

Exploring the Mechanical Properties of Muscle Fibers and Fiber Bundles: Implications for Passive Load Measurement

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INTRODUCTION: A comprehensive understanding of the mechanical properties of muscle fibers and fiber bundles (Fs/FBs) is important as they constitute the fundamental building blocks of whole muscle. The passive mechanical properties of Fs/FBs have been determined through ex-vivo tensile testing, where the specimens are stored in a glycerinated physiological storage solution [1] and are immersed in a physiological relaxing solution [1] during the test, effectively deactivating the myosin and actin interactions. However, testing fresh Fs/FBs without the permeabilization process may result in measurements that reflect a combination of both passive and a portion of active mechanical properties. Therefore, the primary objective of this study is to quantify the alteration in stiffness observed in Fs/FBs stored in a glycerinated solution compared against fresh Fs/FBs. The understanding of mechanical properties differences in stored compared to fresh Fs/FBs will subsequently contribute to a better comprehension of the correlation between the mechanical properties of Fs/FBs and the whole muscles.

METHODS: In this study, the mechanical properties of Fs/FBs from 6 Tibialis Anterior (TA) muscles in 3 male Sprague-Dawley rats (344 ± 11.7 g; mean \pm SD) were investigated using tensile testing. This study was approved by the University of British Columbia Animal Care Committee. Under anesthesia, fresh TA muscle biopsies were obtained, and each biopsy was divided into two portions. One half of the biopsies were promptly used for dissecting Fs/FBs (FRESH), which were immediately subjected to tensile testing. The other half of biopsies were placed in a glycerinated storage solution at 4°C for one day and later transferred to a -20°C freezer, where they remained in the storage solution for an additional 13 days (STORED). After this storage period, the preserved samples were dissected into Fs/FBs and subjected to the identical tensile testing as the fresh samples. Each biopsy portion provided three fibers and four fiber bundles for testing, resulting in a total of 84 tests conducted in this study.

For the tensile tests, fibers or fiber bundles were securely affixed at their ends, with one end connected to a sensitive force transducer (400A, Aurora Scientific) and the other end attached to a length controller while the specimen was immersed in relaxing solution. The top and side diameters of each specimen were measured at three different points along the specimen to calculate the oval cross-sectional area (CSA) by the average of the readings. Subsequently, each specimen underwent a series of six cumulative stretches from the slack length, each applying a 10% sarcomere strain in one second, followed by a 4-minute relaxation period. At the end of each stretch increment, the force reading was divided by the CSA to derive the engineering stress. Each fibers' stress-strain data were then fitted to a linear curve, while the data of fiber bundles were fitted to a 2nd order polynomial curve in order to calculate their respective stiffnesses. A two-way ANOVA, considering muscle's condition (fresh or stored) and strain levels (10%, 20%, and 30% strain) as independent factors, was employed to assess the variations in fiber bundles' elastic modulus.

RESULTS SECTION: The collected average stress-strain data for individual fibers demonstrated a strong linear correlation ($R^2=0.99$) between stress and strain (Fig. 1a), resulting in a strain-independent elastic modulus of 92 kPa for fresh and 42 kPa for stored fibers (Fig. 2a). However, for fiber bundles the data implied a polynomial correlation between stress and strain (Fig. 1b). There were significant differences in elastic modulus between the fresh and stored fiber bundles ($p<0.001$) and also between strain levels ($p<0.001$) (Fig. 2b), but there was no significant interaction between the two ($p=0.29$). Overall, the stiffness of both individual fibers and fiber bundles is more than a twofold greater in fresh samples when contrasted with their preserved counterparts within the glycerinated storage solution.

DISCUSSION: The lower stiffness within stored muscle specimens can likely be attributed to the permeabilization process allowing for the disruption of interactions between myosin and actin due to the presence of ATP and EGTA in the storage and relaxing solutions [2]. The relatively diminutive size of the stored muscle fragments permits the storage solution to effectively permeabilize and infiltrate the fibers, leading to the breaking and disabling of their myosin-actin connections, which results in the measured stiffness representing solely the passive load bearing structures within the Fs/FBs [3]. Alternatively, tensile testing fresh muscle Fs/FBs results in the measured properties reflecting some combination of passive and active contributions. This consideration is significant when comparing the mechanical properties of Fs/FBs with the mechanical properties of fresh whole muscles in order to calculate scaling relationships.

SIGNIFICANCE/CLINICAL RELEVANCE: Storing fibers and fiber bundles of muscles in glycerinated storage solution is necessary to ensure that measured properties reflect solely the contribution of passive load bearing structures. The storage method and preparation before tensile testing is crucial for evaluating solely passive properties and needs to be considered carefully in muscle fibers and fiber bundles mechanical testing.

REFERENCES: [1] Eastwood AB, et al., 1979. Tissue and cell. Jan 1;11(3):553-66. [2] Zwambag, DP, et al., 2019. Journal of Biomechanics, 88, pp.173-179. [3] Wood DS, et al., 1975. Science. Mar 21;187(4181):1075-6.

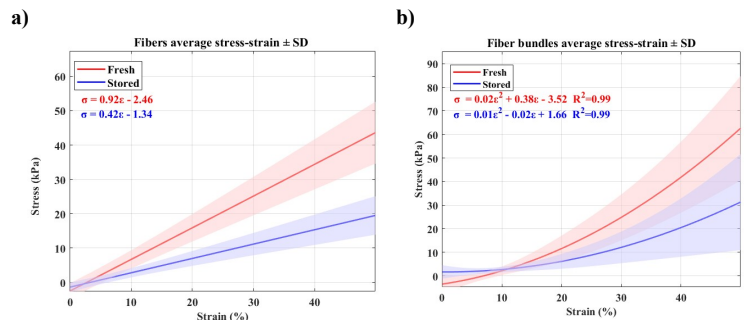


Fig. 1, The average stress \pm standard deviation, along with strain data from the tensile testing for: a) fibers; and b) fiber bundles, in fresh (red) and stored (blue) specimens.

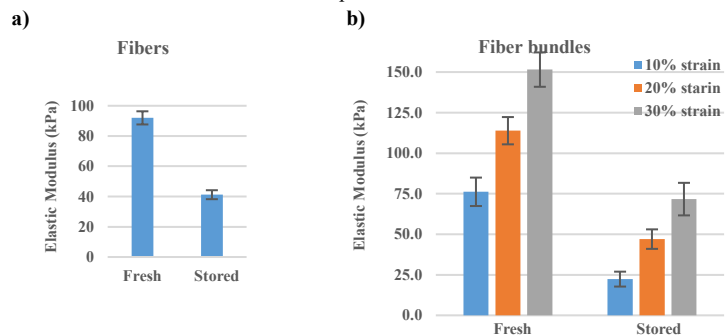


Fig. 2, The average Elastic modulus (slope of the stress-strain curve) \pm standard error of the mean for a) fibers and b) fiber bundles at strain levels of 10%, 20%, and 30% for fresh and stored specimens.