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Lecture

Biaxial quantification of deep layer transverse carpal ligament elastic properties by sex and region

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A B S T R A C T

Background: The transverse carpal ligament is a major component of the carpal tunnel and is an important structure in the etiology of carpal tunnel syndrome. The current study aimed to quantify biaxial elastic moduli of the transverse carpal ligament and compare differences between sex and region (Radial and Ulnar).
Methods: Biaxial testing of radial and ulnar samples from twenty-two (thirteen male, nine female) human fresh frozen cadaveric transverse carpal ligaments was performed. Elastic moduli and stiffness were calculated and compared.
Findings: Biaxial elastic moduli of the transverse carpal ligament ranged from 0.76 MPa to 3.38 MPa, varying based on region (radial and ulnar), testing direction (medial-lateral and proximal-distal) and sex. Biaxial elastic moduli were significantly larger in the medial-lateral direction than the proximal-distal direction ($P < 0.001$). Moduli were significantly larger ulnarly than radially ($P = 0.001$). No significant differences due to gender were noted.
Interpretation: The regional variations in biaxial elastic moduli of the transverse carpal ligament may help improve non-invasive treatment methods for carpal tunnel syndrome, specifically manipulative therapy. The smaller biaxial elastic moduli found in the radial region suggests that manipulative therapy should be focused on the radial aspect of the transverse carpal ligament. The trend where female transverse carpal ligaments had larger stiffness in the ulnar location than males suggests that that the increased prevalence of carpal tunnel syndrome in women may be related to an increased stiffness of the transverse carpal ligament, however further work is warranted to evaluate this trend.

1. Introduction

Carpal tunnel syndrome (CTS) is a widespread peripheral neuropathy, thought to affect approximately 4% of the general population, with symptoms ranging from minor tingling to a burning sensation in areas innervated by the median nerve (Atroshi et al., 1999). It has been consistently noted that CTS is more than twice as likely to develop in women than in men, however, reasons for this are unclear (Atroshi et al., 1999)–(de Krom et al., 1992).

The TCL makes up the volar boundary of the carpal tunnel. It has four bony attachment points, ulnarly at the pisiform and the hook of the hamate and radially at the tuberosity of the scaphoid and ridge of the trapezium (Cobb et al., 1993). The TCL fixates and stabilizes the thenar and hypothenar muscles of the hand, acts as a pulley for flexor tendons within the carpal tunnel and helps maintain the carpal arch (Kline & Moore, 1992; Kung et al., 2005; Xiu et al., 2010). With these major roles in mind, an accurate characterization of the TCL, specifically regarding its material properties, would improve finite element models of the wrist, our understanding of CTS etiology and non-invasive treatment options for CTS. Unfortunately, determining accurate material properties of the TCL is difficult due to the complexity of the ligament. Recent studies of the TCL have shown its properties vary between different regions and depths, suggesting that the TCL experiences multidirectional loading (Holmes et al., 2012; Isogai et al., 2002; Prantil et al., 2012; Stecco et al., 2010).

A range of material testing techniques have been used to evaluate mechanical properties of the TCL, including indentation testing (Brett et al., 2014b, Main et al., 2012), uniaxial testing (Holmes et al., 2011) and biaxial testing (Holmes et al., 2012). As the TCL is loaded in multiple directions simultaneously in vivo, biaxial testing offers a similar loading scenario, however, it can only be used on small tissue samples (Garcia-Elias et al., 1989). While this size limitation means that the TCL cannot be biaxially tested as a whole, this modality facilitates precise regional mechanical property characterization. Due to difficulties in ensuring equal fibre recruitment when clamping thicker tissues, sample thickness for biaxial tissue testing should not exceed 3 mm, with under

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1 mm being optimal to obtain valid results (Garcia-Elias et al., 1989). As TCL thickness can approach 3 mm, biaxial testing of this ligament can become problematic.

Histological studies of the TCL have found that arrangement and density of collagen fibres, vary between TCL depths (Isogai et al., 2002; Prantil et al., 2012; Stecco et al., 2010). Fibres within superficial layers of the TCL are less uniform than deeper layers as they mingle with a layer of fibroadipose tissue which separates the TCL from the antebrachial fascia (Stecco et al., 2010). As a result, deeper layers of the TCL provide greater mechanical strength than superficial layers (Holmes et al., 2012). By thinning TCL samples prior to biaxial testing, discarding uneven superficial layers, samples can approach the recommended 1 mm thickness for biaxial testing while still encompassing deep layers of the TCL thought to be responsible for the majority of TCL strength (Brett et al., 2014a).

A recent uniaxial study of the TCL by Brett et al. (Sacks & Sun, 2003), found that the TCL strains significantly more radially than ulnarly at failure under a medial-lateral uniaxial load. The study also suggested regional differences in strain may lead to improvements in manipulative treatment of CTS, however regional differences in tissue strain at failure may not be present at lower, more physiological, strains experienced during manipulative therapy. Additional work evaluating regional differences in elastic properties of the TCL at more physiologically relevant, pre-failure strains is warranted. Given the in-vivo loading scenario of the TCL and the increased incidence of CTS in women, further work evaluating this trend, specifically a comparison of regional and sex based differences in material properties, tested biaxially, is warranted.

The purpose of the current study was to quantify deep TCL layer biaxial moduli to evaluate differences between males and females, and radial and ulnar regions. The results of this work could have impact related to our understanding of the etiology of carpal tunnel syndrome, help improve upon current computational models of the wrist and may offer improvements in CTS treatment options, specifically non-surgical manipulative therapy.

2. Methods

2.1. Sample preparation

22 (13 male (mean 71 SD (14.3) years) and 9 female (mean 81 SD(2.8) years)) fresh frozen cadaveric wrists were used in this experiment. There was no significant difference between male ($P = 0.070$) (95% CI: 62.1, 80.2 years) and female specimen ages (95% CI: 78.6, 83.2 years).

Human ethics approval to conduct the study was obtained from the University of Toronto as well as the University of Guelph. The specimens were visually inspected to ensure that no discernable pathologies were present. Samples were stored at −20 °C prior to testing, as such conditions have been shown not to alter the tensile material properties of ligaments (Sucher et al., 2005). Each wrist was thawed at room temperature and dissected to expose the TCL. Two, 7 × 7 mm squares of the TCL were obtained from each specimen, centered along the proximal-distal midline, on either side of the medial-lateral midline of the ligament (Fig. 1). Sample squares were cut such that the predominant fibre orientation, running in the medial-lateral direction, was parallel to two edges of the square. The square was thinned, cutting away half of the TCL thickness of the tissue sample from the volar side. Thickness measurements in five locations were taken and subsequently averaged representing the final sample thickness. Throughout the preparation process, the sample was kept hydrated by a 0.9% phosphate buffered saline solution.

2.2. Testing procedure

Biaxial testing was performed using a CellScale BioTester (Waterloo, ON, CA). Samples were mounted using four, 5 mm wide rakes, with the rake tines penetrating the tissue roughly 1 mm from each edge. The sample was mounted such that the predominant medial-lateral fibres within the sample were aligned with the X axis of the BioTester. Prior to the testing procedure, samples were preloaded to ensure that no slack was present. The mounting rakes were displaced equally along each axis until a load of approximately 10 mN was experienced by the tissue in both directions. Samples were then preconditioned over 10 cycles to 9% strain along both axes at a rate of 1%/s to account for tissue viscoelastic effects, as this protocol resulted in a stable tissue response during pilot testing. Samples then underwent biaxial strain to 12% at a rate of 1%/s. A strain rate of 1%/s was selected to allow for better comparison with previous biaxial testing, while minimizing the time samples had to dry (Holmes et al., 2012).

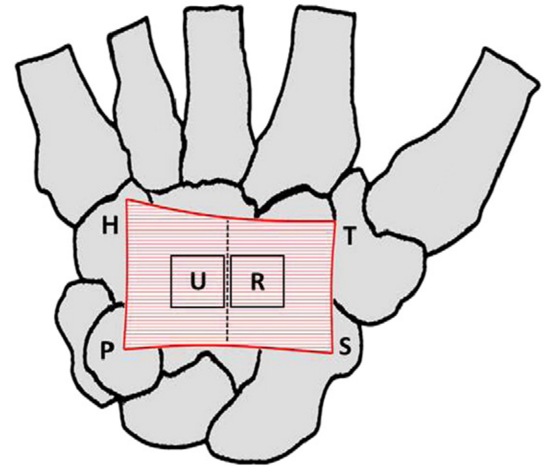

Fig. 1. Radial and Ulnar sample collection regions. Red shaded area represents TCL region. H - hamate bone, P - pisiform bone, T - trapezium bone, S - scaphoid bone, R - radial sample, U - ulnar sample.

2.3. Data analysis

Force and displacement data were collected at a rate of 15 Hz. The cross sectional area of the samples was calculated based on tissue thickness and the initial distance between rakes after preloading but prior to testing. Axial force measurements and cross sectional areas were used to determine stress experienced by the samples in each direction. Sample strains in each direction were determined using axial displacements measured by the BioTester. Two stress strain relationships were plotted for each sample, one for each tested direction. Two force displacement relationships were also plotted for each sample, one for each direction.

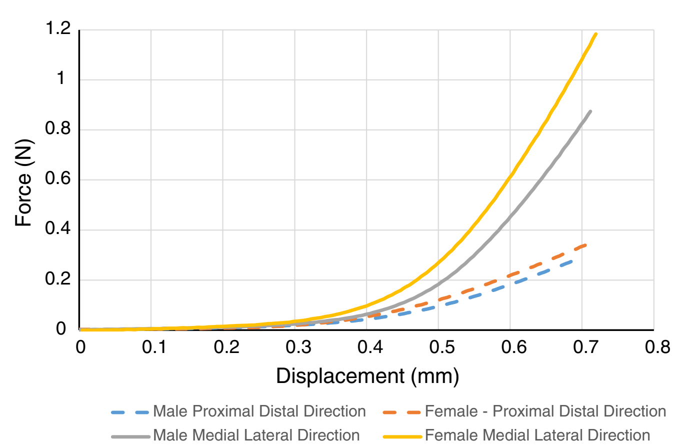

Fig. 2. Averaged stiffness curves by sex ($n = 13$ male, 9 female) and direction of loading (results from the ulnar and radial regions are averaged).