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Short communication

How does tissue preparation affect skeletal muscle transverse isotropy?

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ABSTRACT

The passive tensile properties of skeletal muscle play a key role in its physiological function. Previous research has identified conflicting reports of muscle transverse isotropy, with some data suggesting the longitudinal direction is stiffest, while others show the transverse direction is stiffest. Accurate constitutive models of skeletal muscle must be employed to provide correct recommendations for and observations of clinical methods. The goal of this work was to identify transversely isotropic tensile muscle properties as a function of post mortem handling. Six pairs of tibialis anterior muscles were harvested from Giant Flemish rabbits and split into two groups: fresh testing (within four hours post mortem), and non-fresh testing (subject to delayed testing and a freeze/thaw cycle). Longitudinal and transverse samples were removed from each muscle and tested to identify tensile modulus and relaxation behavior. Longitudinal non-fresh samples exhibited a higher initial modulus value and faster relaxation than longitudinal fresh, transverse fresh, and transverse rigor samples ($p < 0.05$), while longitudinal fresh samples were less stiff at lower strain levels than longitudinal non-fresh, transverse fresh, and transverse non-fresh samples ($p < 0.05$), but exhibited more nonlinear behavior. While fresh skeletal muscle exhibits a higher transverse modulus than longitudinal modulus, discrepancies in previously published data may be the result of a number of differences in experimental protocol. Constitutive modeling of fresh muscle should reflect these data by identifying the material as truly transversely isotropic and not as an isotropic matrix reinforced with fibers.

1. Introduction

The human body relies on skeletal muscle, supported by other orthopedic tissues, for locomotion and posture. Passive properties of muscle are governed by two components of the tissue: the protein titin at the sarcomere level which gives muscle fibers passive stiffness (Magid and Law, 1985; Tskhovrebova and Trinick, 2002), and the collagen rich extracellular matrix which organizes muscle fibers in a hierarchical structure and dominates passive stiffness at the tissue level (Brown et al., 2012; Meyer and Lieber, 2011). In the case of skeletal muscle these passive properties have a multifaceted purpose: allowing for the transmission of internal force generated at muscle fibers to tendons (Gindre et al., 2013; Huijing, 1999), storing energy during locomotion (Cavanagh and Komi, 1979; Ettema, 1996), and maintaining proper resting length for maximum force generation (Fridén and Lieber, 1998). Muscle fiber alignment results in tissue transverse isotropy (Morrow et al., 2010; Pietsch et al., 2014; Takaza et al., 2012; Van Loocke et al., 2006) as the material properties of the aligned fibers differ from those of the organized extracellular matrix (Meyer and Lieber, 2011).

Finite element analyses of biological soft tissues provide important insight into tissue behavior for clinical recommendations and observations. However, inaccurate constitutive models could present erroneous data, thus hampering clinical relevance. Some modeling studies of passive skeletal muscle assume the longitudinal direction is stiffer than the transverse direction (Calvo et al., 2010; Grasa et al., 2011; Hernández-Gascón et al., 2013; Lu et al., 2010). While this is supported by some experimental work (Morrow et al., 2010), there is also data which identifies a stiffer transverse response as compared to the longitudinal direction (Nie et al., 2011; Takaza et al., 2012). These differences may be the result of disparities in experimental protocol and anatomical or species variations, although they are more likely the result of rigor mortis, which results in a stiffening of the tissue (Van Ee et al., 2000; Van Loocke et al., 2006). As rigor mortis is a complex

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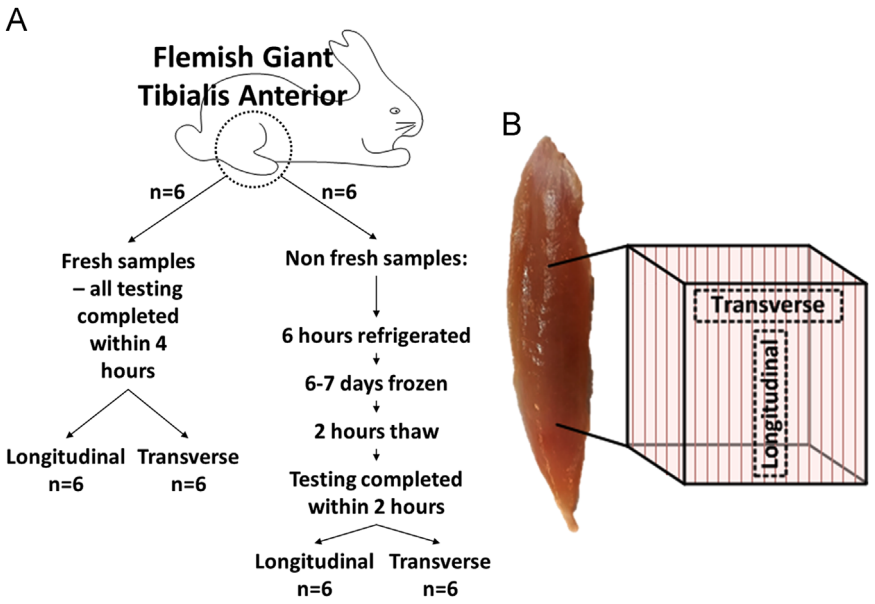

Fig. 1. (A) Specimen groupings and testing timeline, showing the fresh testing group and the group subject to non-fresh conditions and a freeze/thaw cycle. Each of these groups yielded longitudinal and transverse samples for a total of four groups. (B) Dissection orientations show that each muscle yielded two samples, one in the longitudinal direction and one in the transverse direction.

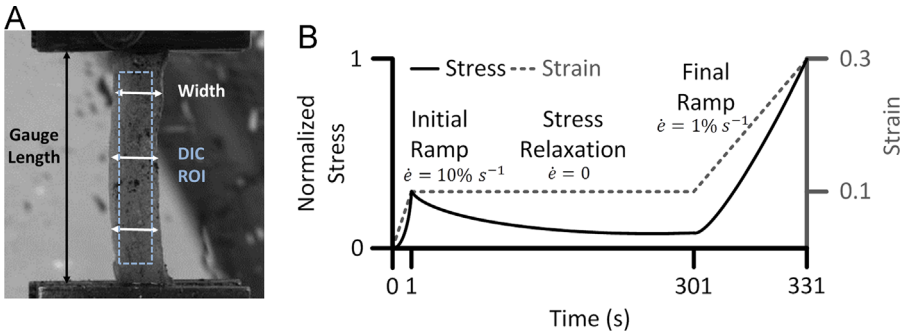

Fig. 2. Specimen testing procedures. A: Experimental setup showing speckled sample with gauge length (black arrow), three width measurement locations (white arrows), and digital image correlation region of interest (DIC ROI – light blue dotted box). B: Testing outline (not to scale) highlighting initial ramp phase, relaxation phase, and final ramp phase to failure (strain of 0.3 given as an example), with representative stress shown as solid black line and strain in dotted gray line. For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.

phenomenon related to the actin-myosin complex (Huff Lonergan et al., 2010; Roth et al., 2006), it most likely influences the longitudinal mechanics to a greater extent than in the transverse direction. Thus, it is hypothesized that the large observed differences in passive transversely isotropic skeletal muscle behavior is a function of non-fresh testing conditions and/or experimental protocol. Data supporting this hypothesis would provide two important recommendations for future studies of skeletal muscle: 1) all mechanical testing should be conducted on fresh, never frozen tissue, and 2) computational models of passively stretched muscle should reflect the true transverse isotropy in that the longitudinal direction is less stiff than the transverse direction.

The goals of this work were thus to evaluate the effects of orientation and post mortem handling on the material properties of skeletal muscle. Specifically, we aim to answer the question: "How does tissue preparation affect the transversely isotropic stiffness and time dependence of skeletal muscle?".

2. Methods

Six Giant Flemish Rabbits were obtained with Colorado State University Institutional Animal Care and Use Committee approval. Following euthanasia, whole tibialis anterior muscles were isolated from each hind limb and stored in a refrigerator for either fresh testing (left or right limb randomly) or to allow for the onset of rigor mortis (contralateral limb). As rigor mortis begins 6–8 h post mortem (Van Ee et al., 2000; Van Loocke et al., 2006) fresh muscles were tested within four hours to reduce these effects, while the contralateral muscle was subject to non-fresh testing following a freeze-thaw cycle (Fig. 1A). Each tibialis anterior yielded two samples, one longitudinal, and one transverse (Fig. 1B). As the pennation angle of the New Zealand White Rabbit is very low (Lieber and Blevins, 1989), the longitudinal direction was assumed to be parallel with the axis of force transmission.

Tensile tests were conducted on a servo hydraulic material test system (MTS, Eden Prairie, MN). Cross sectional area and gauge length were measured optically with a 1.4 megapixel monochrome camera and ImageJ software (National Institutes of Health, Bethesda, MD). Graphite powder was used to track strain with digital image correlation (DIC) software (Matlab, Mathworks, Natick, MA) on a region of interest (ROI) (Fig. 2A). All specimens underwent an initial ramp phase of 10% strain at $10\%\ s^{-1}$ followed by 300 s of relaxation and finally a constant ramp pull at $1\%\ s^{-1}$ until specimen failure (Fig. 2B). Cauchy (true) stress and Euler strain were converted from force-displacement data and used to calculate tangent moduli. For specifics on the testing procedure and data analysis see Supplementary Materials. Tangent moduli were calculated at the initial peak, end of the relaxation phase, and at 20% strain, which is the higher end of the physiological range for the rabbit tibialis anterior (Davis et al., 2003; Winters et al., 2009). Relaxation ratio was calculated as the fraction of stress relaxation over three separate time periods following the initial ramp: 0–5 s, 5–50 s, and 50–300 s. The raw relaxation data were fitted to a power law equation (Eq. 1, where σ is Cauchy stress, t is relaxation time, and a and b are constants which characterize the relative stress level and rate of