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SCALING OF PASSIVE MECHANICS: FROM FIBER TO WHOLE MUSCLE

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INTRODUCTION

Passive tension is borne by a muscle when it is lengthened beyond its slack length. Longer lengths are associated with exponentially larger tensions and result in resistive forces, even in the absence of muscle activation. An understanding of passive mechanical properties is necessary for describing the function of different muscles [1], differentiating between healthy and pathologic muscle [2], and characterizing muscle adaption [3]. Identifying the sources of passive tension and the size scale at which they are functionally relevant is thus prerequisite to adequately understanding muscle.

Passive tension is attributed in part to the parallel action of the extracellular matrix (ECM, primarily collagen) and intra-myofibrillar proteins (primarily, titin). Although the ECM and titin both contribute to passive tension, the relative contribution of each is muscle [4] and size-scale [5] dependent. Titin has been suggested to bear passive load in fibers, while ECM is thought to dominate at large scales. However, the relative contribution of titin and collagen in predicting passive properties is unclear. Furthermore, if passive tension is modulated by different sources at different size scales, then extrapolating whole muscle function from fiber or bundle mechanics may not appropriate. Unfortunately, this idea has never been systematically tested.

Thus, the purpose of the current study was to quantify the passive mechanical properties of single muscle fibers, fiber bundles, fascicles, and whole muscles. The resulting experimental data were then used to determine predictors of passive modulus at each size scale.

METHODS

The tibialis anterior (TA), extensor digitorum longus (EDL), and extensor digitorum II (EDII) muscles of New Zealand White rabbits were chosen for study due to their varied muscle architecture.

The distal tendon of the muscle of interest was transected and clamped to a servomotor (Cambridge Model 310B; Aurora Scientific, Aurora, ON, Canada) at the muscle-tendon junction. Passive stretch was imposed and the corresponding force was measured after three-minutes of stress relaxation.

After whole muscle measurements, muscle fibers, fiber bundles (~20 fibers), and fascicles (~300 fibers) were dissected from the muscle and secured by sutures to a custom testing apparatus (Figure 1). The bundle was passively stretched while real-time sarcomere length measurements were carried out via laser diffraction. From the stress-strain cuve, a tangent modulus at 90% of the anatomic operating range for each muscle was used to quantify *in vivo* stiffness of each muscle at each size scale.

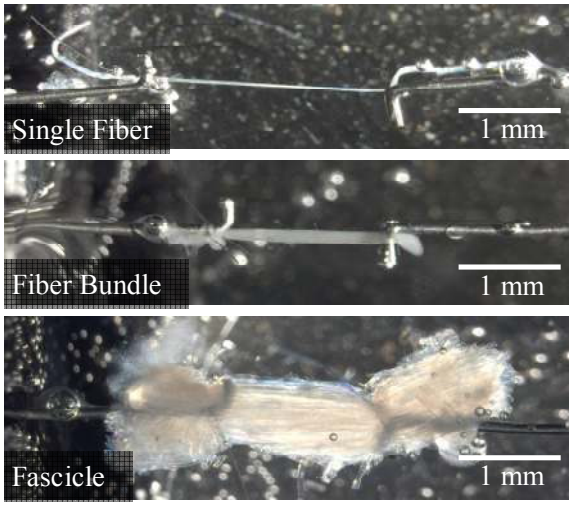

Figure 1: Mechanical testing of a single fiber, fiber bundle, and fascicle segments.

After mechanical testing, titin size and collagen content were analyzed from SDS-VAGE gels and hydroxyproline assays, respectively, for single fibers (~2 mg of fibers grouped together for hydroxyproline), fiber bundles, fascicles, and whole muscle sections (~5-mg wet weight). Muscle architecture was measured to determine the contribution of fiber organization to whole muscle passive mechanics.

Muscle and size scale were compared using a two-way repeated measures ANOVA, and pair-wise comparisons

were used to compare among levels (SPSS, Chicago, IL). Correlation between passive moduli at a given size scale and the measured parameters was evaluated using multiple regression analysis.

RESULTS AND DISCUSSION

Qualitatively, fibers exhibited a linear stress-strain curve. With increasing size scale, this relationship became increasingly non-linear (Figure 2). This phenomenon may be the result of additional layers of ECM at each larger size scale.

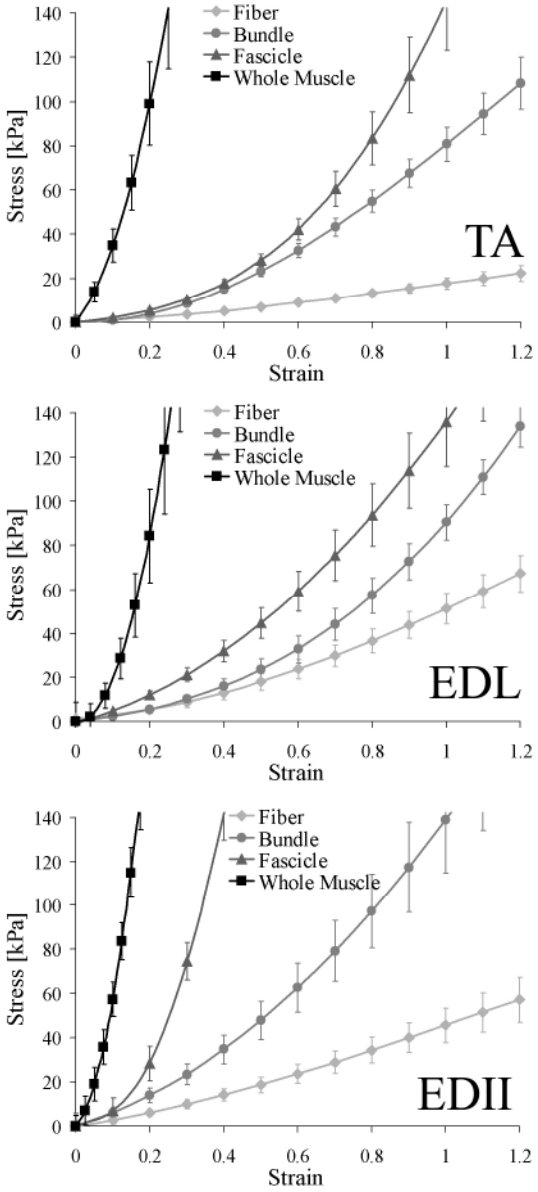

Figure 2: Passive stress-strain curves for (top) TA, (middle) EDL, and (bottom) EDII with each curve representing a different size scale: fibers, bundles, fascicles, and whole muscle.

Passive moduli increased nonlinearly from fiber to whole muscle ($p < 0.001$), and this scaling relationship was muscle-specific ($p < 0.001$) (Figure 3). Muscle fibers and bundles were not significantly different from one another ($p = 0.137$); differences across size scales were not observed until the fascicle level ($p < 0.001$). However, the muscle specific differences observed at the fascicle level did not predict changes at the whole muscle level, suggesting that smaller size scales were unable to capture passive mechanical properties at the whole muscle level.

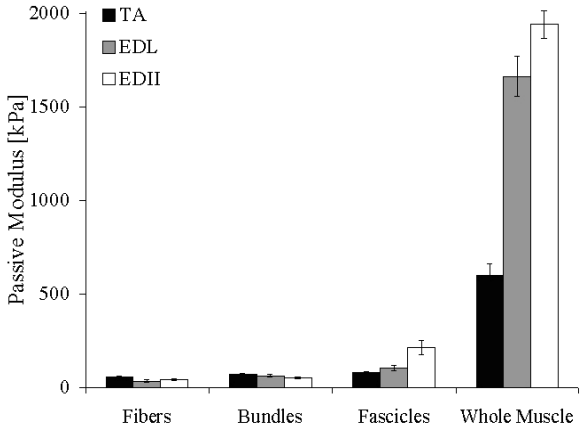

Figure 3: Passive tension moduli scale nonlinearly across size scales in a muscle-specific manner.

Multiple regression analysis examined the contribution of titin, collagen content, and muscle architecture to passive tension at each size scale. At the single fiber level, titin explained 57% of the variation in the passive tension moduli at this size scale ($r^2 = 0.57$, $p < 0.001$). However, at the bundle level, titin size only explained 10% of the variation ($r^2 = 0.10$, $p = 0.009$), revealing our limited understanding of bundle mechanics. At the fascicle level, titin was eclipsed by collagen content, which predicted over 50% of the variation in the modulus ($r^2 = 0.53$, $p = 0.001$). Finally at the whole muscle level, normalized fiber length dominated the regression model ($r^2 = 0.88$, $p < 0.001$), with collagen adding small explanatory power ($r^2 = 0.04$, $p = 0.023$).

CONCLUSIONS

These data demonstrate that passive moduli scale nonlinearly from the fiber to the whole muscle. In addition, the structures responsible for mediating passive tension appear to be fundamentally different across size scales and are poorly understood. Therefore, investigators are cautioned against extrapolating from muscle biopsies to predict whole muscle passive mechanical properties.

ACKNOWLEDGEMENTS

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