

Fresh cadaveric human tissue is a valuable resource that is used to address important clinical questions. However, it is unknown how post-mortem time impacts skeletal muscle mechanical and biochemical properties. We simulated morgue conditions in rabbits and tested the passive mechanical properties of muscle bundles, and the degradation of myosin heavy chain, collagen, and titin at specific intervals up to 7 days post-mortem. While a great deal of inter-specimen variability was observed, it was independent of post-mortem time. Passive mechanics, myosin heavy chain, and collagen content were all unaffected while the titin protein degraded up to 80% over 7 days post-mortem. These data indicate that fresh cadaveric tissue may be used for passive mechanical testing and that certain biochemical properties are unchanged up to 7 days after death.

Published by Elsevier Ltd.

1. Introduction

Procuring human tissue for mechanical and biochemical research has become increasingly important in order to answer clinically relevant questions. Restrictions in the use of primates in research have made human tissue even more valuable. Typically, human muscle tissue is obtained during a surgical procedure or through cadaveric donation programs. It is particularly difficult to obtain "normal" human muscle since muscle obtained in a surgical setting is often compromised due to disuse or pathology. Often, muscles that may be of interest are not physically accessible during surgery. Cadavers provide a valuable alternative source of human muscle tissue, but if the cadaver is fixed, the tissue is unusable for many biomechanical and biochemical tests. Fresh cadaver tissue thus often represents the best option, but it is unknown how the mechanical and biochemical properties of skeletal muscle are altered with time post-mortem. The purpose of this study was to measure changes in passive mechanics of skeletal muscle bundles, titin degradation, myosin heavy chain composition, and hydroxyproline content over 7 days post-mortem using rabbit muscle under simulated morgue conditions.

* Correspondence to: Department of Orthopaedic Surgery, University of California San Diego, 9500 Gilman Drive, Mail Code 0863, La Jolla, CA 92093, USA. Tel.: +1 858 822 1344; fax: +1 858 822 3807.
E-mail address: rlieber@ucsd.edu (R.L. Lieber).

http://dx.doi.org/10.1016/j.jbiomech.2014.02.019
0021-9290 Published by Elsevier Ltd.

2. Methods

Three New Zealand White rabbits (*Oryctolagus cuniculus*) were anesthetized with a subcutaneous injection of a ketamine–xylazine cocktail (50 and 5 mg/kg body mass, respectively). Animals were euthanized with pentobarbital (Euthasol; Virbac AH, Fort Worth, TX) and then stored at a constant temperature of −4 °C for the remainder of the study. Biopsies (approximately 2 cm × 1 cm × 0.5 cm) were obtained from the tibialis anterior muscle of each rabbit ($N=6$) immediately post-mortem and then every 4 h for the first 24 h and then every 12 h up to 7 days post-mortem. Tissue was placed immediately into a glycerinated storage solution and refrigerated at −20 °C until further testing (Friden and Lieber, 2003). Additional biopsies were obtained at the initial time point and at 20 h post-mortem to specifically test for proposed rigor effects. Unilateral tibialis anterior muscle was biopsied sequentially before samples were taken from the contralateral leg (Fig. 1) in order to minimize the effects of skin incision and tissue exposure. Muscle was covered by the overlying skin as much as possible between biopsies in order to minimize tissue drying.

2.1. Passive mechanics

Passive mechanics of muscle bundles was performed using previously reported methods (Friden and Lieber, 2003). Briefly, biopsies were removed from storage solution and placed in relaxing solution. Muscle bundles (approximately 10 muscle fibers per bundle) were dissected from the biopsy ($N=3$ bundles per biopsy) and secured in a custom apparatus using 10-0 monofilament nylon suture on one end to a force transducer and on the other end to a titanium wire rigidly attached to a rotational bearing. Sarcomere length was measured by laser diffraction (Lieber et al., 1984). The bundle was brought to slack length and bundle dimensions were measured with a cross-hair reticule mounted on a dissecting microscope and micromanipulators. The bundle was deformed to strains of approximately 0.25 μm sarcomere^{-1} at 100 fiber lengths s^{-1}. Each stretch was held for 3 min

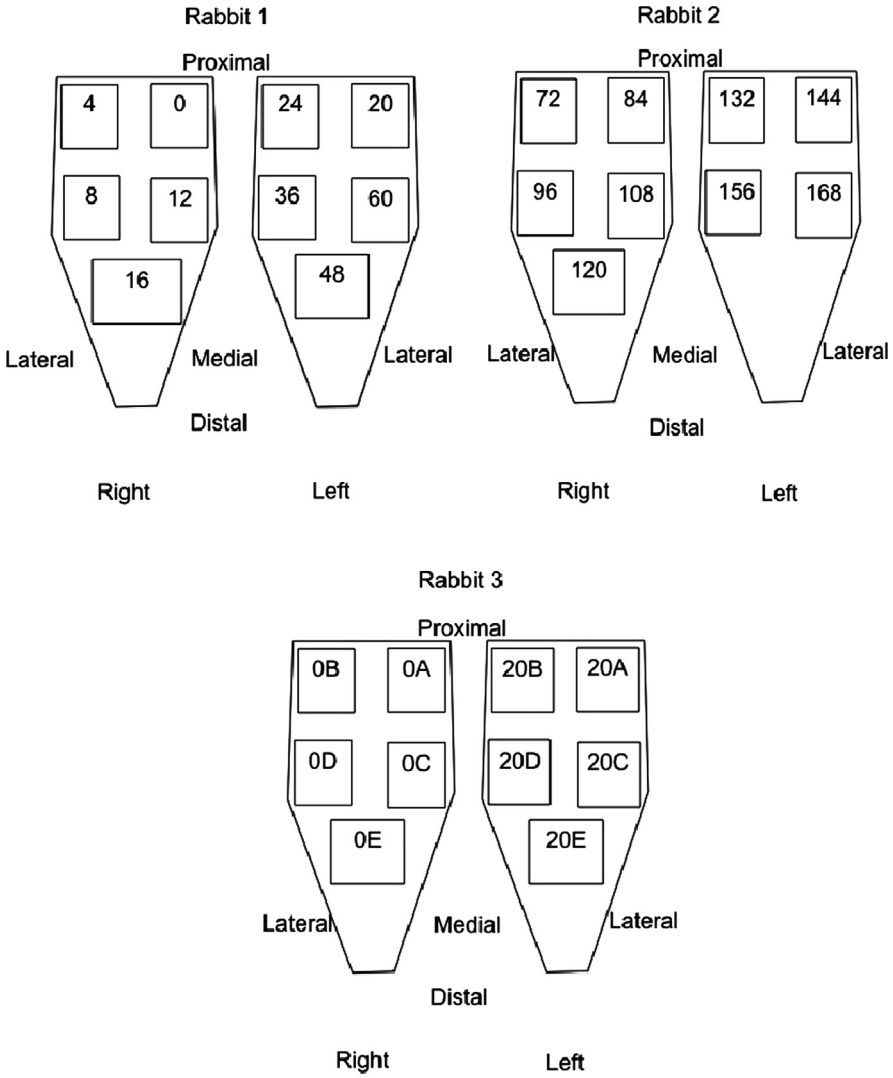

Fig. 1. Schematic representation of sampling procedures. Numbers indicate hours post-mortem.

during which stress relaxation was measured (Friden and Lieber, 2003). Force was converted to stress by dividing force by the baseline cross-sectional area determined assuming a cylindrical sample with an average diameter determined from three separate points along the bundle. Tangent stiffness of the nonlinear fit to the stress–sarcomere length relationship (in units of kPa/μm) at sarcomere length 3.5 μm is reported.

2.2. Titin degradation

Titin molecular mass was determined from muscle bundles tested for passive mechanics using SDS-VAGE as previously described (Warren et al., 2003). Relative mobility and intensity of each band was quantified using a GS-800 Calibrated Densitometer and Quantity One 1-D Analysis software. Relative mobility of protein on the gel is linearly related to the log of its molecular mass; this relationship was used to calculate molecular mass of titin based on its positions relative to rat cardiac and human soleus titin standards. As titin degrades, T1 and T2 bands appear, with the T2 band representing a degradation product (Ali et al., 2010). Thus, titin results are expressed in units of %T1 where 100% represents no degradation.

2.3. Collagen content

Hydroxyproline content was used to determine collagen percentage using a modification of a previously validated protocol (Edwards and O'Brien, 1980). A small tissue sample (2 mg) was taken from each muscle biopsy and hydrolyzed in 6 N HCl at 110 °C for 24 h. Samples were then pipetted with standards into 96 well plates and were incubated with a chloramine T solution for 20 min at room temperature, followed by the addition of a p-dimethylaminobenzaldehyde solution and incubated at 60 °C for 30 min. Hydroxyproline concentration was determined based on the extinction coefficient of hydroxyproline at 550 nm and normalized to the mass of the original tissue sample. Values are reported as μg collagen/mg tissue.

2.4. Myosin heavy chain composition

Myosin heavy chain bands were identified and quantified with densitometry as previously described (Talmadge and Roy, 1993). The progression of the bands was compared and identified based on their relative molecular weight to that of a human protein standard prepared from a normal semitendinosus biopsy that shows all three human MHC bands (types IIa, IIx and I). Values are presented as percent IIA and percent IIX.

2.5. Statistical analysis

A two-way analysis of variance (ANOVA) was used to determine the effect of leg and biopsy location (laterality). Linear regression was used to determine if slopes were different from 0 for muscle bundle tangent stiffness, titin degradation, collagen content, and myosin heavy chain content over time.

3. Results and discussion

Prior to analysis of the time data, we first determined that there was no systematic difference in measures between rabbits or due to the biopsy location within the muscle (proximal, distal, medial, and lateral portions of the muscle). While muscle bundle mechanics were variable (average standard deviation of 17 kPa/μm), this variability was