The Effect of Physiological Relaxing and Storage Solutions on the Ability to Measure Passive Mechanical Properties of Whole Muscle

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INTRODUCTION: The mechanical properties of muscles are fundamentally important when analysing the musculoskeletal system. The passive mechanical properties of individual muscle fibers and fiber bundles have been determined through the application of ex-vivo tensile testing, wherein muscle samples were stored in glycerinated physiological storage solution [1] and then immersed in physiological relaxing solution [1] during testing to deactivate myosin and actin connections to ensure only passive properties were measured. Most investigations on whole muscle properties have been conducted on fresh muscles, thereby including both active and passive aspects of muscle behavior, and the methodology for assessing only the passive properties of whole muscle remains challenging [2]. Consequently, our study aimed to investigate how testing solutions and storage solutions affect the mechanical properties of whole muscle in ex-vivo tensile testing. We first examined how different solutions (i.e. physiological relaxing and Tyrode's [3]) during tensile testing affected the mechanical behavior of fresh whole muscles. Relaxing solution aims to eliminate active contractions by the presence of ATP and EGTA, while Tyrode's solution aims to maintain muscle viability and functionality during testing. We next examined the effect of storing whole muscles in a glycerinated storage solution which aims to preserve muscle integrity and viability over a period of time.

METHODS: The mechanical properties of the Tibialis Anterior (TA) muscles in 12 male Sprague-Dawley rats (349.7 ± 18.7 g; mean ± SD) were investigated using tensile testing. This study was approved by the University of British Columbia Animal Care Committee. To perform the tests, the bone-tendon-TA muscle unit of each rat's hindlimb was carefully dissected immediately following euthanasia and its bony sides were securely held by two custom-made holders, with one holder fixed and the other attached to a 20 N load cell and a servomotor. Then, the muscle was lengthened in ~7% muscle strain increments from 0% to ~50% and held for 4 minutes at each step. Force and displacement at the end of each hold were recorded, and the normalized stress was calculated by dividing the force by the physiological cross-sectional area of the muscle [4]. The stress-strain data were then fitted to a 2nd order polynomial curve to compare the muscles' elastic modulus.
In the first phase of this study, six rats were used. For each rat, one TA muscle, either from the left or right leg, was freshly immersed in a physiological relaxing solution, while the corresponding TA muscle of the other leg was immersed in Tyrode's solution during the tensile testing. This allowed us to determine if the presence of ATP in the relaxing solution during the test had any effect on the mechanical properties of the fresh whole muscle.
In the second phase, an additional six rats were used. One TA muscle from the left or right leg of each rat was tested fresh, directly in Tyrode's solution; and the other TA muscle was stored in glycerinated solution at 4°C for one day and then transferred to a -20°C freezer, where it remained in storage solution for an additional 13 days. On the day of testing, the muscle was placed in relaxing solution at 4°C for 2 hours before the tensile testing, which was also conducted in relaxing solution. Following the tests, the muscles were flash frozen for histological evaluation with hematoxylin and eosin (H&E) staining. A two-way ANOVA, with the independent factors of solution types (relaxing and Tyrode's) and strain levels (10%, 20%, and 30% strain) in the first study phase, and muscle conditions (fresh and stored) and strain levels in the second phase, was employed to assess the variations in muscles' elastic modulus.

RESULTS SECTION: In the first phase, there were no significant differences in the elastic modulus between the fresh muscles tested in Tyrode's versus relaxing solutions ($p=0.66$); however, the effect of strain levels on elastic modulus was statistically significant ($p<0.001$) and there was no significant interaction between the two ($p=0.99$) (Fig.1a). In the second phase, notable variations were observed in elastic modulus between fresh and stored muscles ($p=0.002$), strain levels ($p=0.003$), and interaction between the two ($p=0.004$). Stored whole muscles illustrated a linear association between stress and strain, resulting in a strain-independent elastic modulus. In contrast, fresh whole muscles showed an increase in elastic modulus with higher muscle strain, characterized by a parabolic stress-strain curve (Fig.1b). Also, histological evaluation of the stored muscles indicated abnormality in the center region of the muscles (Fig.2).

DISCUSSION: Comparing fresh whole muscle testing results in a relaxing versus Tyrode's solutions suggests that the presence of ATP in the relaxing solution during the testing period is insufficient to permeate the muscle tissue and disrupt the interactions between actin and myosin. Therefore, there is no notable difference in testing whole muscle in relaxing or Tyrode's solutions. Also, the results of stored whole muscles testing indicate that the glycerinated storage solution does not work for preserving the passive properties of muscles of this size [5]. Histological examination shows that the storage solution is unable to penetrate the central regions of the rat's TA muscles, leading to damage induced by freezing at -20°C. This emphasizes the need for innovative storage methods and devising a novel approach for preparing whole muscles before testing, preserving mechanical properties, and ensuring that tensile testing solely measures the passive mechanical properties of whole muscle.

SIGNIFICANCE/CLINICAL RELEVANCE: In fresh whole muscle, relaxing solution is inadequate for inducing muscle relaxation and suppressing active contractions during tensile testing. Additionally, the application of glycerinated storage solution proves insufficient in preserving the mechanical properties of muscles at this size. Therefore, further exploration and alternative storage methods are necessary for comprehensive investigations in whole muscle studies.

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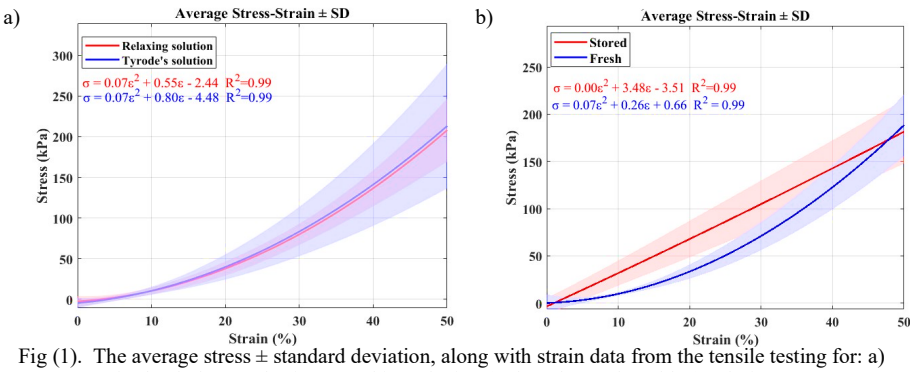

Fig (1). The average stress ± standard deviation, along with strain data from the tensile testing for: a) fresh whole muscles immersed in Relaxing (red) and Tyrode's (blue) solutions; and b) fresh (blue) and 14-day stored (red) whole muscles.

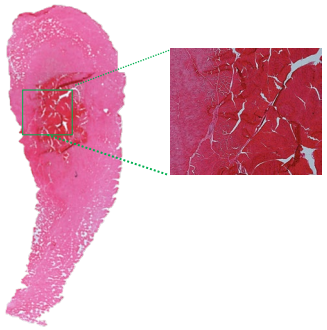

Fig (2). Transverse section of H&E stained central area of a stored TA muscle in a glycerinated storage solution.