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Title:

Velocity of the muscle tendon unit is sex-dependent and not altered with acute static stretch

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Abstract

Contraction velocity of a muscle tendon unit (MTU) is dependent upon the interrelationship between fascicles shortening and the tendon lengthening. Altering the mechanical properties of these tissues through a perturbation such as static stretching slows force generation. Females, who have inherently greater compliance compared with males have slower velocity of MTU components. The addition of a static stretch might further exacerbate this sex difference. The purpose of this study was to investigate the velocity of fascicle shortening and tendon lengthening in males and females during isometric maximal voluntary contraction (MVC) of the plantar flexors prior to and following an acute static stretch. The MTU was imaged with ultrasound and voluntary activation tested with twitch interpolation for the 5-second plantar flexion MVC which proceeded and followed an acute stretch. For the 3-minute stretch the ankle was passively rotated to maximal dorsi-flexion. The males were stronger (128.71 ± 7.88 Nm) than the females (89.92 ± 4.70 Nm) but voluntary activation did not differ. Tendon lengthening velocity ($p=0.001$) and fascicle shortening velocity ($p=0.01$) were faster in males than females. Tendon velocity was positively and significantly correlated with fascicle velocity, ($r^2=0.307$, $p=0.02$). Although sex was significant as a predictor ($p=0.05$) time was not independently significant. Thus, stretch did not alter this relationship in either sex ($p=0.6$). The velocity of the individual components of the MTU are slower in females when compared to males; however, acute stretch does not alter the relationship between these components in males or females.

Keywords: ACHILLES TENDON, MEDIAL GASTROCNEMIUS, ULTRASONOGRAPHY, PLANTAR FLEXORS, FASCICLE SHORTENING, TENDON ELONGATION

Introduction:

The production of maximal force is sensitive to the velocity of the contraction where an increase in velocity decreases force output for shortening contractions through the relationship described in the force velocity curve (Abbott and Wilkie 1953, Bottinelli et al. 1996). During isometric contractions the elastic properties of the tendon allow it to lengthen and the muscle fascicles to shorten (Griffiths 1991, Ito et al. 1998) although the joint angle remains constant. Thus, to produce rapid maximal force during an isometric contraction the velocity in which muscle fascicles shorten must be echoed by the velocity that the tendon lengthens. With the use of ultrasound these non-isometric movements of the muscle tendon unit (MTU) can be visualized (Herbert et al. 2004, Muramatsu et al. 2001, Narici et al. 1996, Reeves and Narici 2003), and the velocity of the individual components of the MTU determined.

During isometric contractions muscle fascicles shorten and stretch the tendon until the fascicles reach maximum force and cannot contribute further to elongating the tendon (Griffiths 1991). If a tendon is compliant it will stretch further per unit of force applied by the sarcomeres, thus delaying the attainment of maximal force until the stretch of the elastic structure is fully applied. This is demonstrated through a negative correlation between the rate of torque development (RTD) and the compliance of the tendinous structures in a muscle tendon unit (MTU) (Bojsen-Moller et al. 2005). Placing the muscle in a lengthened position can utilize the contribution of passive tension to maximal force production and increase RTD, compared to the muscle being placed in a shortened position where slack can be introduced to the tendon and connective tissue in and around the muscle fascicles (Herbert et al. 2011), delaying maximal force attainment. Thus, the optimal model for rapid force production requires a stiffer tendon and slightly longer muscle fascicles (Lichtwark and Wilson 2008; Lichtwark et al. 2007), which

likely disadvantages females who generally present more compliant tendons when compared to males (Bryant et al. 2007). Thus, the optimal model for rapid isometric force production involves a stiff tendon (Bojsen-Moller et al. 2005) which more effectively transmits force to the bone but this likely disadvantages females, who present more compliant tendons when compared with males (Bryant et al. 2007). These differences in mechanical behaviour between males and females may contribute to tendon lengthening velocity becoming uncoupled from the fascicle shortening velocity, specifically in populations that have inherently greater tendon compliance such as females. Males, with relatively low connective tissue compliance, are likely to have equal velocities of the fascicles and tendon during contraction as the fascicle velocity does not need to compensate for increased tendon compliance. This potential sex difference has yet to be investigated in relation to the velocity of the components of the individual element of muscle and tendon that comprise the MTU.

Static stretching of a sufficient magnitude and duration prior to force production can further exacerbate the potential negative effects of compliant connective tissue and tendon in females producing high velocities of shortening and lengthening of the muscle and tendon, respectively. Common to athletic events, acute static stretching performed prior to explosive movements is detrimental to rate and magnitude of force development (Cramer et al. 2004, Young and Behm 2003). Five repeated bouts of 120 seconds of passive stretching of the plantar flexors decreased maximal torque post stretching (Weir et al. 2005), while five, one minute static stretches, reduced MTU passive stiffness and increased range of motion and muscle tendon junction displacement (Morse et al. 2008). However, when static stretching was applied to the plantar flexors in 3 repeated bouts of 45 seconds there were no significant changes in torque or range of motion observed (Power et al. 2004). These equivocal findings, Simpson et al. (2016)

suggested culminated from the intensity of the stretch. To alter the properties of a MTU, stretch durations and intensities need to be of sufficient magnitude and differences between studies likely arise from the stretching paradigm.

The purpose of this study was to examine the relationship of the velocity of medial gastrocnemius muscle fascicle shortening and Achilles tendon lengthening in males and females for isometric plantar flexion contractions, and to determine whether stretch alters the velocity of these MTU components. We hypothesized that an acute bout of passive static stretching would slow the velocities of each respective component of the MTU. However, irrespective of stretch males would reach a higher overall velocity of the individual MTU components than females, and the muscle fascicles and tendon would not differ in their respective velocities during contraction in males. In females, the velocity of the muscle fascicles shortening would be less than the tendon lengthening resulting in a sex-related differences in the velocity relationship between muscle and tendon that would not be influenced by stretch.

Materials and Methods:

Experimental Protocol

Ten males (24.0 ± 3.0 years, 180.6 ± 7.8 cm, 90.7 ± 17.4 kgs) and 11 females (22.0 ± 3.0 years, 168.7 ± 5.2 cm, 64.8 ± 7.8 kgs) gave informed written consent for the experiment that received ethics approval from the University of British Columbia Behavioural Research Ethics Board. Laying prone with the knees fully extended and the left foot hanging free of the plinth, ultrasound was used to measure the length of the Achilles tendon (AT) from the insertion on the calcaneus to the muscle tendon junction (MTJ) of the Gastrocnemius medialis (GM). After the procurement of this measure, an orthosis was fitted to the foot and the participant was positioned and secured to the device. The customized foot plate was passively rotated to 15° plantar flexion

Running Head: Muscle fascicle and tendon velocity during contraction

for the three to four familiarization maximal voluntary contractions (MVC) performed with electrical stimulation to assess voluntary activation (VA). Electrical stimuli (200 μ s pulse width) was applied to the tibial nerve with the subject at rest, with amperage incremented until a maximal twitch was achieved, and then the intensity was increased 10%. The participant then performed a 5 second MVC of the plantar flexors while electrical stimulation was applied prior to, during, and following the MVC. The subject was instructed to contract as fast and as hard as possible and maintain the same level of contraction throughout the MVC. During the MVC and stretch a camera (Canon Power Shot ELPH 100HS, Canon Canada Inc., Brampton, Ontario, Canada) was used to record heel excursion from the footplate. Video was monitored on-line during the experiment to ensure heel lift did not occur. Immediately after the conclusion of the torque production, the ankle was passively rotated to maximal dorsi flexion at 5°/sec (Kay and Blazevich, 2009) as indicated by the subject's point of discomfort (Kay and Blazevich, 2009). The ankle was maintained in this stretched position for 3-minutes, after which the ankle was rotated back to 15° of plantar flexion at 5°/sec and the MVC procedure repeated with stimulation.

Experimental Set-Up

The chair of the Biodex dynamometer (Biodex Medical Systems, Shirley, New York, USA) was placed in a reclined position for participants to lay prone on a plinth which was fastened to the Biodex chair. Both feet extended beyond the plinth so that approximately 15 cm of the distal portion of the shank was unsupported. The left foot was placed into a soft foot orthosis which was laced up the front and adjusted for foot size. The left foot was placed onto the custom built foot plate with the ankle joint centred to the axis of rotation of the Biodex and the foot secured to the plate using Velcro® (Velcro Companies, Manchester, New Hampshire, USA) straps. A harness was fitted around the participant's waist above the iliac crest and tie down straps were

used to restrict forward movement of the participant during plantar flexion by attaching these straps underneath the plinth and inferior to the hips. Torque was collected from the maximal plantar flexion contraction against a custom built foot plate and converted from analogue to digital format at 496 Hz (16-bit 1401 plus, Cambridge Electronics Design). Carbon stimulation electrodes (4.5 x 4.5 cm) were placed on the patella and inferior to the popliteal fossa crease to stimulate the tibial nerve in order to induce twitches and measure VA of the MVC.

Muscle architectural changes in the GM including fascicle length, pennation angle and tendon elongation were recorded with Bmode Ultrasound using an ML6-15 probe (4.5-15.0MHz linear array, 15 MHz frequency, 13 x 58 mm footprint, 50 mm field of view, 8 cm depth of field, LOGIQView, GE©, Fairfield, Connecticut, USA) positioned into a custom made housing unit which was placed over and secured to the MTJ area of interest. To ensure positioning the MTJ was initially identified with a RSP6-16 3D/4D probe (5.6-18.4 MHz linear array, 38.4 x 44.5 mm footprint, volume 37.4 mm x 29° field of view). This probe allowed for the MTJ to be visualized in three planes through simultaneous capture of longitudinal, cross sectional and frontal position of anatomical structure. As per prior reports (Simpson et al. 2017), the MTJ was defined as the location in which the muscle receded to tendon in all three of these views and this position was indicated through placement of an echo-absorptive marker between the skin surface and the probe to ensure the probe remained in a static position throughout image acquisition (Figure 1). Aquasonic clear ultrasound transmission gel (Parker Laboratories Inc, New Jersey USA) provided acoustic contact between the probe and skin.

[Insert Figure 1 approximately here]

Data Analysis

Data analysis was performed using pre-programmed ultrasound measurement tools (LOGIQView, GE©, Fairfield, Connecticut, USA) and custom scripts designed for Spike 2 (Version 7, Cambridge Electronic Design, Cambridge, England). MVC was measured as the difference between the peak torque and baseline torque for each contraction. The highest VA score and corresponding MVC was used as maximal. Rate of torque development was considered from where the torque tracing deflected from baseline to the peak MVC. Voluntary activation was calculated from the post twitch and interpolated twitch recording (Jakobi et al. 2001).

Muscle fascicle length was measured as close to the MTJ as possible for fascicles that could be clearly seen both at rest and during contraction. If the fascicle met the upper aponeurosis within the frame of the screen, the length was measured as Lf (full length). If the fascicle extended off of the screen trigonometry was used to estimate the remaining length of the fascicle as described previously (Simpson et al. 2016). The estimated fascicle length was added to the measured fascicle length to give a total fascicle length. Curved muscle fascicles were traced with a spline function until the orientation straightened. The curved fascicle length was added to the linear measure to give a total length of the fascicle. The fascicle length was measured at rest, tracked through the contraction, and measured again at MVC. The original fascicle length minus the contracted length was determined as the measure of change in fascicle length. Tendon length changes were measured as the difference between the original position of the MTJ and its end-point during the contraction. The surface marker was measured to ensure consistent probe positioning.

Duration of fascicle shortening was measured from the ultrasound video as the time between the initiation of fascicle contraction to cessation of fascicle movement (Simpson et al.

2016). The amount of fascicle displacement was divided by the duration of fascicle shortening to quantify velocity. Velocity of the fascicle was also normalized to the resting fascicle length. The time differences method gained from the video tracking of fascicles was also applied to the tendon displacement to ascertain tendon velocity (measured in millimeters per second (mmm/s)). All values of tendon and fascicle velocity were normalized to MVC.

Statistical Analysis

A two way time (pre stretch, post stretch) by sex (male, female) repeated measures ANOVA was used to ascertain if RTD, MVC, VA, tendon elongation, tendon and fascicle velocities were altered for males and females after an acute stretch. Male and female fascicle velocities were compared to their respective tendon velocities using a paired sample T-test. Pearson's Correlation Coefficient (r) and multiple regression analyses were conducted to examine the relationship between tendon and fascicle velocity with time (pre stretch, post stretch) in both males and females. All statistical procedures were performed using SPSS (v. 19, Chicago, IL). An α level of $p \leq 0.05$ was used for statistical significance.

Results:

Males ($128.71 \text{ Nm} \pm 7.88$) were stronger than females ($89.92 \text{ Nm} \pm 4.70$) but this sex main effect ($F_{(1,29)}=10.799$, $p=0.003$) was not due to differences in VA ($p>0.05$; males $97.0 \pm 2.1\%$; females $94.8 \pm 3.4\%$). There was no effect of stretching on MVC or VA. Rate of torque development did not differ between sexes ($F_{(1,18)}=3.619$, $p=0.07$; males $52.3 \text{ Nm/s} \pm 19$; females $39.5 \text{ Nm/s} \pm 17$) or change following stretch ($F_{(1,18)}=0.012$, $p=0.92$; pre stretch $45.9 \text{ Nm/s} \pm 19$; post stretch $46.2 \text{ Nm/s} \pm 15$). Fascicle lengths were longer in males ($3.49 \text{ cm} \pm 0.8$) compared to

females ($2.55 \text{ cm} \pm 0.7$) ($p < 0.02$). There was a significant time by sex interaction ($F_{(1,29)} = 4.725$, $p = 0.04$) for tendon elongation as during the MVC the female tendon elongated more after the stretch when compared to pre stretch ($p = 0.02$), while tendon elongation in the males did not differ pre to post stretch during MVC ($p = 0.89$). Males had a higher tendon lengthening velocity ($p = 0.001$) and higher fascicle shortening velocity ($p = 0.01$) during the MVC compared to females (sex main effect $p = 0.002$) (Figure 2). The female tendons lengthened at a significantly faster velocity during contraction than the female fascicles shortened ($p = 0.002$), whereas in males the tendon lengthening and fascicle shortening did not differ ($p = 0.32$) (Figure 2). There was no interaction or main effects of the normalized fascicle velocity; however males (1.34 ± 0.2) had a faster velocity than females (0.4 ± 0.2) ($p = 0.02$).

[Insert Figure 2 approximately here]

Tendon velocity ($10 \text{ mm/s} \pm 4$) was positively and significantly correlated with fascicle velocity ($7.4 \text{ mm/s} \pm 8$, $r^2 = 0.307$, $p = 0.02$, $n = 56$). The multiple linear regression with sex and time as predictors was significant ($p = 0.005$; $r^2 = 0.214$, $F_{(3,56)} = 4.822$). However, time was not independently significant to the prediction ($p = 0.66$), suggesting the addition of an acute stretch does not alter the tendon or fascicle velocity relationship; while sex was significant ($p = 0.008$, males, $y = 0.8626x + 0.3183$; females $y = 0.2549x + 3.0864$) (Figure 3).

[Insert Figure 3 approximately here]

Discussion:

The purpose of this study was to examine the velocity of muscle fascicle shortening and tendon lengthening during the generation of an MVC. The primary findings were that males reached a higher overall velocity in both the fascicle and tendon compared with the females. The independent elements of muscle fascicle and tendon velocity did not differ in males but in

females, the velocity of tendon lengthening was significantly faster than the velocity of muscle fascicle shortening. Regardless of sex, the tendon and fascicle velocity have a positive linear relationship that is not influenced by an acute stretch.

In previous studies involving muscle biopsy males had a greater area of fast twitch muscle fibres than females in the gastrocnemii (Costill et al. 1976; Glenmark et al. 1992) and, utilizing the examination of twitches, male contractile times were faster than females but the muscle was not as resistant to fatigue suggesting muscle in males contains a greater proportion of type II fibres (Hamada et al. 2003; Wust et al. 2008). Results here, which are the first to compare velocity of fascicle shortening resultant to an acute stretch in males and females support contractile property literature (Hunter 2014, Wust et al. 2008), thus it is likely that the sex-related differences in velocity of fascicle shortening is in-part related to fibre types. An additional factor is the architecture differences of size and length of muscle in males. The males in this study had longer muscle fascicles ($3.55 \text{ cm} \pm 0.5$) than females ($2.55 \text{ cm} \pm 0.7$), suggesting the male fascicles were comprised of a greater number of sarcomeres in series and this anatomical difference between males and females can increase the velocity of muscle contraction (Fukunaga et al. 2002, Spector et al. 1980). Further support for this sex-related difference is the normalized velocity between males and females differed. Thus, in accounting for the sarcomeres in series this study provides support for a true sex-related difference in the velocity of shortening. It is unsurprising then, that given the anatomical factors of sarcomeres in series, muscle length, fibre type and absolute strength, that males have a faster velocity of fascicle shortening than females.

Previously, we have reported through the application of video ultrasound, the duration of fascicles shortening is faster in males than females (Simpson et al. 2016). This sex-related difference in the time of shortening was attributed to greater compliance of the connective tissue

in and around the muscle fascicles and tendon of females (Bryant et al. 2007, Heitz et al. 1999, Wojtys et al. 2002). Greater compliance of the connective tissue induces a time delay between force generation of the contractile components of the muscle fascicle and force transfer through the passive components. Previous work has identified this sex-related difference in compliance persists through submaximal and maximal contractions (Onambélé et al. 2007) and this study resulted in the female tendon stretching further before maximal force was reached, thus lengthening the time over which the tendon motion was recorded and decreasing the tendon velocity in females when compared to males. The female tendon excursion was greater post stretch during the MVC as well, but this did not alter the relationship between tendon and fascicle velocity. Yet, what remains interesting is the female tendon velocity was uncoupled from the muscle fascicles in that the tendon reached a significantly higher velocity than the fascicles; an effect not noted in males where the tendon and fascicle velocities were synchronous.

The uncoupling observed in the females could arise from the MVC at 15° of plantar flexion. This ankle position creates slack in the tendon and in the resting muscle fascicles and regardless of sex, the degree of slack in fascicles is non-uniform over a muscle (Herbert et al. 2011). Muscle fascicles, when the tendon is seemingly in series with the aponeurosis, generates force progressively as those with lesser slack are the first to transmit the contractile output (Herbert et al. 2004, 2011). In males, where fascicles shorten at a velocity approximately paralleling the lengthening of the tendon, the net contraction velocity and force is perhaps high because the slack is minimal. However, in females the progressive generation of force from individual fascicles throughout the muscle with a more compliant tendon slowed RTD. However, RTD was unchanged pre and post stretch, and the tendon lengthened with a faster velocity

There is a positive linear relationship between the velocity of the fascicles shortening and the tendon lengthening. Contrary to the hypothesis, this relationship was unaltered by the addition of a static stretch. It was anticipated that the stretch would increase the tendon compliance and therefore slow the lengthening velocity of the tendon for both sexes, augmenting the relationship between tendon and fascicle velocity, but this was not evident. We have recently shown that with progressive overload of a static stretch fascicle length increases (Simpson et al. 2017) but the intensity of the stretch is seemingly paramount when utilizing this modality to alter muscle architecture and connective tissue compliance. Potentially, the stretch tolerance limit was insufficiently high or the duration of stretch was too short to alter compliance and in-turn influence the velocity of contraction. Previous work involving acute stretch failed to alter tendon properties (Morse et al. 2008) and instead suggested that connective tissue elements such as compliance of the aponeuroses and the perimysium were the malleable elements of the MTU. However, these elements either require greater stretch, longer duration or do not influence the velocity of the individual components of the MTU. Along with the tendon velocity and fascicle velocity relationship, MVC and RTD were not influenced by static stretch. Power et al. (2004) reported non-significant changes in MVC of the plantar flexors after static stretching that were attributed to the intensity of the stretch protocol and fibre characteristics of the gastrocnemius. Moreover, Wilson et al. (1994) suggested that a more compliant MTU is inversely related to isometric contraction performance and RTD. The non-significant changes in MVC and RTD post stretching are further evidence that stretching of sufficient intensity is necessary to facilitate altering compliance and in-turn RTD and MVC.

During maximal isometric contraction at 15° plantar flexion the velocity of Achilles tendon lengthening and MG fascicles shortening is more rapid in males than females. In males,

Running Head: Muscle fascicle and tendon velocity during contraction

the tendon and muscle velocities correspond while in females the Achilles tendon lengthening velocity was significantly faster than the fascicle shortening velocity. This uncoupling of components of the MTU is likely due to compliance of the tendon and compensation within the fascicles to remain within an optimal velocity range for producing maximal force. The addition of an acute and self-regulated static stretch does not alter tendon and fascicle velocities for either sex.

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Conflicts of Interest:

The authors report no conflicts of interest associated with this manuscript.

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Figure Captions:

Figure 1: Representation of muscle fascicles at the muscle tendon junction at rest (a) and during contraction (b). The muscle tendon junction was identified using an RSP6-16 3D/4D probe with a 5.6-18.4 MHz linear array, 38.4 x 44.5 mm footprint, volume 37.4 mm x 29° field of view, and data was collected with an ML6-15 probe 4.5-15.0MHz linear array, 15 Hz frequency, 13 x 58 mm footprint, 50 mm field of view, 8 cm depth of field.

Figure 2: Fascicle and tendon velocity for males and females. Significant difference between male and female tendon velocity and fascicle velocity ($\dagger$). *, significant difference between fascicle and tendon velocity in females. Values are mean $\pm$ SE, $p < 0.05$

Figure 3: Fascicle and tendon velocity relationship pre and post stretching for males and females. The multiple linear regression for males ($y = 0.8626x + 0.3183$) and females ($y = 0.2549x + 3.0864$). Male data is the open circle and dashed line, female is the solid circle and line.

Representation of muscle fascicles at the muscle tendon junction at rest (a) and during contraction (b). The muscle tendon junction was identified using an RSP6-16 3D/4D probe with a 5.6-18.4 MHz linear array, 38.4 x 44.5 mm footprint, volume 37.4 mm x 29° field of view, and data was collected with an ML6-15 probe 4.5-15.0MHz linear array, 15 Hz frequency, 13 x 58 mm footprint, 50 mm field of view, 8 cm depth of field.

108x60mm (300 x 300 DPI)

Fascicle and tendon velocity for males and females. Significant difference between male and female tendon velocity and fascicle velocity (†). *, significant difference between fascicle and tendon velocity in females. Values are mean $\pm$ SE, $p < 0.05$

108x60mm (300 x 300 DPI)

Fascicle and tendon velocity relationship pre and post stretching for males and females. The multiple linear regression for males ($y = 0.87x + 0.32$) and females ($y = 0.26x + 3.09$). Male data is the open circle and dashed line, female is the solid circle and line.

108x60mm (300 x 300 DPI)