INFLUENCE OF BICEPS BRACHII TENDON MECHANICS ON POSITION-DEPENDENT ELBOW FLEXOR FORCE STEADINESS

by

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Abstract

Elbow flexor force steadiness (FS), measured as coefficient of variation (CV) of force, depends on forearm position and strength. Achilles tendon mechanical properties are associated with standing balance and plantar flexion FS; however, little is known about how tendon mechanics contribute to FS of an upper limb muscle such as the elbow flexors. The purpose of this study was to determine the influence of distal biceps brachii tendon mechanics on elbow flexor FS across supinated, neutral and pronated forearm positions. It was hypothesized that maximal voluntary contraction (MVC), stiffness, tendon force and stress would be higher in supinated and neutral, while strain would be higher in pronated, contributing to enhanced FS, as previously observed for supinated and neutral compared with the pronated position. Eleven males performed isometric elbow flexion tasks at low (5, 10% MVC) and high (25, 50, 75%) forces. Tendon elongation and cross-sectional area were recorded with ultrasound during contraction to quantify tendon mechanics among positions. MVC, FS, tendon force and stress were less in pronated ($p<0.01$). Tendon strain was greater in neutral compared to pronated at 25, 50 and 75% MVC, and compared to supinated at 75% MVC ($p\leq0.05$). Tendon stiffness did not differ among positions ($p>0.05$). The associations and influence of MVC and tendon mechanics on CV of force were analyzed using Pearson's correlations and forward multiple regressions, respectively, for low and high force levels. Associations of MVC ($-0.61<r<-0.72$), tendon force ($-0.65<r<-0.83$), and stress ($-0.64<r<-0.78$) with CV of force were significant across positions at low forces ($p<0.05$). At high forces, MVC ($-0.431<r<-0.726$) was associated for all positions and stiffness ($r=0.35$) was associated for the neutral position ($p<0.05$). Variance in CV of force was explained by MVC ($-0.330<\beta<-0.722$, $p<0.01$) in all positions at low forces, as well as stress ($\beta=-0.432$, $p<0.05$) in neutral and tendon force ($\beta=-0.698$, $p<0.01$) in pronated. At high forces, MVC explained CV variances for supinated ($\beta=-0.651$, $p<0.01$) and neutral ($\beta=-0.726$, $p<0.01$) positions, while CSA ($\beta=-0.379$, $p<0.05$) explained CV variances in the supinated position. Tendon mechanics differ across supinated, neutral and pronated positions, which in turn contribute to position-dependent FS of the elbow flexors.

Preface

Ethics approval for this study was granted by the University of British Columbia's Behavioral Research Ethics Board on June 20th, 2016. The certificate of approval number for this ethics is H16-00948. To date, no part of this study has been published or presented in conference format.

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List of Abbreviations

ANOVA: analysis of variance
BB: biceps brachii
cm: centimeters
CSA: cross-sectional area
CV: coefficient of variation
EMG: electromyography
FS: force steadiness
GPa: gigapascals
Hz: hertz
kg: kilograms
LH: long head
mm: millimeters
mm^2: millimeters squared
MPa: megapascals
MRI: magnetic resonance imaging
MTJ: muscle-tendon junction
MTU: muscle-tendon unit
MU: motor unit
MVC: maximal voluntary contraction
N: Newtons
SD: standard deviation
SH: short head
VA: voluntary activation
YM: Young’s Modulus
yrs: years

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Dedication

To my Family

Chapter 1: Introduction

1.1 Muscle structure

An individual muscle is composed of multiple structurally organized sections (Figure 1.1). The sarcomere is the basic unit of striated muscle; they are aligned in series and in parallel and tightly bound together to form a myofibril. Multiple myofibrils are grouped together and are held in place by a sarcolemma to form a single muscle fiber. Surrounding the myofibrils, within each muscle fiber, is a sarcoplasmic matrix through which dense vascular networks and mitochondria penetrate to provide the necessary nutrients and energy to the myofibrils. These single muscle fibers are surrounded by endomysium and are grouped together within the perimysium to form a fasciculus. Multiple fasciculi grouped together are surrounded by the epimysium to form the outer most layer of the muscle. The epimysium is the fascial layer that surrounds the entire muscle belly, maintaining the shape of the muscle as well as structural integrity. At the proximal and distal ends of the muscle, the epimysium forms strong connective tissue sheaths that join the muscle to the tendon forming the muscle-tendon junctions (MTJ).

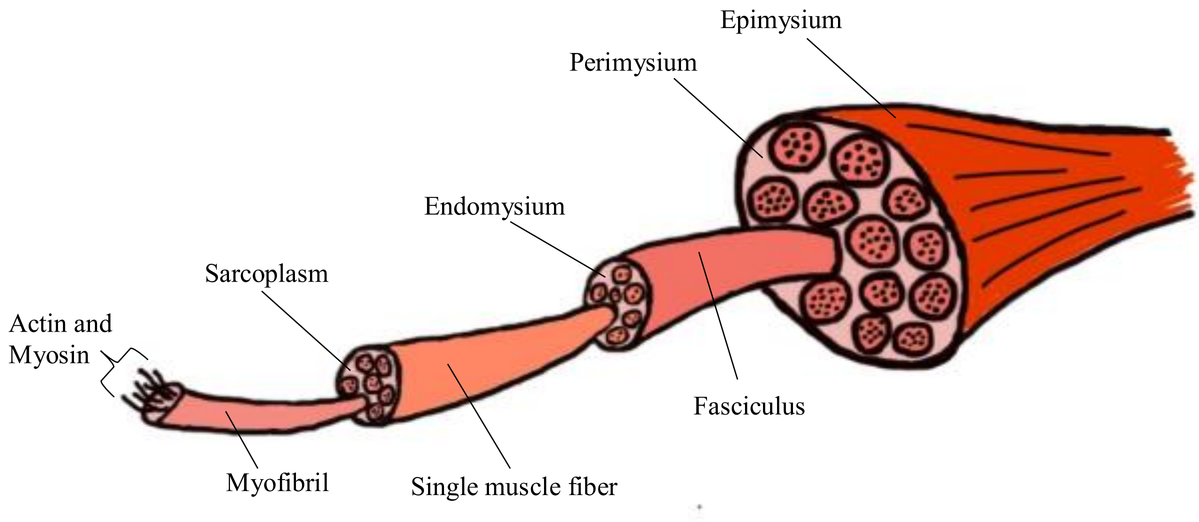

Figure 1.1: Structural organisation of muscle tissue

Beginning at the level of the sarcomere, force production is initiated through the cyclical interaction of actin and myosin. Myosin filaments are anchored to the middle of the sarcomere, while the actin filaments are anchored at the end of the sarcomere. Both project out from their points of attachment and overlap with each other to create areas where cross-bridges form between the proteins (Vandenboom, 2016). Formation of cross-bridges between actin and myosin is the basis of muscle contraction whereby shortening of sarcomeres lead to the entire muscle decreasing in length during contraction.

1.2 Tendon Structure

Much like muscle, tendons are composed of progressively larger groupings of fibers that culminate in the whole tendon (Figure 1.2). The fundamental units of a tendon are collagen fibrils intertwined together. Surrounding each collagen fibril is a layer of connective tissue called the endotenon that helps bind multiple collagen fibrils together to form a primary fibre bundle, known as a subfascicle. Subfascicles are then grouped together within the endotenon to form a secondary fibre bundle, known as a fascicle. Multiple fascicles are grouped together and surrounded by the last layer of endotenon to form a tertiary fiber bundle. Continuous with the endotenon, a second layer of connective tissue known as the epitenon surrounds multiple tertiary fiber bundles to complete a tendon (Kannus, 2000). The peritenon provides an outermost layer of loose areolar connective tissue around the tendon that acts as an elastic sleeve for the tendon, preventing direct friction between the tendon and surrounding tissues (Hess, Cappiello, Poole, & Hunter, 1989; Kannus, 2000).

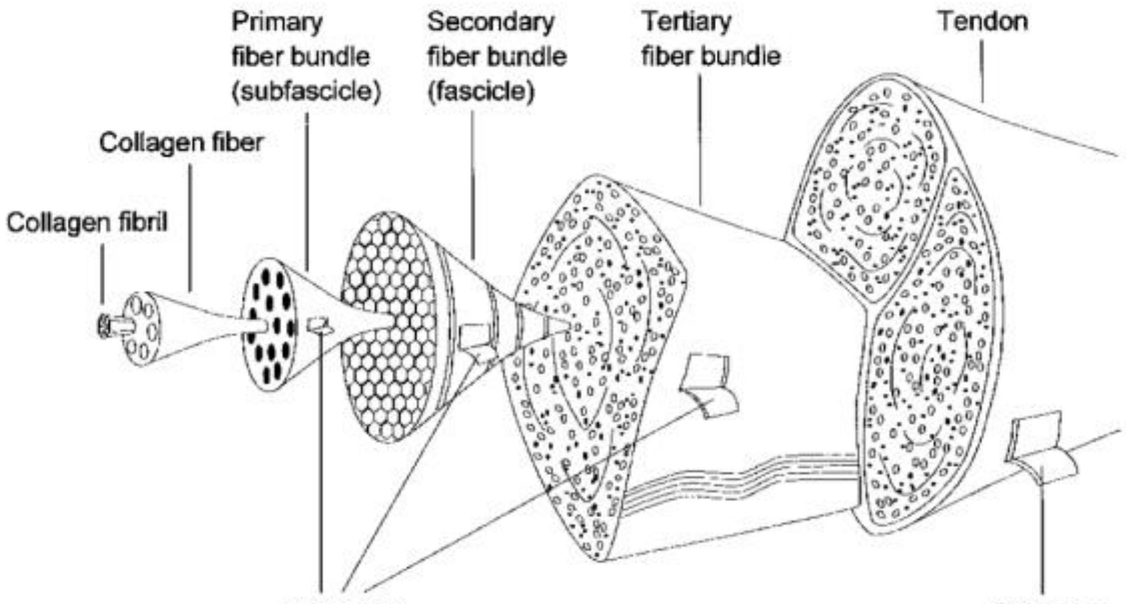

Figure 1.2: Structural organisation of tendon (Kannus et al. 2000).

The two primary elements found in tendons are collagen, making up 65% of the dry tendon mass, and elastin, making up ~1-2% of the dry mass. The remainder of the tendon's dry mass is composed of proteoglycans that combine with water to form a matrix in which the collagen and elastin are embedded (Hess et al., 1989; Józsa, Lehto, Kvist, Bálint, & Reffy, 1989; Kirkendall & Garrett, 1997; O'Brien, 1997). The collagen and elastin are produced by tenoblasts and tenocytes found between the collagen fibers of the tendon (Hess et al., 1989). Insoluble collagen molecules are formed by molecules of soluble tropocollagen, and these insoluble collagen molecules aggregate into microfibrils and progressively into the collagen fibrils (Kannus, 2000). The elastic and collagen composition of the tendon causes it to display viscoelastic properties allowing it to elongate beyond its resting length and return to normal resting length after a stress (Maganaris & Paul, 2000). It has been proposed that the stretch-recoil ability of the tendon may be a function of the elastin filaments; however, this has not been clearly demonstrated in humans (Kannus, 2000; Thorpe, Birch, Clegg, & Screen, 2013). The resistance of the tendon to elongate allows for the force produced by the muscle to be transferred through the tendon to the bone, causing movement of the corresponding limb. The composition of the tendon coupled with the viscoelastic nature not only provides structural integrity to the tendon, but also give rise to the tendon's anatomical properties that can be visualized and quantified using ultrasound.

Ultrasound imaging uses high frequency sound waves emitted from a probe that are reflected off internal structures and conveyed on a spectrum of white to black based upon the echo frequency allowing structures to be distinguished using a grey scale. This technology has been increasingly used in both clinical and research settings for real-time evaluation of internal body structures. Tendons appear on ultrasound as hyperechoic (white) structures when viewed in the longitudinal orientation, and as circular structures with a hyperechoic border when viewed in cross-section. The hyperechoic nature of the tendon arises from the thick exterior epitenon encasing the other tendon components. Due to the real-time ability of ultrasound image acquisition tendon length and cross-sectional area (CSA) can be measured from resting to contracted states. From the measures of elongation and CSA during contraction, the mechanics of a tendon can then be quantified.

1.2.1 Tendon Mechanics

A number of parameters have been established to describe the mechanical properties of tendinous structures when they are placed under load during muscle contraction (Heinemeier & Kjaer, 2011). These parameters include stress (σ), strain (ε), Young's modulus (YM) (E), and stiffness (K) of the tendon, and have been quantified using ultrasound during both electrically evoked (Maganaris & Paul, 1999) and voluntary (Johannsson, Jakobi, Duchateau, & Baudry, 2015; Kubo, Kanehisa, Miyatani, Tachi, & Fukunaga, 2003; Onambélé, Narici, Rejc, & Maganaris, 2007; Onambélé, Narici, & Maganaris, 2006; Onambélé, Burgess, & Pearson, 2007; Smart et al., 2016; Smart, Richardson, & Jakobi, 2017; Stenroth et al., 2012, 2016) contractions. Stress is the quotient of tendon force to tendon cross sectional area (CSA) during contraction, and strain is the quotient of the change in tendon length during contraction to the resting tendon length (Figure 1.3). Tendon force is obtained from the quotient of the muscle moment (which is the product of the force produced and lever arm length) to the tendon moment arm (Figure 1.3). Tendon stiffness is the slope of the linear portion of the tendon force-elongation relationship (Stenroth et al., 2012, 2016) (Figure 1.3). Stiffness is the slope of the change in tendon length to the force applied on the tendon and is dependent on both the CSA and length of the tendon, in which a greater CSA and smaller displacement results in a stiffer tendon (Heinemeier & Kjaer, 2011). Young's modulus, also referred to as tendon modulus (Heinemeier & Kjaer, 2011) or tendon tensile modulus (Onambélé et al., 2007), is obtained by calculating the slope of the linear portion of the tendon stress and strain relationship at high force levels up to 100% of the participants maximal voluntary contraction (MVC) (Johannsson et al., 2015; Stenroth et al., 2012, 2016). Young's modulus represents the material properties of the tendon normalized to the dimensions of the tendon (CSA) (Heinemeier & Kjaer, 2011). Stiffness and YM of the tendon are coupled in that a stiffer tendon elongates less, leading to reduced tendon strain and hence increased YM. These properties in turn determine how the force exerted by the muscle acts on the corresponding bone.

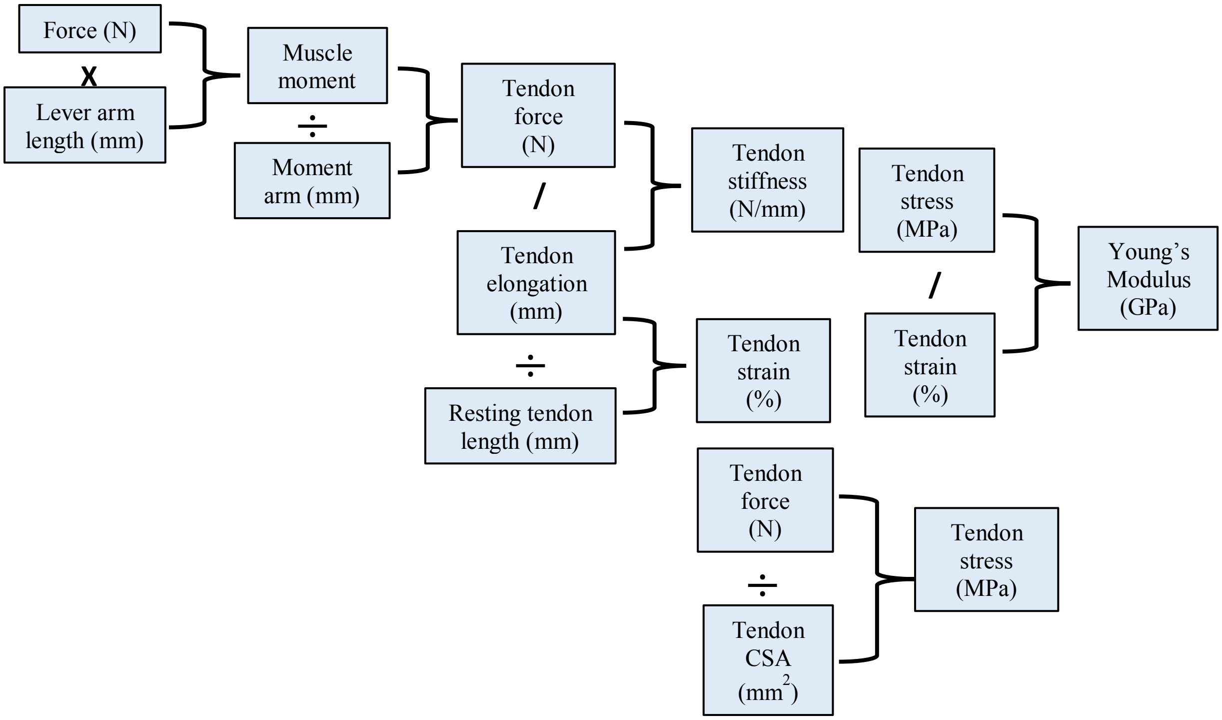

Figure 1.3 Description of methods used to determine tendon mechanics. N, Newton; mm, millimetre; %, percent; mm^2, millimetre squared; MPa, Megapascal; GPa, Gigapascal; /, indicates calculation of variable obtained from slope of relation at high force levels.

1.3 Biceps brachii Anatomy

The biceps brachii (BB) is a fusiform muscle composed of two muscle bellies, the long (LH) and short (SH) heads. The proximal tendon of the LH originates at the supraglenoid tubercle of the scapula and passes laterally before turning anteriorly to travel between the greater and lesser tubercles of the humerus to lie in the intertubercular (bicipital) groove on the anterior humerus. The tendon then joins the muscle belly of the LH by means of connective tissue at its proximal muscle-tendon junction (MTJ) located at approximately 1/3 the length of the humerus. The muscle belly of the LH passes along the lateral aspect of the anterior humerus until it reaches the level of the coronoid fossa where connective tissue forms the distal MTJ. The proximal tendon of the SH originates at the coracoid process of the scapula and the proximal MTJ of the SH is generally located just inferior to the proximal MTJ of the LH. The SH muscle belly forms at approximately one third of the humerus length and travels along the medial aspect of the anterior humerus until the coronoid fossa where connective tissue at the distal MTJ joins the connective tissue of the LH to form a common distal BB tendon that inserts onto the radial tuberosity. Along the length of the humerus, the muscle belly of the LH and SH are connected through a common septum, thus allowing both muscles to work in conjunction with each other to produce movements around the elbow. However, it has been noted that in some individuals the LH and SH remain as two distinct muscle bellies without a common septum (Cucca, McLay, Okamoto, Ecker, & McMenamin, 2010), yet in others the muscle fibers of the two heads intertwine along the length of the muscle bellies (Eames, Bain, Fogg, & van Riet, 2007). Another anatomic variation of the BB is the presence of two distinct distal tendons which insert onto the radial tuberosity from each of the LH and SH muscle bellies (Cho et al., 2011; Cucca et al., 2010; Eames et al., 2007; Tagliafico, Michaud, Capaccio, Derchi, & Martinoli, 2010). Two separate muscle bellies, along with separate insertions of the distal tendon might allow the heads of the BB to work as two distinct muscle-tendon units (MTU). Overall, the insertional arrangement of the distal BB tendon(s) onto the radial tuberosity allows the BB to act in forearm flexion, as well as supination. The multi directional movement ability of the BB about the elbow joint makes it a key muscle to study in regards to force production and control of forearm movement.

1.4 Force production of the elbow flexors

Elbow flexion force is produced through the combined activity of the LH and SH of the BB, brachialis and brachioradialis. Contribution of these muscles to elbow flexion force is largely dependent on the moment arms of these muscle, which vary according to elbow flexion angles. The largest moment arms of the BB, brachialis and brachioradialis occur at angles ranging from 80-110^{o} of elbow flexion (Akagi et al., 2012; An, Hui, Morrey, Linscheid, & Chao, 1981; Gonzalez, Hutchins, Barr, & Abraham, 1996; Koo, Mak, & Hung, 2002; Murray, Delp, & Buchanan, 1995; van Zuylen, van Velzen, & Denier van der Gon, 1988; Winters & Kleweno, 1993). This range of optimal joint angles for the BB moment arm is also supported by its length-tension relationship that indicates the optimal muscle length for force production occurs between 90° and 100° of elbow flexion (Hansen, Lee, Barrett, & Herzog, 2003; Ismail & Ranatunga, 1978; Leedham & Dowling, 1995). This occurs regardless of the upper arm being placed in a neutral position in line with the torso or in a flexed position with the humerus in the same line as the shoulder, thereby highlighting that 90-100° of elbow flexion is the optimal angle for force production regardless of differences in arm position. Knowledge of the moment arm of a muscle and its tendon, in this case the BB, is crucial for the measurement of tendon mechanics as it allows for determination of the forces acting through the tendon (tendon force) at various force levels.

Additional to the effect of elbow joint angle, forearm position has also been shown to affect maximal elbow flexion force, with supinated and neutral positions having higher MVC force compared to the pronated position (Brown, Edwards, & Jakobi, 2010; Harwood, Edwards, & Jakobi, 2010). Position-dependent force production may be influenced by reduced surface EMG of the BB (Barry, Riley, Pascoe, & Enoka, 2008; Buchanan, Rovai, & Rymer, 1989) and increased motor unit recruitment threshold of the BB SH (Harwood et al., 2010) in the pronated position. Coupled to the observation of differential changes in EMG between the LH and SH across positions, Brown et al. (2010) and Hardwood et al. (2010) have hypothesized that altering forearm position between supinated, neutral and pronated may independently alter the length of each head of the BB muscle but this has yet to be determined. Alterations in muscle length due to forearm position may place the BB at different positions on the length-tension relationship, thereby influencing absolute force production.

As the forearm moves through supinated, neutral and pronated positions (Figure 1.4) the BB muscle likely lengthens from the pull of the distal MTJ as the tendon is internally rotating with the radius through its attachment on the radial tuberosity (Koch & Tillmann, 1995). Shear-wave ultrasound elastography studies indicate that the BB muscle belly remains slack up to lengths corresponding to elbow flexion angles of ~95°, after which the slack is reduced at longer muscle lengths and the shear-wave signal increases (Lacourpaille et al., 2014; Lacourpaille, Hug, & Nordez, 2013). The angle at which the BB muscle belly is slack is known as the slack length.

Slack lengths differ between the muscle and tendon of the gastrocnemius-Achilles tendon MTU (Hug, Lacourpaille, Maïsetti, & Nordez, 2013), but little is known about the slack length of the BB and its distal tendon. By marginally lengthening the BB muscle as the forearm is placed in the pronated position, any lengthening of the muscle may also result in reduced strain on the distal BB tendon, leading to changes in tendon slack. In contrast to the pronated position, placing the forearm in the supinated position with the radius rotated externally would likely cause shortening of the BB muscle belly and subsequent pull on the distal BB tendon, reducing slack within the tendon. Changes in tendon slack across forearm positions at one joint angle could affect the compliance of the tendon, and subsequently influence the ability to maintain steady forces, as has been previously demonstrated in the Achilles tendon (Johannsson et al., 2015; Onambélé et al., 2007, 2006). At the elbow differences in tendon slack between forearm positions may contribute to the previously observed differences in force steadiness between supinated, neutral and pronated forearm positions (Brown et al., 2010).

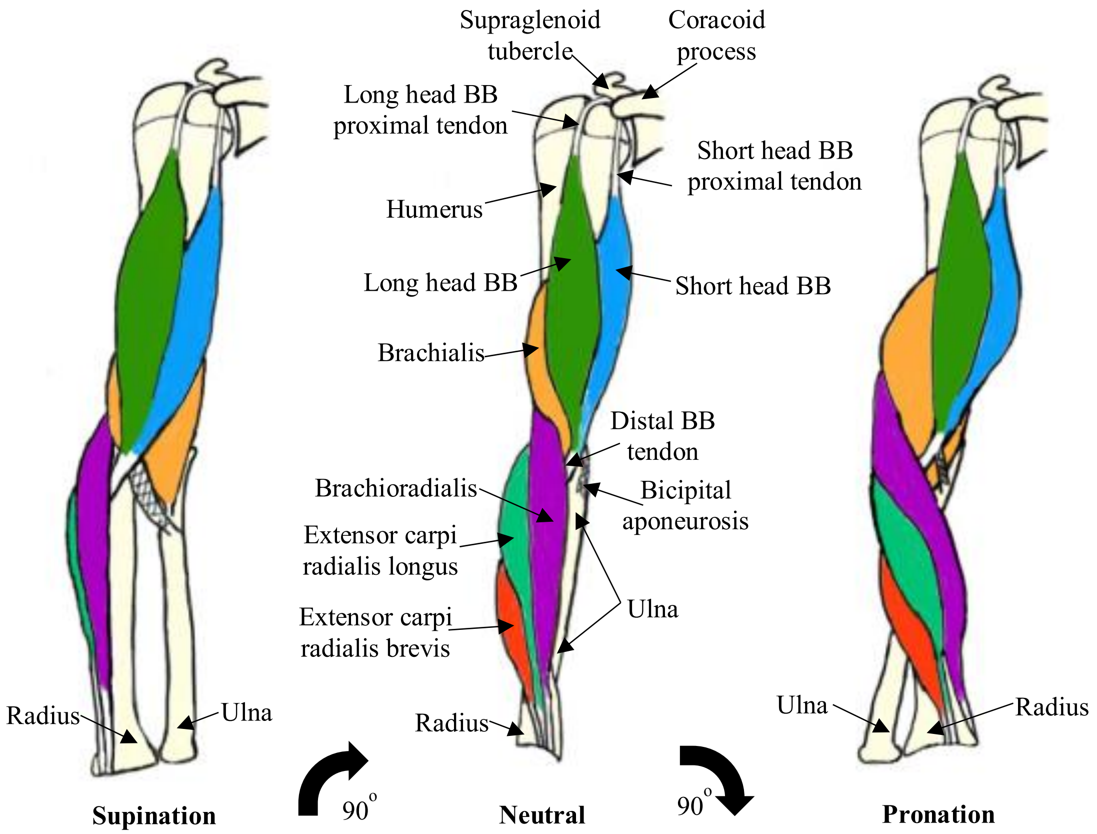

Figure 1.4: Changes in position of arm and forearm muscles as forearm is rotated from supination to neutral to pronation.

1.5 Force Steadiness

Changing muscle length, and hence force production according to the length-tension relationship (Hansen et al., 2003; Ismail & Ranatunga, 1978; Leedham & Dowling, 1995) influences both absolute strength as well as force steadiness (FS). Higher absolute strength is a known contributor to increased FS (Brown et al., 2010; Enoka et al., 2003; Smart et al., 2016). Force steadiness for isometric contractions is the ability to maintain a steady contraction at a target force and is generally measured using the coefficient of variation (CV) of force around a given submaximal target level (Brown et al., 2010; Pereira et al., 2015), which normalizes the deviations in force output to the absolute force produced in the contraction. A lower CV in force represents a steadier contraction, as there is less fluctuation in force around the target force.

Force steadiness is dependent on a number of factors including motor unit (MU) properties (Enoka et al., 2003), strength (Brown et al., 2010; Pereira et al., 2015), forearm position (Brown et al., 2010) and the type and intensity of contraction (Enoka et al., 2003). Intermediate force levels (20%-40% MVC) are the steadiest, followed by high force levels (>50% MVC) being less steady, and low force levels (2.5% MVC) being the least steady (Enoka et al., 2003). This results in an inverted J-shaped plot when CV of force is plotted against increasing submaximal force levels (Figure 1.5).

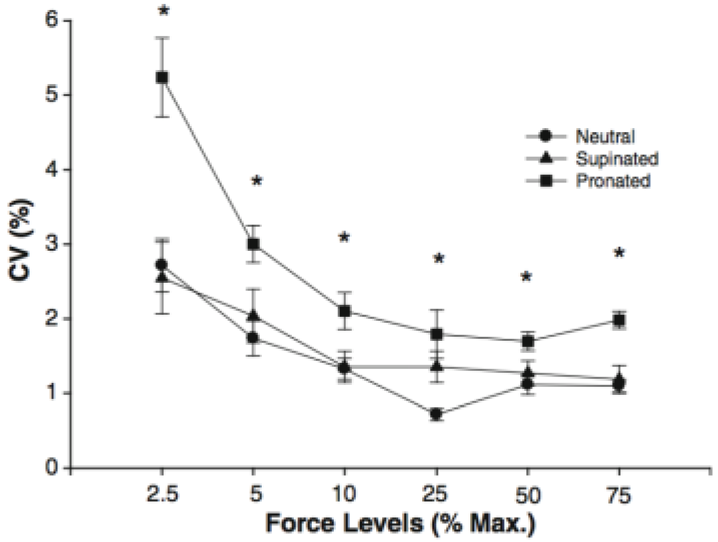

Figure 1.5: Position dependent FS of the elbow flexors (Brown et al. 2010). CV, coefficient of variation in force; % Max, Percent of maximal voluntary contraction force;. *: pronated greater than supinated and neutral (Brown et al. 2010).

In addition to position-dependent strength of the elbow flexors, FS of the elbow flexors also depends on forearm position, with the supinated and neutral positions being steadier than the pronated position and this has been observed in males and females (Brown et al., 2010) (Figure 1.5). Increased SH recruitment threshold in males (Harwood, Cornett, Edwards, Brown, & Jakobi, 2014), and for the pronated compared to supinated positions (Harwood et al., 2010), may contribute to sex- and position-dependent FS of the elbow flexors (Brown et al., 2010) by reducing strength in this position. Hypothesized changes in fascicle length across forearm positions (Harwood et al., 2010) may contribute to position-dependent FS, as previous observations indicate that longer fascicle length increases MU recruitment threshold in the tibialis anterior (Pasquet, Carpentier, & Duchateau, 2005). In addition to muscle length influencing motor unit properties, corticospinal excitability, measured using motor evoked potentials of the BB, is reduced as the forearm is moved across supinated, neutral and pronated forearm positions (Forman, Richards, Forman, Holmes, & Power, 2016; Mitsuhashi, Seki, Akamatsu, & Handa, 2007; Mogk, Rogers, Murray, Perreault, & Stinear, 2014) and this reduction in descending drive may also influence FS; however, this remained to be studied. The studies of Harwood et al. (2010) and Brown et al. (2010) provide evidence of position-dependent MU properties and force production influencing FS. However, to produce force the muscle must act through the tendon, a factor that has only recently been considered when evaluating FS.

The tendon is a key component in force production as it forms the link between muscle and bone enabling muscular contraction to result in limb movement. As tendons are viscoelastic materials capable of elongation and recoil, their innate properties likely contribute to precise adjustments required to produce steady contractions. Studies by Johannsson et al. (2015) and Onambélé et al. (2007, 2006, 2007) have suggested that a stiffer tendon is able to transfer force to bone with less dampening of the force compared to a compliant tendon and this likely facilitates precise adjustments of force required to maintain a steady contraction and balance. Recent data from our laboratory (Smart et al., 2016) showed that increased distal BB tendon stiffness leads to reduced CV of force in young males, supporting previous findings of increased Achilles tendon YM contributing to increased torque steadiness of the plantar flexors (Johannsson et al., 2015). As

YM represents the stiffness of the tendon normalized to its CSA studies of stiffness and YM can be compared. Moreover, because tendon stiffness is associated with strength (Folland & Williams, 2007), and because strength is a significant contributor to elbow flexion FS across three positions of the forearm (Brown et al., 2010), it is likely that mechanical properties of the distal BB tendon would vary across forearm positions contributing to position dependent FS.

Chapter 2: Purpose and Hypothesis

2.1 Purpose

The purposes of this study were to 1) quantify mechanical properties of the distal BB tendon across supinated, neutral and pronated forearm positions, and 2) determine whether position-dependent FS of the elbow flexors is associated with tendon mechanics in young males.

2.2 Hypotheses

It was hypothesized that tendon mechanics will be position dependent, with supinated and neutral having less strain than pronated, but higher levels of tendon force, stress and stiffness. Secondly, it was hypothesized that across supinated, neutral and pronated forearm positions, increases in strength, tendon force, stiffness and stress will be associated with reduced CV of force, while increased strain will be associated with increased CV of force.

Chapter 3: Methods

3.1 Participants

Eleven males (23 ± 3 yrs, 175.6 ± 8.2 cm, 72.9 ± 7.5 kg) volunteered to participate in the present study. Each participant visited the lab for three experimental sessions separated by a minimum of 48 hours between visits. All participants were right-hand dominant. Exclusion criteria included: 1) active tendinopathy, 2) systemic diseases affecting collagenous tissue, 3) those who have had an injury or orthopaedic surgery to the right arm or shoulder in the prior 6 months, 4) Involvement in high levels of upper-body strength training, 5) history of training in fine motor tasks (i.e. musicians), 6) nerve damage to right arm. Ethics were gained from the University of British Columbia Okanagan Behavioural Research Ethics Board (H16-00948).

3.2 Experimental Setup

Participants were seated in a custom-built dynamometer chair with the knees and hips positioned at 90°, the shoulder of the dominant arm abducted ~10°, and the elbow placed at 110°. The dynamometer chair was adjusted to achieve the appropriate joint angles. Participants grasped the force device with their dominant hand, with the forearm resting on a padded support. The force transducer was located immediately below the participant's hand to measure elbow flexion force with the forearm in a supinated, neutral or pronated position. Force was displayed in real-time on a 52 cm (20.5 inch) monitor located 1 meter in front of the participant, with the middle of the monitor level with the participant's eyes to ensure consistent visual feedback between participants and sessions (Figure 3.1) force signals were amplified (100×), and grounded through a Coulbourn instrument unit (Allentown, Pennsylvania, USA). Force signals were sampled at 2381 Hz using a 16-bit 1401plus analog to digital converter (Cambridge Electronic Design

(CED), Cambridge, England) and stored for offline analysis using Spike 2 version 7 (CED, Cambridge, England). Two 4cm × 4cm carbon-carbon stimulation electrodes coated in electrode gel were placed proximally and distally on the BB muscle belly to provide square wave pulse stimuli to induce twitches for assessment of voluntary activation (VA) during MVC attempts. During the tracking tasks, the ultrasound probe was secured, using a customized probe holder, to the arm in the longitudinal plane to visualize the distal BB tendon MTJ in long-axis. For measures of tendon CSA, the ultrasound probe was secured to the arm in the transverse plane to view the distal BB tendon in short axis.

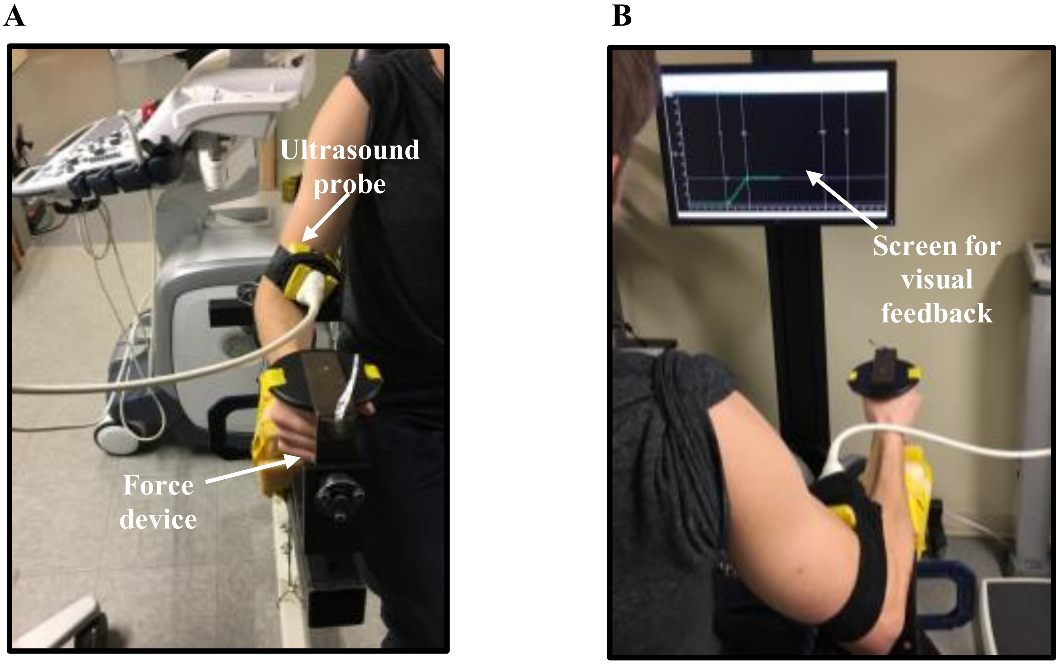

Figure 3.1: Experimental set-up. A) Anterior view showing placement of the arm in the force device and positioning of the ultrasound probe in the longitudinal orientation to record tendon elongation. B) Posterior view showing visual feedback of force tracing on screen.

3.3 Resting Anatomical Measures

Resting muscle length and CSA of the BB, as well as resting distal tendon length were recorded at 110° using an ML6-15 B-mode ultrasound probe (GE LOGIQ E9; General Electric, Fairfield, CT, USA) with the LOGIQView® function allowing panoramic scans of the structure of interest.

Muscle length was recorded from the proximal to distal MTJs of the BB, allowing the entire muscle to be viewed in a single image. Muscle CSA was recorded at the midpoint of the muscle. BB distal tendon length was recorded from the distal MTJ of the BB muscle to the tendon's insertion onto the radial tuberosity. The distal BB tendon CSA was recorded at the largest CSA. These measures were performed in the supinated, neutral and pronated positions. Lever arm length was measured as the distance from the head of the radius to the force transducer located immediately below the participant's hand grasping the wrist apparatus. The perpendicular distance from the lateral epicondyle of the humerus to a linear edge placed between the distal MTJ of the BB and the insertion of the BB tendon onto the radius was measured and considered as the moment arm of the BB.

3.4 Protocol

Three sessions, each separated by a minimum of 48 hours, were undertaken to avoid possible effects of fatigue from the cumulative number of contractions. The first session consisted of obtaining resting ultrasound measures and establishing MVC force level with VA. The second and third session consisted of recording either distal BB tendon elongation or CSA during submaximal force levels, and was randomized for each participant. The three sessions are described in more detail below.

Testing session 1

Following resting ultrasound anatomical measures, supramaximal stimulation intensity for the BB was established in the neutral position by applying a 100μs square-wave pulse (DS7AH, Digitimer Ltd., Welwyn Garden City, UK) to the BB muscle belly and increasing the stimulation intensity until a plateau in twitch force amplitude occurred; followed by increasing the stimulation intensity a further 15%. Participants then performed 2-3 five-second MVCs in the neutral position with three twitches applied before, during and after the MVC to measure VA. Participants were given 2-3 minutes rest between each contraction to prevent muscle fatigue. Establishment of supramaximal stimulation intensity followed by execution of MVCs were repeated for supinated and pronated positions.

Testing session 2

MVC was re-established for each forearm position on Day 2. Participants were required to achieve within 5% the force attained during assessment of VA during session 1. Participants then performed submaximal tracking tasks at 5, 10, 25, 50 and 75% MVC force to assess FS. Ultrasound was recorded throughout the duration of the submaximal tracking tasks and the order in which tendon elongation (Figure 3.2) and cross-section (Figure 3.3) were imaged was randomized between testing sessions 2 and 3 for each participant. Submaximal tracking tasks consisted of increasing force in a ramp fashion to the target force level over three seconds, maintaining that force as best possible for ten seconds, then decreasing force in a ramp fashion over three seconds back down to baseline (Figure 3.4). The order of submaximal target forces within each forearm position and the order of forearm positions were randomized; however, all force levels were completed in one forearm position prior to changing positions in order to maintain visualisation of the distal MTJ. Contraction levels were repeated twice in each forearm position to obtain two measures of tendon elongation and CSA, from which an average was taken and reported. Following all submaximal tracking tasks, participants performed a final MVC to ensure no fatigue occurred as a result of the protocol. The force value was required to be within 5% of the maximal value achieved prior to the submaximal tracking protocol.

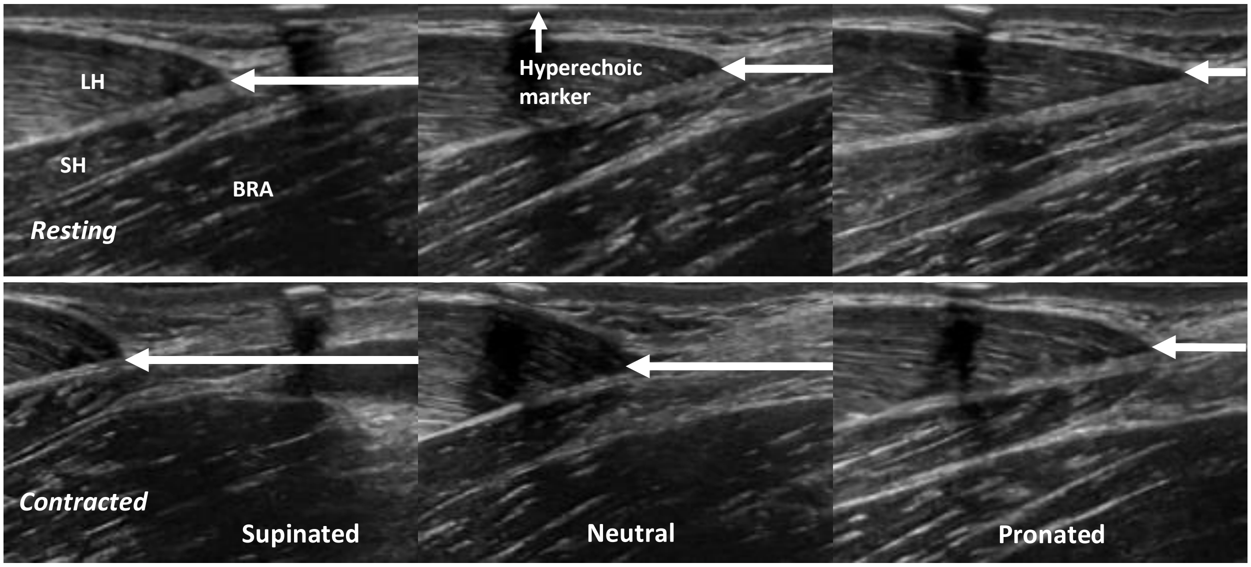

Figure 3.2: Representative ultrasound images of distal biceps brachii tendon elongation in the supinated, neutral and pronated forearm positions. LH, Long head biceps brachii; SH, Short head biceps brachii; BRA, Brachialis

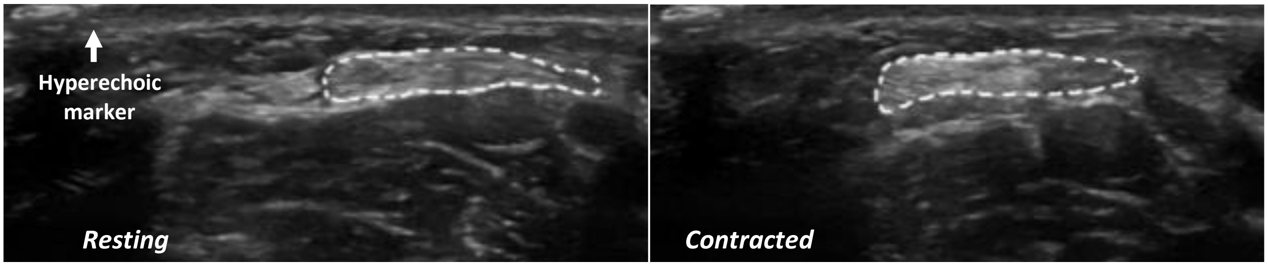

Figure 3.3: Representative ultrasound images of distal biceps brachii tendon cross-sectional area.

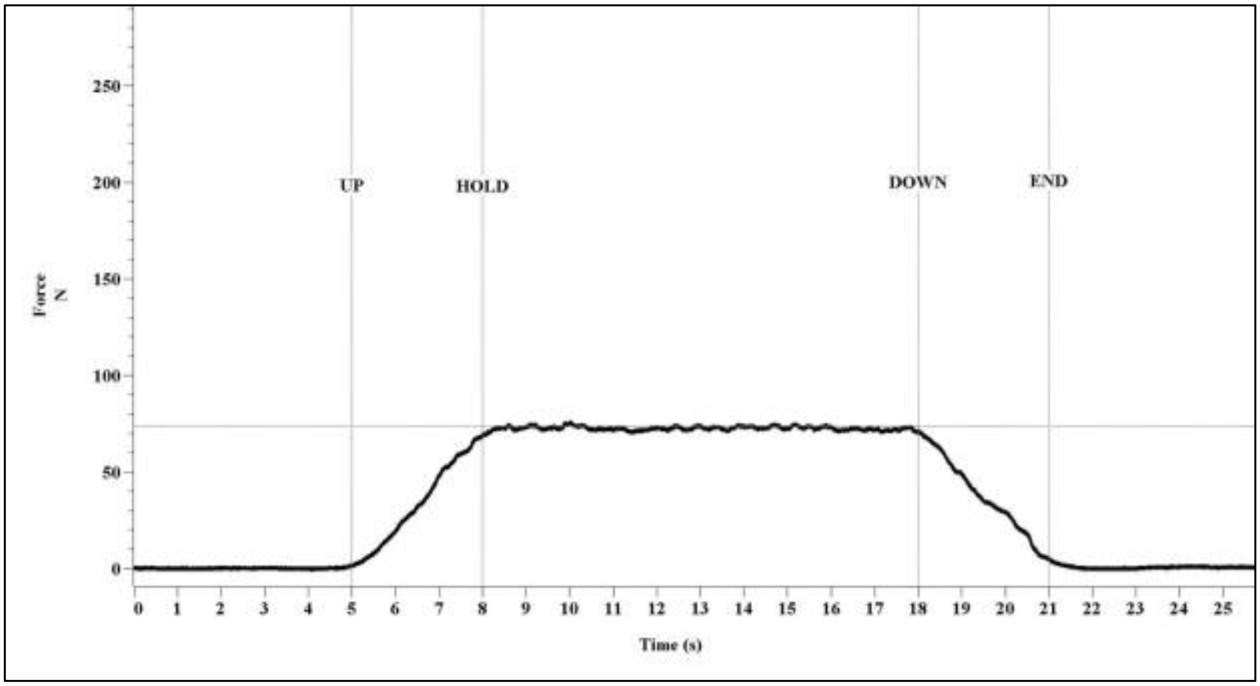

Figure 3.4: Representative image of a 25% MVC submaximal tracking task performed in the neutral forearm position. N, newtons; s, seconds.

Testing session 3

MVC was re-established. Participants then performed submaximal tracking tasks as described in testing session 2, and ultrasound of the distal BB tendon was recorded in the view not previously obtained in session 2 (longitudinal or cross-sectional).

3.5 Data Analysis

Ultrasound Analysis

All ultrasound measures were performed using the built-in analysis platform contained on the ultrasound machine (GE LOGIQ E9, General Electric). Muscle CSA of the BB was measured by tracing the outer border of the muscle in cross-section using a free-hand tracing tool. Muscle length was measured as the straight-line distance from the proximal MTJ to the distal MTJ. Resting tendon length was measured from the distal BB MTJ to the insertion of the tendon onto the radial tuberosity using the open spline trace. Tendon elongation during contraction was measured as the difference in position of the distal MTJ from resting to contracted states. Tendon CSA at rest and during contraction were measured by tracing the outer border of the tendon using the free-hand tracing tool.

Tendon Mechanics

Tendon mechanics were calculated across all force levels and forearm positions. Tendon force was calculated as the quotient of muscle moment to tendon moment arm of the BB. Muscle moment was obtained from the product of the submaximal force level (contraction specific) and lever arm length measured from the head of the radius to the force transducer. Tendon stress was calculated as the quotient of tendon force to tendon CSA during contraction. Tendon strain was calculated as the quotient of tendon elongation during contraction to the resting tendon length. Tendon stiffness was calculated as the slope of the tendon force-elongation relationship from 25-75% MVC.

Force Analysis

Voluntary activation during the MVC attempts was calculated using the interpolated twitch technique (Belanger & McComas, 1981; Gandevia, Herbert, & Leeper, 1998; Jakobi & Rice, 2002). The interpolated twitch technique measures voluntary activation using the following formula: [1-(interpolated twitch/resting twitch) × 100]. Force steadiness was analyzed as the CV in force around the given target force level for 5 second epochs during the middle of the plateau phase of the contraction. Force steadiness for each force level in each forearm position was averaged across two trials.

3.6 Statistical Analyses

Statistical analysis was conducted using Statistical Package for Social Sciences (SPSS) version 24 (IBM, Amrok, NY, USA). MVC force, resting anatomical ultrasound measures, and stiffness at low and high force levels were compared across forearm positions using a one-way ANOVA with Bonferroni post-hoc tests to correct for multiple comparisons. A 3 (position: supinated, neutral, and pronated) × 5 (force level: 5, 10, 25, 50, and 75% MVC) repeated measures ANOVA was used to compare CV of force and tendon mechanics (strain, tendon force, stress, CSA) across forearm position and force levels. When significant interactions were present, one-way ANOVAs with Bonferroni corrections were used to compare values across positions. Pearson's correlations were conducted between MVC and tendon mechanics as independent variables and CV of force as the dependent variable. Forward multiple linear regressions were used to determine which combination of variables best predict variances in CV of force. Standardized beta weights coefficients are also reported, because they explain the relative contributions of each independent variable. Correlations and regressions were performed separately for both low (5 and 10% MVC) and high (25, 50, 75% MVC) force levels across supinated, neutral and pronated forearm positions. Significance was set at an alpha value of $p \leq 0.05$. Values are reported as mean ± SD in tables and in text, and mean ± SE in figures.

Chapter 4: Results

4.1 Subject Characteristics

MVC force was less in the pronated position (113.6 ± 21.3 N) compared to supinated (213.6 ± 49.6 N) and neutral (243.5 ± 48.0 N) positions ($p<0.01$), while supinated and neutral did not differ ($p>0.05$). Voluntary activation was lower in pronated (70.9 ± 20.4 %) compared to supinated (93.0 ± 5.24 %) ($p<0.01$) and neutral (96.1 ± 3.24 %) ($p<0.01$) positions, while supinated and neutral did not differ ($p>0.05$). Resting muscle length (176.8 ± 17.5 mm) and muscle CSA (1030.33 ± 252.4 mm^2) did not differ among positions ($p>0.05$). Resting tendon length was shorter in the pronated position (64.2 ± 7.90 mm) compared to supinated (76.4 ± 6.81 mm, $p<0.01$) and neutral (72.3 ± 6.60 mm, $p<0.05$), while supinated and neutral did not differ ($p>0.05$). Resting tendon CSA (23.8 ± 2.18 mm^2) and moment arm (56.2 ± 1.98 mm) did not differ among positions ($p>0.05$) (Table 4.1).

Table 4.1: MVC, voluntary activation, resting measures and tendon stiffness across positions.

	Supinated	**Neutral**	**Pronated**
MVC (N)	213.6 ± 49.6	243.5 ± 48.0	113.6 ± 21.3*
VA (%)	93.0 ± 5.2	96.1 ± 3.2	70.9 ± 20.4*
Muscle CSA (mm^2)	1170.2 ± 246.0	984.9 ± 242.0	935.9 ± 226.4
Muscle length (mm^2)	167.9 ± 17.4	178.3 ± 16.7	184.4 ±15.8
Tendon CSA (mm^2)	23.3 ±2.1	24.1 ± 1.6	24.2 ± 2.8
Tendon length (mm)	76.4 ± 6.8	72.3 ± 6.6	64.2 ± 7.9*
Tendon stiffness (N/mm)	347.8 ± 170.7	264.2 ± 141.2	190.1 ± 115.4

MVC, Maximal voluntary contraction; N, newtons; VA, voluntary activation; CSA, cross-sectional area; mm^2, millimetres squared. *, $p<0.05$.

4.2 Force steadiness

A force by position interaction was observed for CV of force (F=20.200, $p<0.01$). Supinated and neutral did not differ in CV of force ($p>0.05$), but were both less than pronated across all force levels (position main effect: F=25.632, $p<0.01$). At 5 and 10% MVC, CV of force was higher than all other force levels ($p<0.0001$), while 25 and 50% MVC had the lowest CV of force across force levels and 75% MVC differed from all other force levels ($p<0.05$) (Figure 4.1 A).

4.3 Tendon Mechanics

Force by position interactions were observed for tendon elongation (F=7.603, $p<0.01$), strain (F=7.061, $p<0.01$), tendon force (F=24.826, $p<0.01$) and tendon stress (F=22.177, $p<0.01$). Tendon elongation increased with increasing force level (force main effect: F=49. 207, $p<0.01$) and was greater in neutral than pronated at 25, 50 and 75% MVC ($p<0.05$). Tendon strain increased with increasing force level (force main effect: F=48.587, $p<0.01$), and was greater in neutral compared to pronated at 25, 50 and 75% MVC ($p<0.05$), as well as compared to supinated at 75% MVC ($p=0.05$) (Figure 4.1 B). Tendon force increased with increasing force level (force main effect: F=582.407, $p<0.01$), and supinated and neutral did not differ from each other ($p>0.05$) but were greater than pronated at all force levels ($p<0.01$). Tendon CSA had a main effect of force (F=21.977, $p<0.01$), but no interaction. Tendon CSA at 5 and 10% MVC was greater than all other force levels ($p<0.01$) and decreased from 25-75% MVC ($p<0.05$), but did not differ across the three positions ($p>0.05$) (Figure 4.1 C). Tendon stress increased with increasing force level (force main effect: F= 463.015, $p<0.01$), and supinated and neutral did not differ from each other ($p>0.05$) but were greater than pronated at all force levels ($p<0.01$) (Figure 4.1 D). Tendon stiffness did not differ across supinated (347.8 ± 170.7), neutral (264.2 ±

141.2) and pronated (190.1±115.4) positions ($p>0.05$), however supinated approached greater stiffness than pronated ($p=0.06$). It is also of note that the slope of the strain value in the neutral position was greater than supinated ($p<0.05$), while pronated did not differ from supinated or neutral.

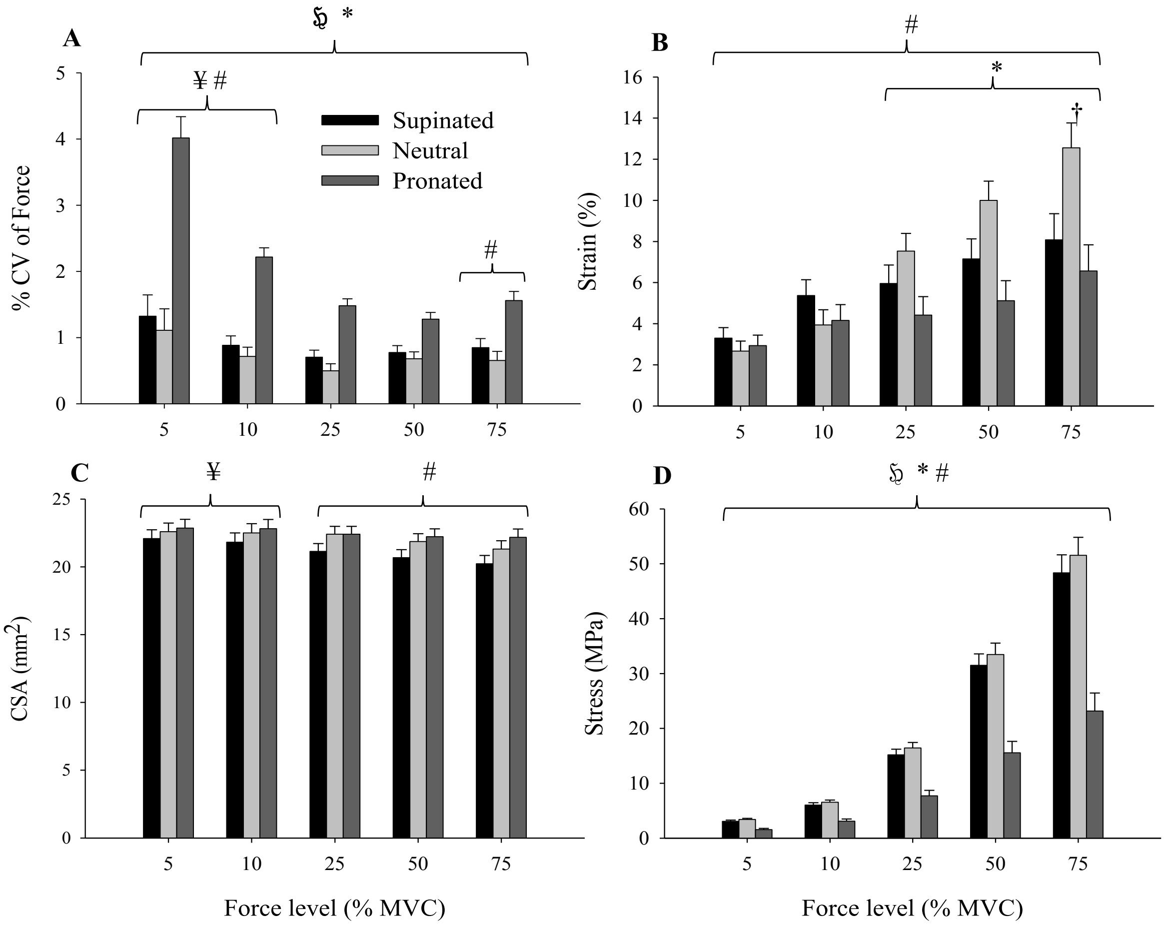

Figure 4.1: A) CV of force, B) tendon strain, C) CSA and D) stress for supinated, neutral and pronated forearm positions across submaximal force levels. CV, coefficient of variation; CSA, cross-sectional area; MVC, maximal voluntary contraction; %, percent; mm^2, millimetres squared; MPa, megapascals. §, neutral differs from supinated; *, neutral differs from pronated; †, neutral differs from supinated; ¥, greater than all other force levels; #, differs from all other force levels for all positions.

4.3.1 Correlation and Regression Analysis

Pearson's correlations and multiple forward linear regression analysis were performed separately for the low (5 and 10% MVC) and high (25, 50, 75% MVC) forces due to the rapid increase in tendon strain above 10% MVC observed for the neutral position, as well as 5 and 10% MVC being less steady than all other force levels causing the steep inverted J representation of CV of force (Brown et al., 2010; Enoka et al., 2003).

Pearson's correlations revealed that at low force levels, CV of force was negatively associated with MVC, tendon force and tendon stress in the supinated ($r = -0.722, -0.652, -0.641$; $p<0.01$), neutral ($r = -0.708, -0.679, -0.670$; $p<0.01$) and pronated ($r = -0.617, -0.834, -0.782$; $p<0.01$) positions, respectively (Table 4.2). At high force levels, CV of force was negatively associated with MVC in the supinated ($r = -0.594$, $p<0.01$), neutral ($r = -0.726$, $p<0.01$) and pronated ($r = -0.431$, $p<0.05$) positions. CV of force was also positively associated with tendon stiffness ($r = 0.352$, $p<0.05$) in the neutral position at high force levels (Table 4.3).

From the multiple forward linear regressions at low force levels, MVC was a significant predictor of reduced CV of force in both the supinated and neutral positions, while tendon stress was a significant predictor of reduced CV in neutral, and tendon force was a significant predictor of reduced CV of force in the pronated position ($p<0.05$) (Table 4.2). At high force levels, MVC was a significant predictor of reduced CV of force in the supinated and neutral positions, while CSA was also a significant predictor of reduced CV of force in the supinated position. There were no predictors of CV of force in the pronated position at high force levels (Table 4.3).

Table 4.2: Pearson’s correlations and multiple regression analysis of tendon mechanics and force steadiness at low force levels

Low Forces

	MVC	Strain	Tendon force	CSA	Stress
Supinated	-0.722$^{\#}$	-0.416	-0.652$^{\#}$	0.202	-0.641$^{\#}$
	β= -0.722$^{\#}$				
	$y=3.145\ -0.010(MVC)$				
	r^2= 0.521, *adjusted* r^2= 0.496				
Neutral	-0.708$^{\#}$	-0.071	-0.679$^{\#}$	0.066	-0.670$^{\#}$
	β=-0.503$^{\#}$				β=-0.432*
	$y= 2.852\ -0.006(MVC) - 0.114(Stress)$				
	r^2= 0.646, *adjusted* r^2= 0.609				
Pronated	-0.617$^{\#}$	-0.099	-0.834$^{\#}$	-0.239	-0.782$^{\#}$
	β= -0.330*		β= -0.698$^{\#}$		
	$y=8.646\ -0.053(Tendon\ force)\ -0.024(MVC)$				
	r^2= 0.786, *adjusted* r^2= 0.760				

Values are Pearson’s r coefficients of the respective associations, and beta weights of variables predicting variance in coefficient of variation in force. #, $p<0.01$; *, $p<0.05$.

Table 4.3: Pearson’s correlations and multiple regression analysis of tendon mechanics and force steadiness at low force levels

High Forces

	MVC	Strain	Tendon force	CSA	Stress	Stiffness
Supinated	-0.594$^{\#}$	0.107	0.103	-.281	-0.111	0.325
	β= -0.651$^{\#}$			β= -0.379*		
	$y=2.092\ -0.003(MVC)\ -0.037(CSA)$					
	r^2= 0.493, *adjusted* r^2= 0.445					
Neutral	-0.726$^{\#}$	-0.236	-0.152	-0.271	-0.127	0.352*
	β=-0.726$^{\#}$					
	$y= 1.597\ -0.004(MVC)$					
	r^2= 0.527, *adjusted* r^2= 0.510					
Pronated	-0.431*	0.073	0.135	-0.091	0.046	-0.123

Values are Pearson’s r coefficients of the respective associations, and beta weights of variables predicting variance in coefficient of variation in force. #, $p<0.01$; *, $p<0.05$.

Chapter 5: Discussion

The present study aimed to determine whether distal BB tendon mechanical properties differ between supinated, neutral and pronated forearm positions, and contribute to position-dependent FS of the elbow flexors. Strength and FS were less in the pronated compared with supinated and neutral positions and these positions did not differ in MVC or CV of force. Tendon force and stress increased with increasing force levels and were less in pronated than supinated and neutral at all force levels. Strain increased with increasing force levels for all positions, but at high forces was greater in neutral compared to supinated and pronated. At low force levels, greater forces acting on the distal BB tendon contributed to reduced CV of force for all positions. At high force levels, increased tendon CSA acted to reduce CV of force in the supinated position, possibly through reducing the amount of stress experienced by the tendon, while an increase in tendon stiffness increased CV of force in the neutral position. Strength was positively related to reduced CV of force across all positions at both low and high forces, suggesting that positional differences in maximal force have a strong influence on FS. These findings support the hypothesis that positional differences in tendon mechanics influence FS across forearm positions.

The reduction in CV of force followed by the plateau between 25-50% MVC and the subsequent increase in CV was previously observed for the elbow flexors (Brown et al., 2010; Smart et al., 2016) and other muscles (Dideriksen, Negro, Enoka, & Farina, 2012; Enoka et al., 2003; Jesunathadas, Klass, Duchateau, & Enoka, 2012; Moritz, Barry, Pascoe, & Enoka, 2005). This inverted J curve for CV of force has been attributed to reductions in MU discharge rate variability with increasing force levels (Dideriksen et al., 2012; Enoka et al., 2003; Jesunathadas et al., 2012; Moritz et al., 2005). The prior reports of position-dependent FS of the elbow flexors

(Brown et al., 2010), as well the inverted J shape of the CV of force curve (Brown et al., 2010; Enoka et al., 2003) are further supported by mechanics of the tendon.

5.1 Resting tendon length and strain

Resting tendon length in the pronated position was significantly shorter than both the supinated and neutral positions despite equal resting muscle lengths across positions. This may be due to inherent differences in the compliance of the muscle and tendon (Abellaneda, Guissard, & Duchateau, 2009; Herbert et al., 2011), allowing for the distal BB tendon to rest at it's true slack length in the pronated position, resulting in the shorter resting tendon length. Differences in the muscle and tendon's responses to changing joint angle have been observed in the gastrocnemius-Achilles tendon unit whereby the slack length of the muscle and tendon occur at different joint angles (Hug et al., 2013). A similar phenomenon may be occurring in the BB and its distal tendon. In addition to the muscle and distal tendon, the proximal tendons of the BB may also be contributing to the dissociation between muscle and tendon length at the distal end. Albeit unlikely, the proximal tendons of the LH and SH through their unique and independent origin on the scapula might independently accommodate to length changes induced by rotation of the radius at the distal end permitting changes in distal tendon length without a change in muscle length. To my knowledge compliance and mechanics of the entire BB muscle-tendon unit inclusive of proximal tendons have not been investigated. This is likely due to the proximal tendon of the LH passing under the acromion process of the scapula and changing course to insert onto the supraglenoid tubercle of the scapula, preventing ultrasound acquisition of resting tendon length required for measures of proximal tendon strain. While anatomical constraints may prevent the study of proximal tendon contributions to tendon mechanics of the BB, changes in forearm muscle position (see below) as the forearm rotates might explain the reduced levels of strain observed in the pronated position.

These changes in anatomy may include lateral and posterior muscles of the forearm such as the brachioradialis, extensor carpi radialis longus and extensor carpi radialis brevis rotating overtop of the distal BB tendon, as well as the tendon being pulled medially around the radius (Koch & Tillmann, 1995) into a reduced radioulnar space (Bhatia, Kandhari, & DasGupta, 2017). These combined constraints may reduce the ability of the BB and its distal tendon to work at an optimal position, thereby limiting the tendon's ability to elongate and reducing its strain compared to the neutral position at force levels above 10% MVC. While tendon stiffness did not differ among positions, supinated trended towards having higher stiffness than pronated (p=0.06). This will also contribute to strain levels not differing between the supinated and pronated positions, even with MVC and tendon force being higher in the supinated position. These findings contradict the second hypothesis of tendon strain being greater in pronated compared to supinated and neutral. Assuming the distal BB tendon experiences the greatest amount of slack in the pronated position, greater muscle force acting on the tendon would be required to overcome the increased slack prior to the tendon lengthening. Additional force would then be required to achieve strain levels equivalent to those observed in the supinated and neutral positions at high force levels. The ability for the BB to achieve this required level of force may not be possible due to reflex inhibition between positions.

Studies examining reflex and MU properties of the BB and brachioradialis across forearm positions have observed that due to type 1 non-reciprocal pre-synaptic inhibition from the brachioradialis in the pronated position at low force levels (3-15% MVC), BB motor unit discharge rates are reduced during voluntary contractions and interspike intervals following radial nerve stimulation are increased (Barry et al., 2008), while recruitment threshold of SH BB MUs is also increased (Harwood et al., 2010). The inhibition of the BB observed by Barry et al. (2008) also confirmed previous findings of a spinal mechanism through which the brachioradialis inhibits MU activity of the BB (Naito, Shindo, Miyasaka, Sun, & Morita, 1996). Inhibition of the BB in the pronated position, coupled with increased recruitment thresholds of SH MUs likely contributes to current and previous (Brown et al., 2010) findings of reduced MVC force in the pronated position, as well as reduced tendon strain. In addition to reflexes, reduced MVC force in pronation also increases MU discharge rate variability for this position (Harwood et al., 2010), and although elevated MU discharge rate variability increases CV of force (Dideriksen et al., 2012; Enoka et al., 2003; Jesunathadas et al., 2012; Moritz et al., 2005), it might not be the primary contributor as MU activity is directly influenced by strength (Vila-Cha & Falla, 2016), which is position-dependent (Brown et al., 2010) While reflex and motor unit activity may explain strain differences between the pronated position compared with supinated and neutral, other factors within the MTU may result in strain differing between the supinated and neutral positions.

The difference in strain between supinated and neutral at 75% MVC was not expected as tendon force, resting tendon length and elongation did not differ statistically between supinated and neutral positions. The 4.1 mm difference in tendon length and 2.9 mm difference in elongation of the distal BB tendon during contraction in the neutral compared to the supinated position, coupled with the 125 N difference in tendon force, likely contributed to significantly greater strain values for the neutral compared to supinated position at 75% MVC. These small and non-statistical differences in tendon length and elongation indicate that subtle changes between positions are additive and alter mechanics at high force levels. The greater tendon strain at 75% MVC, as well as the steeper slope in neutral relative to supinated ($p<0.05$) (Figure 4.1 B), suggest an uncoupling in the strain response to the forces acting on the tendon between positions at high forces. This uncoupling may be due to alterations within the tendon, such as the behavior of the perimysium and collagen fibres under load observed using microscopes for in situ (Franchi et al., 2007) and in vitro (Abrahams, 1967; Purslow, 1989) animal tendons, as well as the human Achilles tendon in vitro (Abrahams, 1967).Changes in the perimysium and collagen fibers have been speculated to explain in vivo observations of the Achilles tendon-gastrocnemius MTU using ultrasound (Herbert et al., 2011). The interaction of forearm position and strain in the supinated and neutral positions suggests subtle differences between positions can affect the measured mechanics of the tendon between positions.

5.2 Tendon Force and stress

To generate increasing levels of force about a joint, the muscle must increase its contractile force to generate pull on the tendon resulting in movement of the respective limb. As tendon force is the quotient of the muscle force acting on the limb and the tendon's moment arm, it is largely derived from absolute strength. Although relative force levels were matched between participants and positions, the absolute force exerted at submaximal levels depended on MVC force. This resulted in the increase in tendon force from 5-75% MVC across all positions. Changes in the force acting on the tendon also lead to changes in the tendon's dimensions, altering how that force is distributed through the tendon. Similar to previous observations in a neutral forearm position (Smart et al., 2016; Smart et al., 2017), CSA of the tendon decreased with increases in force, and this was also observed for the supinated and pronated positions. The combination of an augmentation in tendon force and attenuation of CSA as relative force increased culminated in greater distal BB tendon stress with increasing force levels as more force is required to act through a smaller CSA. This has also been observed for the Achilles tendon (Obst, Renault, Newsham-West, & Barrett, 2014). These forces acting on the tendon will also cause it to elongate from its resting length, thereby increasing the strain placed on the tendon as was observed in the present study and others (Smart et al., 2017). The results of strain, tendon force, CSA, and stress demonstrate that regardless of forearm position, the architectural response of the distal BB tendon to increased force is a decrease in area and increase in length.

Positional differences in isometric elbow flexion MVC whereby pronated is weaker than supinated and neutral positions were expected (Brown et al., 2010; Harwood et al., 2010). This in turn led to the higher tendon forces in the supinated and neutral positions compared to the pronated position regardless of force level, suggesting that the positional differences in tendon force are caused by the forces acting on the tendon due to absolute strength differences between positions. Because tendon CSA did not differ among positions, albeit smaller at higher forces, it was the higher tendon force acting on the same size tendon across positions that contributed to stress being greater in supinated and neutral than pronated. Thus, the results of the present study suggest that absolute strength differences among positions are critical contributors to elbow flexor FS as adaptations in the distal BB tendon directly alter the mechanical property of stress. The findings of greater tendon force and stress in the supinated and neutral positions support the first hypothesis. Thus, with strength influencing both the previously mentioned MU properties as well as tendon mechanics, it might be the primary factor shaping FS through its indirect influence on all previously associated neural factors (Harwood et al., 2010; Vila-Cha & Falla, 2016), and as demonstrated in this study its effect on tendon mechanics.

5.3 Tendon mechanical properties and force steadiness

At both low and high force levels, increased MVC force was negatively associated with CV of force across supinated, neutral and pronated positions. The negative association of MVC with CV of force in the elbow flexors has also been observed by Brown et al. (2010) for males and females, as well as for young and old adults (Pereira et al., 2015; Smart et al., 2016). The mechanism through which strength acts to reduce CV of force may arise from a reduction in MU discharge rate variability with increased strength (Vila-Cha & Falla, 2016), as increases in MU discharge rate variability lead to increases in CV of force (Jesunathadas et al., 2012; Moritz et al., 2005) and for all three positions in this study the multiple forward regression analysis also revealed that MVC was a significant predictor of CV of force. This has been previously shown in young and old (Smart et al., 2016) as well as males and females (Brown et al., 2010) for the elbow flexors in the neutral position, as well as in the plantar flexors of young and old in relation to balance (Onambélé et al., 2007, 2006). While the importance of MVC in force steadiness across forearm positions and force levels has been confirmed in the present study, the differences in tendon mechanics between positions also adds insight into why FS varies according to force levels and position beyond absolute strength.

For low force levels, the mechanical properties of tendon force and tendon stress were both negatively associated with CV of force in addition to MVC. The negative association between tendon force and CV of force suggests that a greater force acting on the tendon helps to maintain steadier contractions. Submaximal target forces are dependent upon the MVC and this absolute force will dictate how much force is acting on the tendon. Thus, the association between tendon force and CV of force is partly attributed to mechanical factors that are directly linked with absolute strength. With increasing forces acting on the tendon, the distribution of force through a smaller tendon CSA leads to higher stress levels (Obst et al., 2014; Smart et al., 2016). The association of tendon stress and CV of force was previously shown for low forces in young and old adults (Smart et al., 2016), and the present study highlights this relationship across forearm positions. However, measures from the present study indicate that the negative association of stress and CV is due to the force acting on the CSA of the tendon. Although the supinated and neutral positions experienced greater tendon stress due to higher tendon forces, tendon CSA did not differ across positions for any force level, thus placing a larger amount of force on a smaller tendon CSA may allow for better transmission and control of force at low levels. The associations of tendon mechanics with CV of force at low force levels suggest that in addition to MVC, the amount of force acting on the tendon from the muscle, and the tendon's ability to distribute that force through its CSA all contribute to reduced CV of force at low force levels regardless of forearm position.

At high force levels, increased tendon CSA was a predictor of reduced CV of force in the supinated position, while increased stiffness was associated with increased CV of force in the neutral position. These were present alongside the previously mentioned associations of MVC and CV of force. Increased tendon CSA was a predictor of reduced CV of force in the supinated position despite no differences in CSA across positions. This is suggestive of the tendon's dimensions acting to reduce the stress placed on the tendon by increasing the distribution of high forces across the tendon (Magnusson et al., 2003; Stenroth et al., 2012, 2016) which likely reduces CV of force. The association of increased stiffness and increased CV of force in the neutral position is contrary to previous observations of increased tendon stiffness leading to reduced CV of force in the elbow flexors (Smart et al., 2016) and plantar flexors (Johannsson et al., 2015), as well as decreasing postural sway (Onambélé et al., 2007, 2006). This might result from the tendon acting as a rigid force transducer at high force levels (Earp, Newton, Cormie, & Blazevich, 2014; Rack & Ross, 1984), allowing the fluctuations in force output from the BB to be transferred to the radius causing increased CV of force. These findings suggest that in addition to the influence of MVC across positions at high force levels, increased CSA of the tendon may act to reduce CV of force in the supinated position by reducing the amount of stress placed on the tendon, while increased stiffness of the tendon in the neutral position increases CV of force by transferring force fluctuations from muscle to bone.

The contribution of distal BB tendon mechanics to force control of the elbow flexors in the supinated, neutral and pronated forearm positions was evaluated. The position-dependency of FS can be largely attributed to strength, as MVC was associated with increased FS across all positions at low and high force levels, and pronated had lower MVC and FS compared to supinated and neutral. Differences in forces produced also translate to lower tendon force and stress in the pronated position; but tendon force and stress were negatively associated with increased FS across positions only at low force levels. Tendon strain was higher in the neutral position at high force levels, but it was not associated with FS. In addition to the contribution of MVC to FS at high force levels, tendon CSA was a predictor of increased FS for supinated, while tendon stiffness was associated with reduced FS in neutral. The results of the current study suggest that regardless of differences in tendon mechanics across positions, strength remains the largest contributor to increased FS at low and high forces, while the forces acting on the tendon influence FS at low force levels, and contributions from the tendon’s dimensions and stiffness also affect FS at higher force levels.

Chapter 6: Conclusion

6.1 Conclusions

The present study aimed to evaluate the contribution of distal BB tendon mechanical properties to elbow flexor force steadiness across supinated, neutral and pronated forearm positions in young males. Tendon force and stress were greater in supinated and neutral compared to pronated, supporting the first hypothesis of position-dependent tendon mechanics. Tendon strain was greater in neutral compared to supinated and pronated at high force levels, which did not support the first hypothesis of greater strain in the pronated position. At low force levels, MVC, tendon force and tendon stress were all negatively associated with CV of force for supinated, neutral and pronated forearm positions, while MVC was also a predictor of reduced variability in CV of force for all positions, supporting the second hypothesis. At high force levels, MVC was negatively associated with CV of force across all positions and tendon strain was negatively associated with CV of force in the supinated position, while MVC was a predictor of reduced CV of force in the supinated and neutral position, and CSA was a predictor of reduced CV of force in the supinated position. These findings confirm the second hypothesis that MVC, tendon force and tendon stress would be associated with reduced CV of force, while the association of strain and reduced CV of force in the supinated position at high force levels was not expected. The contribution of MVC in all three positions at low and high forces indicate that while the mechanics of the tendon do influence elbow flexor FS, strength continues to be the predominant contributor to increased FS regardless of force level and forearm position. This thesis expands on previous findings of position-dependent strength influencing FS (Brown et al., 2010) to show that these differences in strength directly influence mechanics of the distal BB tendon which also contribute to position-dependent responses in FS of the elbow flexors.

6.2 Limitations

The present study measured tendon mechanics of the distal BB; however, the brachioradialis and brachialis were not examined. These additional elbow flexors contribute primarily to elbow flexion force (van Zuylen et al., 1988), with the brachioradialis having varying activation levels across forearm positions (Harwood et al., 2010) as well as partially contributing to forearm supination and pronation torques (Murray et al., 1995; Zhang et al., 1998). While not examined in the current study, differential contribution of these muscles to elbow flexion across forearm positions may explain some of the observed differences in distal BB tendon mechanics. Moreover, the proximal LH and SH tendons of the BB were not evaluated. These measures would offer insight into mechanics of the entire BB MTU and may provide an explanation for resting muscle and tendon lengths across positions if slack within the system is distributed within the proximal and distal tendons as well as the muscle when the shoulder is kept stable. However, anatomy challenges the capture of these measures with current technology. The resting tendon length of the proximal LH cannot be obtained due to the change in course of the tendon as it passes over the head of the humerus and turns medially under the acromion process to insert onto the supraglenoid tubercle. Thus, the scapula impedes imaging of the entire tendon. In addition to the proximal tendons, fascicle length of the BB was not measured during contraction as an entire fascicle cannot be captured on video from resting to contracted states. Measures of the BB proximal tendons and muscle belly would allow quantification of mechanics of the entire BB MTU.

6.3 Future Research Directions

This thesis examined the contribution of distal BB tendon mechanical properties to elbow flexor FS across supinated, neutral and pronated forearm positions. The results suggest that strength is the largest contributor to increased FS across forearm positions when compared with tendon mechanics; however, changes in neural factors (surface and intramuscular EMG) were not evaluated. To further elucidate potential neural factors of the arm, EMG of all elbow flexor muscles (LH and SH BB, brachioradialis, brachialis) as well as other elbow extensor muscles that may be contributing to the large difference in pronated from the other positions should be examined, and the coherence between these muscles across forearm positions and force levels should be considered. Moreover, inhibition of the BB through reflex activity in the pronated position (Barry et al., 2008), as well as changing wrist and forearm anatomy as the radius is rotated over the ulna to achieve pronation, may play a role in altering elbow flexor FS through reflex changes which are induced by alterations in muscle and tendon slack length.

Additional to neural factors of synergist and antagonist muscle activation, advances in ultrasound techniques that allow for capture of the entire fascicle lengths during contraction through the use of two probes or a LOGIQView® scan should be considered. This approach would allow the entire length of the BB muscle to be determined during contraction. Differences in muscle shortening during active contraction might account for the observed position-dependent strain values of the current study. To gain a better understanding of elbow flexor FS, simultaneous capture of muscle architecture, tendon mechanics and EMG of the primary and secondary contributors to elbow flexion should be evaluated during both forearm rotation and elbow flexion. This study was constrained to one joint angle, thus additional joint angles or dynamic movement requires study.

Other populations such as females or elderly adults could be studied, as tendon mechanics differ between these populations. While ultrasound is an easily accessible and versatile tool for evaluating muscle under many situations, magnetic resonance imaging (MRI) remains the gold-standard of medical imaging. The use of MRI would allow for ease of imaging resting tendon length of the proximal LH BB tendon and other tendons partially obstructed on ultrasound due to surrounding anatomy. This would permit mechanics of strain to be calculated for various other tendons. Albeit, the technology is not yet available for these measures during muscle contraction.

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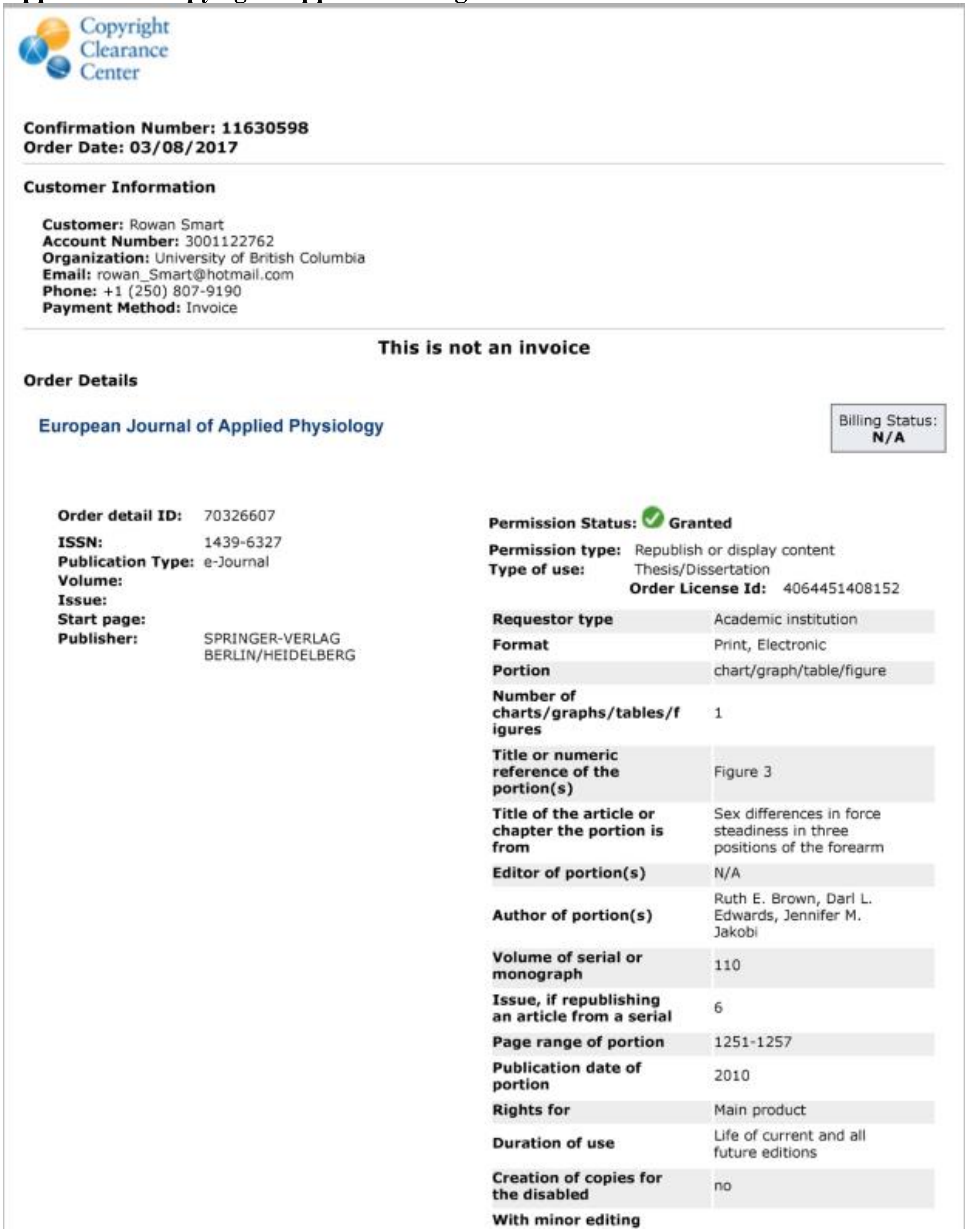

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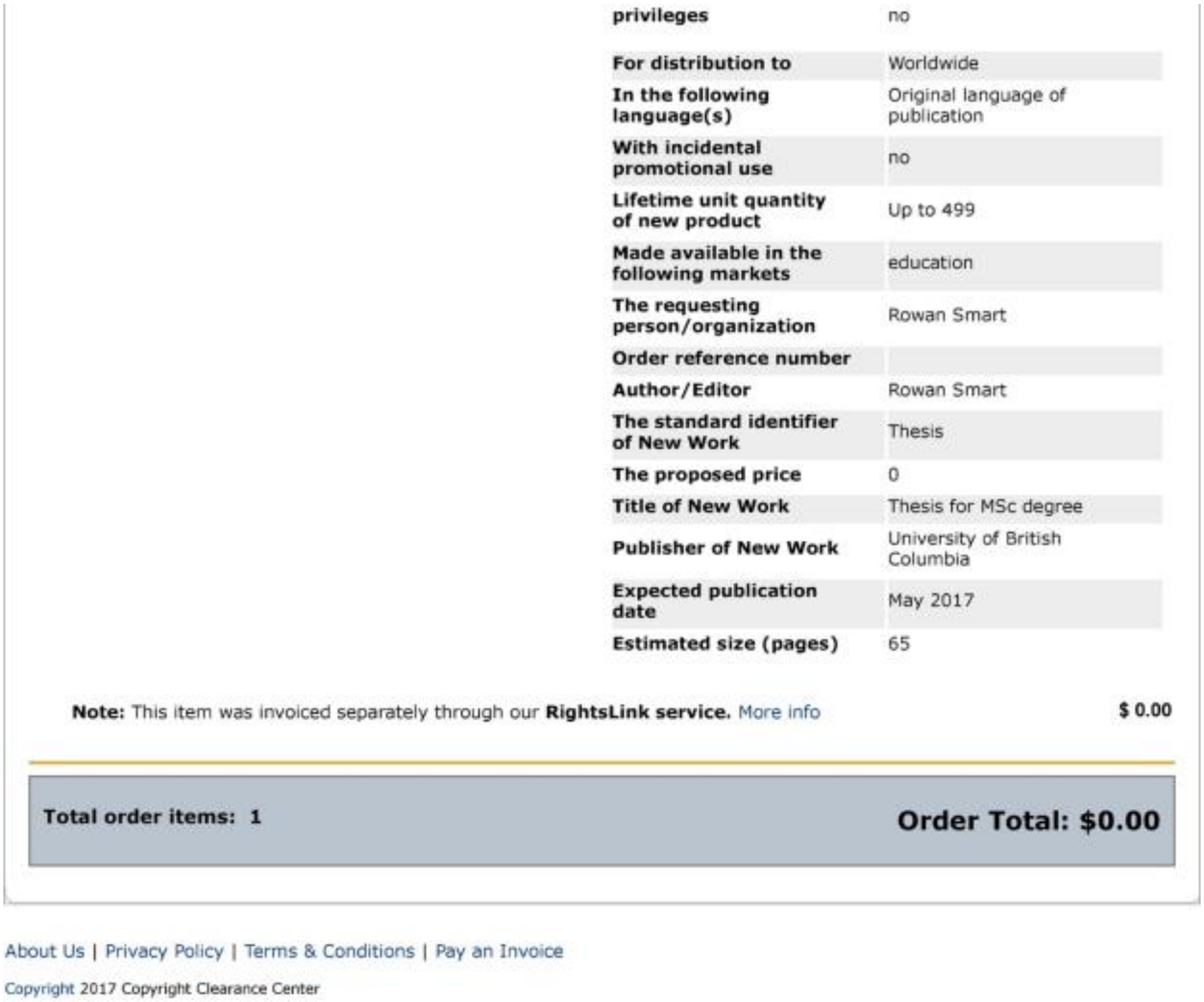

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Appendix C: Ethics Approval

The University of British Columbia Okanagan
Research Services
Behavioural Research Ethics Board
3333 University Way
Kelowna, BC V1V 1V7   Phone: 250-807-8832
Fax: 250-807-8438

CERTIFICATE OF APPROVAL - MINIMAL RISK AMENDMENT

PRINCIPAL INVESTIGATOR:	**DEPARTMENT:**	**UBC BREB NUMBER:**
Jennifer M. Jakobi	UBC/UBCO Health & Social Development/UBCO Health and Exercise Sciences	H16-00948

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Okanagan

CO-INVESTIGATOR(S):
Rowan Smart

SPONSORING AGENCIES:
Natural Sciences and Engineering Research Council of Canada (NSERC) - "Spinal Network Adaptability for the Control of Steady Contractions in Men and Women"

PROJECT TITLE:
Force Steadiness, Tendon Mechanics and Muscle Architecture

Expiry Date - Approval of an amendment does not change the expiry date on the current UBC BREB approval of this study. An application for renewal is required on or before: June 20, 2017

AMENDMENT(S):

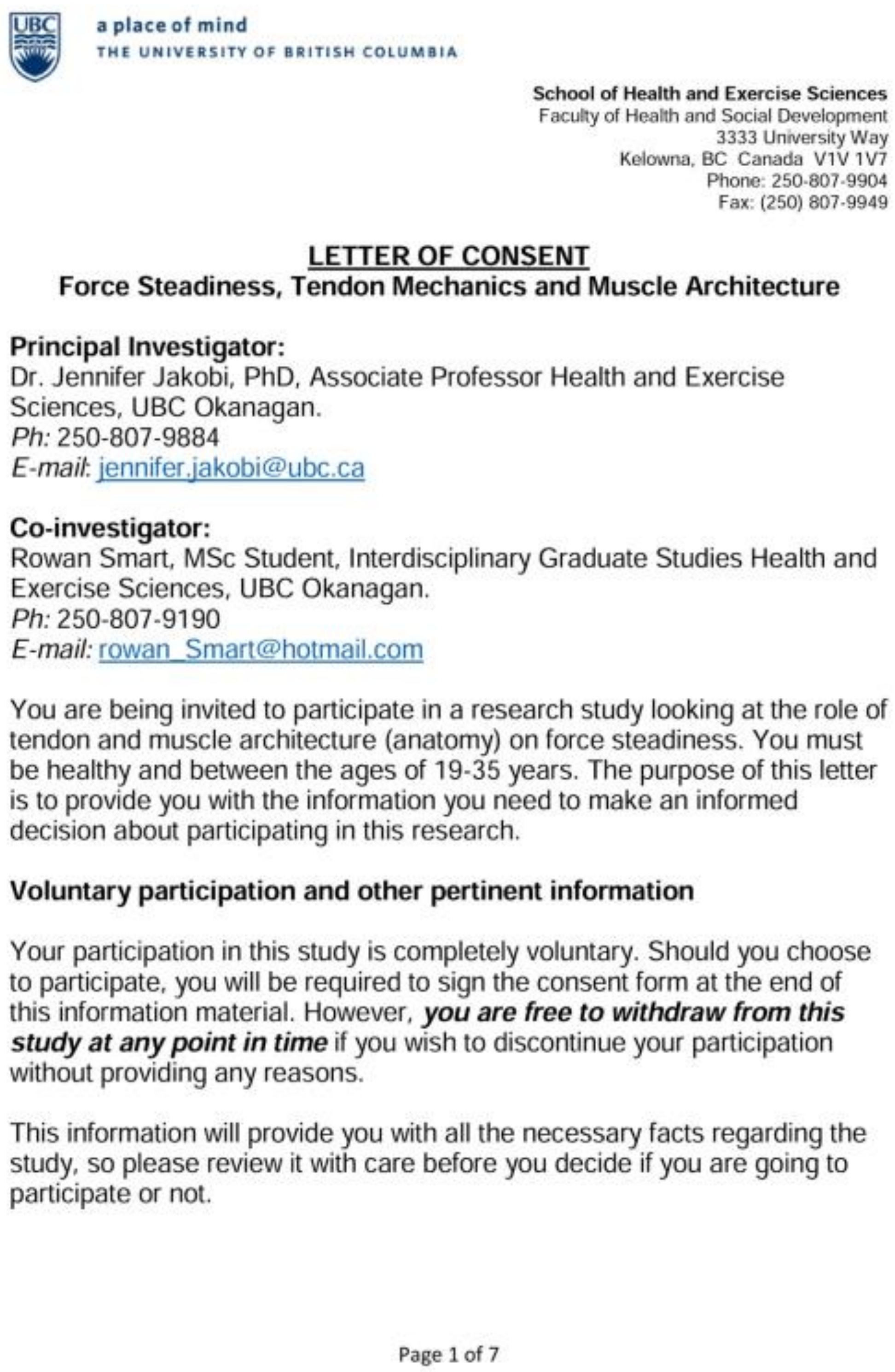
AMENDMENT APPROVAL DATE:
October 21, 2016

Document Name	Version	Date
Consent Forms:		
Consent form with age at 35	2	October 20, 2016
Fixing consent form deletion	N/A	October 21, 2016
Ethics Revision Age	02	October 18, 2016

The amendment(s) and the document(s) listed above have been reviewed and the procedures were found to be acceptable on ethical grounds for research involving human subjects.

This study has been approved either by the full Behavioural REB of the UBC Okanagan or by an authorized delegated reviewer

Appendix D: Consent Form



a place of mind
THE UNIVERSITY OF BRITISH COLUMBIA

School of Health and Exercise Sciences
Faculty of Health and Social Development
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Phone: 250-807-9904
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LETTER OF CONSENT
Force Steadiness, Tendon Mechanics and Muscle Architecture

Principal Investigator:
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Co-investigator:
Rowan Smart, MSc Student, Interdisciplinary Graduate Studies Health and Exercise Sciences, UBC Okanagan.
Ph: 250-807-9190
E-mail: rowan_Smart@hotmail.com

You are being invited to participate in a research study looking at the role of tendon and muscle architecture (anatomy) on force steadiness. You must be healthy and between the ages of 19-35 years. The purpose of this letter is to provide you with the information you need to make an informed decision about participating in this research.

Voluntary participation and other pertinent information

Your participation in this study is completely voluntary. Should you choose to participate, you will be required to sign the consent form at the end of this information material. However, ***you are free to withdraw from this study at any point in time*** if you wish to discontinue your participation without providing any reasons.

This information will provide you with all the necessary facts regarding the study, so please review it with care before you decide if you are going to participate or not.

If you choose not to participate in this study, you will not be penalized in any way, nor do you need to disclose why you have chosen not to participate.

You should only agree to participate if you feel happy that you know enough about the study. If you are participating in another study at this time, please inform the lead study investigator to determine if your participation in this study is appropriate.

Background Information

Ultrasonography is a non-invasive procedure used to measure muscle and tendon structures by imaging through the skin. This study will use ultrasound to measure changes in muscle and tendon of the arm in healthy persons during force production. While your muscle and tendon are being measured you will be producing force over a variety of different force levels ranging from low forces to your highest force. These measures will be made over the course of 2-4 visits, each lasting approximately 120-150 minutes. The time varies between participants as some images are obtained quickly, while differences in individual anatomy can require a longer time to obtain the images.

Purpose of this study

The purpose of this study is to determine the effect of muscle and tendon architecture on force production and whether this differs in males and females, as well as across different arm positions.

Who can participate?

If you are a healthy male or female between the ages of 19-35 years old, are not involved in high levels of upper body strength training, are not trained in fine motor skills and have not had any injuries or orthopaedic surgeries to the right shoulder or arm in prior 6 months, you are welcome to participate in this study. You must be able to speak and read English fluently.

Who should not participate?

You should not participate in this study if you are: 1) under the age of 19 or over the age of 29, 2) participating in high levels of upper body strength training, 3) have a current or previous history of injury or orthopaedic surgery to the right arm or shoulder in prior 6 months, 4) have sever cognitive impairment, or 5) are unable to read and speak English fluently.

Procedure for this study

Should you choose to participate in this study, your participation would involve; completing questionnaires regarding your physical activity and performing maximal voluntary contractions and submaximal contractions. Ultrasound will be used to collect data throughout the duration of this study. This assessment requires 2-4 visits separated by a minimum of 24hrs, with each session lasting approximately 120-150 minutes in duration.

1. You will come to the Neuromuscular Physiology Lab in Arts and Sciences 164 at a predetermined time. Upon arrival you will do a physical activity questionnaire.
2. The graduate student will take initial measurements of your muscle and tendon using ultrasound.
3. You will perform a variety of contractions at different forearm positions.
4. Graduate students will operate the ultrasound to record images of your tendon, and the dynamometer to record force.

Your responsibilities

It is important that you come to the lab dressed in appropriate clothing such as shorts and a t-shirt, which are ideal for comfort. Also, please refrain from exercising on testing days as it may affect the results of the study.

Risks and discomforts of participation

The risks associated with the proposed research are minimal. Minimal muscle soreness may result from maximal voluntary contractions.

Associated benefits of participation

There are no direct benefits to you, except the results of an assessment of your muscles and tendons.

Results could provide useful information in understanding the role of the muscle and tendon in differences in force control between young males and females.

Will I be paid or do I have to pay to participate?

You will not be paid and there is no financial cost to your participation in the described experiments. However, any incurred parking and travel expenses as a result of your participation in this study will be reimbursed, provided receipts are submitted to a member of the research team. **You are free to withdraw from the experiment at any point in time. You do not have to provide a reason for withdrawing from the study if you do not wish to do so.**

What to do if you want to withdraw from this study:

Participation in this study is voluntary. You have the right to refuse to participate, refuse to answer any questions or withdraw from the study at any time with no effect on your future (care/academic status/employment). You do not have to provide reasons to do so. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrollment in the study will be retained for analysis. This data is to be used for MSc thesis work, which will become a publicly accessible document. No individual will be disclosed in this document and there are no identifiers of person. Data not used in the publication(s) are kept on file, for approximately 5-years which approximates the funding cycle.

What happens if something goes wrong during the study?

Any adverse events that should arise will be followed-up thoroughly to ensure your safety and health. Background health history and functional assessment parameters will be collected and stored on a computer, while any clinical symptoms will be recorded in the investigator's laboratory book. All data arising from this study will be archived and stored securely by the investigators through password protected computer systems and remain confidential. You do not wave any legal rights by signing the consent form. The researchers of this study will be readily available if you would like to discuss any problems or concerns that may arise. There will be no feedback to individual participants. Group results for the study will be made publicly available through submission for publication. Additionally, participants can contact the researchers for a summary of the study results.

Can I be asked to leave the study?

If you do not adhere to the study guidelines outlined earlier in this document, you will be asked to leave the study. Also, in the rare event that a medical emergency occurs during the study, you will be automatically withdrawn from the study to ensure your safety and well-being.

After this study is complete:

Results of this study will be published as a manuscript, and will comprise a graduate student's (Rowan Smart) thesis. Thesis documents are publicly available on the internet. No data will be linked to a specific participant. Your data will be assigned a personal identification number to ensure anonymity in both the analysis and documentation of results. The raw data obtained in this study will only be available to the principle investigator (Dr. Jakobi). As stated earlier, you will be provided with a feedback sheet explaining the outcomes and specific findings of this study.

Privacy and confidentiality

Your confidentiality will be respected. No information that disclose your identity will be released or published without your explicit consent to the disclosure. *However, research records and medical records identifying you may be inspected in the presence of the investigator or his or her designate by representatives of the UBC Behavioural Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the investigators' offices.*

Personal descriptors (i.e. names) will be coded to a numeric value and data will be kept in a locked cabinet in ASC 164 at the University of British Columbia Okanagan. The data will be made available online to members of the research team, and destroyed in 5 years after publication. The master copy will be stored separate from the coded data in ASC 164 at the University of British Columbia Okanagan.

If you have any concerns about your rights as a research participant and/or your experiences while participating in this study, you may contact the Research Participant Complaint Line in the UBC Office of Research Services at 1-877-822-8598 or the UBC Okanagan Research Services Office at 250-807-8832. It is also possible to contact the Research Participant Complaint Line by e-mail (RISL@ors.ubc.ca).

Funding source/sponsor

Funding provided through the National Sciences and Engineering Research Council (NSERC) #312038

CONSENT FORM FOR PARTICIPANTS

I have read and understand the information sheet concerning this project. All my questions have been answered to my satisfaction. I understand that I am free to request further information at any stage. I know that:

1. My participation in the project is entirely voluntary, and I am free to withdraw from this study at any time without any disadvantage.
2. The data on which the results of the project depend will be retained in secure storage for five years after publication, after which they will be destroyed.
3. I will be required to complete initial assessments and the protocol consisting of 2-4 visits of approximately 120-150 min in duration.
4. The experimental session will involve the following measurements:
    - Questionnaires
    - Assessment of muscle and tendon using ultrasound
    - Submaximal and maximal force level contractions
5. The results of this project will be published as part of a thesis and may be published as part of a manuscript and will be available in the University of British Columbia Okanagan Library but every attempt will be made to preserve anonymity.
6. I will receive a signed and dated copy of this consent form.

I agree to take part in this project

....
Printed Name of Subject

....
Signature and Date

....
Printed Name of Principle Investigator

....
Signature and Date