

EFFECT OF COMMON EXPERIMENTAL STORAGE TECHNIQUES ON ARTERIAL BIOMECHANICS

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ABSTRACT

Blunt arterial injury experimentally occurred in distraction with intimal subfailure before ultimate failure. Experimental soft-tissues are commonly frozen until testing and may be refrigerated between tests. The present study axially distracted porcine aortas to failure implementing fresh, refrigerated, and frozen storage conditions. Intimal subfailure occurred in 83% of specimens. Elasticity and subfailure and failure stresses decreased in refrigerated compared to fresh and frozen specimens. Fresh and frozen specimens were not significantly different. Clinically relevant intimal failures can be reproduced and injury mechanics determined while adhering to experimental protocols of freezing specimens before testing. However, short-term tissue refrigeration may affect biomechanics.

Keywords: Carotid artery injury, tunica intima, experimental storage techniques, subfailure.

BLUNT ARTERIAL INJURIES were reported to occur during automotive impacts and have affected the thoracic aorta and common and internal carotid arteries (Carrillo *et al.* 1999; Creasy *et al.* 1997; Fabian *et al.* 1996; McKeivitt *et al.* 2002). Delayed symptomatology has been associated with intimal layer subfailure, wherein inner regions of the vessel wall fail and superficial layers remain acutely intact (Cogbill *et al.* 1994; Fabian *et al.* 1996; Punjabi *et al.* 1997). These injuries have been experimentally reproduced (Cohuet *et al.* 2001) with subfailure mechanics quantified relative to ultimate mechanics (Stemper *et al.* 2005a). In particular, under longitudinal vessel distraction, intimal layer subfailures occurred significantly earlier in fresh porcine aorta specimens than in previously frozen human carotid artery specimens. This difference may be attributed to factors including specimen storage (fresh versus frozen), age, and differences in vessel mechanics between species. Although some studies have demonstrated contradictory results, freezing was previously shown to affect biomechanics of some soft tissues including tendons, ligaments, articular cartilage, and spinal motion segments (Callaghan and McGill 1995; Duma *et al.* 2004; Smith *et al.* 1996; Turner *et al.* 1988). However, the effect of common experimental storage techniques on arterial biomechanics remains unclear as a majority of studies have focused on cryopreservation techniques with extremely low freezing temperatures, low freezing rates, and the use of cryoprotectants to preserve physiologic function. To better understand the mechanism of blunt carotid artery injury, the mechanical effects of storage techniques on arterial specimens must be characterized. The purpose of the present investigation was to quantify the effect of common experimental storage techniques of short-term refrigeration and long-term freezing on elastic, subfailure and ultimate failure biomechanics of arterial specimens under longitudinal distraction.

METHODS

Descending thoracic aortas were obtained from adult porcine specimens. Aortas were sectioned into ninety-three 4.0-cm segments from three vessel regions (proximal, mid-region, distal). Loose connective tissue was removed and each segment was immediately placed in lactated Ringer's solution in a separate container and randomly assigned to one of three storage groups: fresh, refrigerated, and frozen. An equal number of segments from each of the three vessel regions was assigned to each storage group. Fresh segments were tested on the day of harvest. Refrigerated segments were stored for 24 h at +4 deg C and tested within one hour after removal from refrigeration. Frozen specimens were stored for three months at -80 deg C, thawed in a 37 deg C water bath, and

tested within one hour of thawing.

The mechanical testing protocol used to identify and quantify intimal layer subfailures was published previously and will be summarized here (Stemper *et al.* 2005b). Aortic segments were opened at the mid-diameter level to expose the intimal side. I-shaped test specimens were created along the longitudinal dimension of the vessel using a custom-designed die. Test specimens were created from regions of the aorta devoid of branching vessels. The inferior end of each specimen was fixed to the test frame through a load cell and the superior end was attached to the piston of an electrohydraulic testing device (MTS Systems Corp., Eden Prairie, MN, USA). Specimens were initially oriented to the *in vitro* length for zero applied stress. Measurements of specimen width, length between fixations, and wall thickness were obtained using a digital callipers. Specimens were then quasi-statically loaded in longitudinal distraction at 1-mm/sec until complete vessel failure. **All specimens were tested at room temperature.** Longitudinal loads were measured using the inferior load cell. Displacement of the superior fixation was measured using a linear variable differential transducer (LVDT). The event was imaged at 125 Hz from intimal and adventitial sides using high resolution videography.

Global strain and engineering stress were obtained by correlating force and displacement data with initial specimen geometry. Previous literature has demonstrated that intimal layer subfailures may not be identifiable as a dip in the stress-strain plot (Stemper *et al.* 2005a). Therefore, the point of initial subfailure was identified using videography. Ultimate failure was defined as the point on the stress-strain curve wherein increasing strain resulted in decreased stress. Elastic modulus was computed as the slope of the linear region of the stress-strain curve. Analysis of variance (ANOVA) was used to determine statistically significant differences ($p < 0.05$) in vessel elastic, subfailure (stress and strain), and ultimate failure (stress and strain) mechanics based on storage technique and vessel region. Fisher's Least Significant Difference (LSD) analysis was used to determine differences between each of the three test groups (fresh, refrigerated, frozen).

RESULTS

Seventy seven test specimens sustained intimal layer subfailure prior to ultimate vessel failure (83%). Multiple subfailures were evident in many specimens. Absence of initial intimal layer subfailure prior to ultimate failure was not dependent upon storage group. Intimal layer subfailures initiated between fixation devices in the 'web' of the test specimen and progressed laterally (circumferentially). Subfailures were identified using high resolution videography, as a majority of subfailures did not result in a noticeable 'dip' in the stress-strain curve. ANOVA analysis demonstrated that stress at initial subfailure was dependent upon storage technique, while strain at initial subfailure was not (Figure 1). Fisher's LSD analysis indicated that subfailure stress was significantly decreased in refrigerated compared to frozen specimens. Fresh and frozen specimen subfailure biomechanics were not significantly different. **Mean wall thickness for the three test groups was 1.8 \pm 0.3 mm for fresh, 1.8 \pm 0.3 mm for refrigerated, and 2.0 \pm 0.4 mm for frozen specimens.**

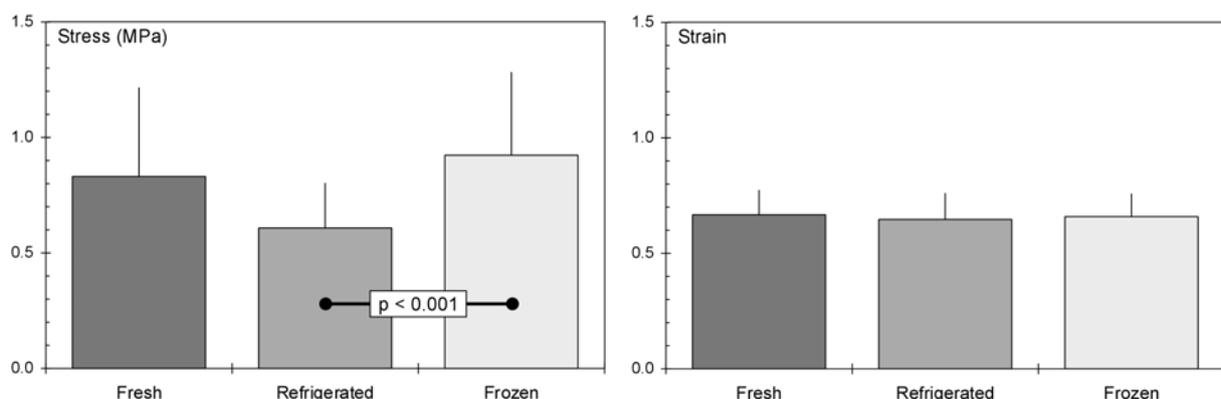


Fig. 1 – Subfailure mechanics (significant differences identified using Fisher's LSD analysis)

Ultimate failure was defined as the peak of the stress-strain curve. Ultimate stress was dependent upon storage technique and refrigerated specimens had a significantly lower stress at ultimate failure than fresh and frozen specimens (Figure 2). Ultimate strain was not dependent upon storage technique. Fresh and frozen ultimate biomechanics were not significantly different.

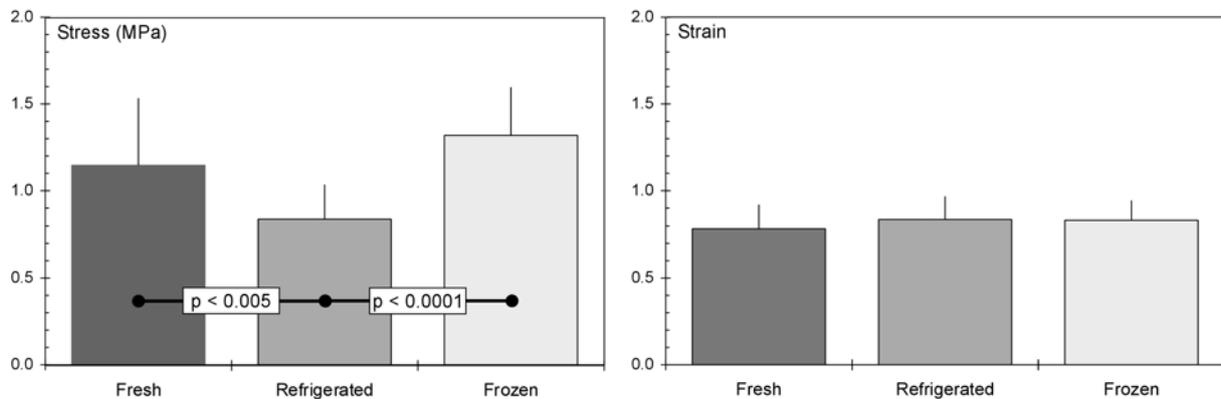


Fig. 2 – Ultimate mechanics (significant differences identified using Fisher’s LSD analysis)

Arterial elasticity was significantly dependent upon storage technique (Figure 3). Modulus of Elasticity was significantly decreased in refrigerated specimens compared to fresh and frozen specimens. Fresh and frozen elastic biomechanics were not significantly different.

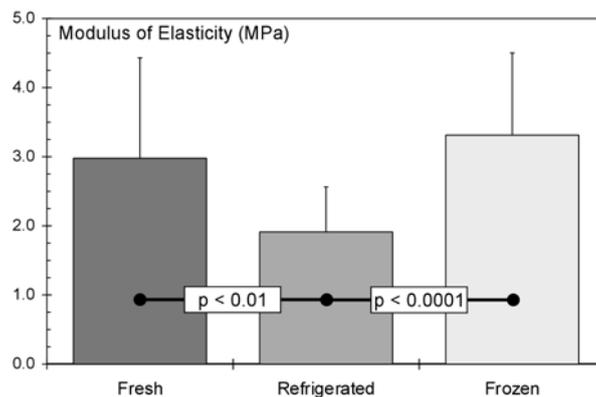


Fig. 3 – Elastic mechanics (significant differences identified using Fisher’s LSD analysis)

DISCUSSION

Present results demonstrated that elastic, subfailure, and ultimate failure mechanics of arterial vessels may be affected by common storage techniques. Mechanics of vessels frozen for three months prior to testing were not significantly different from fresh specimens tested within 24 hours of excision. However, significant differences were noted in the magnitude of stress at initial subfailure and ultimate failure, as well as elasticity, in refrigerated specimens. In particular, subfailure stress, ultimate stress, and modulus of elasticity were lower in refrigerated specimens. Strain to initial subfailure and ultimate failure was not affected by storage technique. These results indicate that short term refrigeration may not be an appropriate storage method for soft-tissue specimens used in biomechanical testing. Although not tested, results of the present study imply that short term freezing of soft-tissue specimens will decrease the likelihood of specimen degeneration.

Studies investigating effects of frozen storage on soft tissue biomechanics produced inconsistent results. Using a similar protocol, Adham et al. (1996) reported that ultimate stress and high strain modulus were not significantly different between fresh, refrigerated, and frozen longitudinally cut human aortic specimens. However, ultimate stress decreased an average of 33.3% over four refrigeration time periods compared to fresh specimens. High strain modulus, an estimate of arterial elasticity, did not demonstrate obvious trends with storage technique. Other studies investigating the

effect of frozen storage on ligament and tendon biomechanics demonstrated no change in ultimate strength and maximum load (Smith *et al.* 1996; Turner *et al.* 1988; Woo *et al.* 1986). However, similar to the present study, modulus of elasticity and stiffness decreased in two studies (Smith *et al.* 1996; Turner *et al.* 1988). The present study demonstrated statistically significant decreases in ultimate stress and modulus of elasticity in refrigerated specimens, while freezing did not affect vessel biomechanics. Ultimate strain was consistent across three storage techniques. In addition, the present study was the first to quantify the effect of storage techniques on arterial subfailure mechanics.

CONCLUSIONS

Experimental testing is an ideal method for studying fundamental and failure biomechanics of soft tissues. However, due to large sample sizes and experimental limitations, short-term refrigeration or long-term freezing is commonly necessary. These storage techniques are implemented to slow the tissue degeneration process, which will likely alter biomechanics. Present results demonstrated that short term refrigeration may not be an ideal method for preserving biomechanical properties of arterial vessels. Decreased failure stresses and elastic modulus are likely the result of continued degeneration, despite cold storage. Future studies will focus on the effect of short-term freezing to slow the biomechanical effects of degeneration.

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