EFFECTS OF SEX AND FATIGUE ON ULTRASOUND-BASED MEASURES OF TENDON STIFFNESS

by

DANIEL SHLESINGER

A THESIS

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Approved: *Damien Callahan, Ph.D.*
Primary Thesis Advisor

Tendons transmit skeletal muscle force to produce joint torque. Insight into tendon stiffness could help better understand the limits of musculotendinous performance and may help predict the risk of soft tissue injuries in response to extreme load. Clinical ultrasound imaging, both traditional B-mode and more recently, shear wave elastography (SWE) have been used to assess muscle and tendon stiffness. Recent studies using SWE have illustrated that acute fatigue can decrease stiffness in skeletal muscle. Others have demonstrated the effects biological sex and age on tendon stiffness using multiple ultrasound-based approaches. However, no studies have used SWE to assess the effect of acute fatigue on tendon stiffness, nor have any studies compared these measures with those produced using B-mode ultrasound. While SWE has shown promise in assessing muscle stiffness, we aimed to evaluate its correlation with more traditional modes of assessing tendon stiffness. **Purpose**: the purpose of the study was to investigate the effects of fatigue and sex on tendon stiffness measured by two ultrasound-based techniques: B-mode and SWE (SWE). **Methods**: In this study, young healthy males (n=15) and females (n=21) were recruited. Subjects performed 3 maximum voluntary isometric contractions (MVIC) of the knee extensors to evaluate maximal torque and rate of torque development (RTD) using an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). During ramped isometric contractions a linear array ultrasound transducer was used to measure elongation of the patellar tendon (PT). In a subset of participants (n=5) Shear wave velocity (SWV) measurements were observed during passive measurements. Participants were then instructed to fatigue their muscles by performing a bout of repeated maximum voluntary knee extensions with isotonic load set to 30% MVIC until task failure (inability to complete more than ~50% range of motion or maintain pace of contractions). **Results**: As measured by B-mode ultrasound, fatigue did not reduce active stiffness in the PT ($p = .101$) or have a fatigue by sex interaction ($p = .075$). Active and passive SWV was not reduced by fatigue ($p = .157$). **Conclusion**: Our data suggest that a dynamic fatiguing exercise of the knee extensors does not alter PT stiffness under active and passive conditions. While B-mode ultrasound demonstrated an increase in stiffness relative to the %MVIC, SWE showed no difference in stiffness as %MVIC increased. Both modalities illustrated an unaltered stiffness response to acute fatigue, suggesting a degree of consistency between both ultrasound methods.

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Background

Tendons transmit skeletal muscle force to produce joint torque. Insight into tendon stiffness could help better understand the limits of musculotendinous performance and may help predict the risk of soft tissue injuries. The common perception of muscular stiffness might bring to mind a tight feeling within muscles, especially after a hard workout. However, in the context of this study, stiffness refers to the relationship between force and muscle deformation (Maciejewska-Skrendo et al., 2020). More specifically, stiffness is the ability of tissue to resist deformation under a constant load. Rate of torque development (RTD) constitutes a crucial metric, representing the slope of the torque-time curve observed during an isometric contraction. Research indicates a strong correlation between RTD and fall occurrences among older adults (Kamo et al., 2019). Our study investigates two distinct stiffness measurements: active and passive. Active stiffness pertains to movement that involves muscle contraction, while passive stiffness involves the state of muscles at rest, without any contraction. The details of musculotendinous properties are key to understanding tendon function and mechanics. When a muscle contracts, it shortens, exerting a pulling force on the tendons, which in turn moves the bones. Tendons act as efficient levers facilitating bone movement as muscle contract and relax. Variations in tendon length can significantly impact force production, thereby potentially influencing physical performance (*Tendon*, n.d.). Notably, increased stiffness within muscle tissue has been correlated with an elevated risk of muscle strain and tendon tears (Ekstrand & Gillquist, 1983). Conversely, diminished muscle strength and tendon stiffness have been linked to compromised balance recovery and joint stability, particularly in older adults (Karamanidis & Arampatzis, 2007). Hence, stiffness emerges as a crucial parameter in the assessment of musculotendinous pathophysiology.

Tendons, composed of connective tissue rich in resilient collagen fibers, exhibit high resistance to tearing but limited elasticity. Moreover, they are comparatively less vascularized than muscles, making them more susceptible to injury when subjected to excessive strain and prolonging the healing process (“In Brief,” 2022). Tendons can be envisioned as connectors allowing for the transmission of muscle movement to bone. The patellar tendon (PT) acts as a crucial link between the patella and the tibia, facilitating knee extension movements. Its involvement in activities like running, kicking, and jumping, compounded by the engagement of the quadriceps muscles, significantly impacts its stiffness. This is because a stiffer tendon can influence the timing between muscle activation and force production. Any delay in transmitting rapidly generated forces could have repercussions for balance and stability. This delay could potentially slow reaction times and increase the risk of falls (Khambalia et al., 2006; Pijnappels et al., 2005; Waugh et al., 2014)

The assessment of tendon stiffness heavily relies on ultrasound technology, due to its non-invasive nature and capacity to deliver precise visualizations. B-mode ultrasound employs soundwaves outside of human hearing to generate 2D images, discerning the tissue density variations under the probe (Carovac et al., 2011). While this non-invasive imaging technique boasts various clinical applications, it serves to visually monitor tissue displacement during active muscle contractions. Simultaneously, understanding tissue load and displacement enables the quantification of tissue stiffness. While traditional B-mode sonography remains significant, recent advancements, such as shear-wave elastography (SWE), may significantly increase diagnostic capabilities. SWE, a technique within ultrasound elastography, can measure tissue stiffness without necessitating the determination of applied stress.

By utilizing shear waves that traverse perpendicular to the tissue, SWE captures particle motion within, allowing assessment of abnormal stiffness levels (Taljanovic et al., 2017).

Introduction

The composition and mechanical properties of tendons play a significant role in transmitting force between muscle to bone, a process essential for movement. Over time, tendons have undergone changes in response to various factors such as energy storage and mechanical advantage, shaping their structure and function. (LaCroix et al., 2013). These adaptations have altered the structure and function of tendons. They have shaped them into strong and flexible connectors capable of facilitating precise and coordinated movements. Recent studies have suggested that despite the significant variation in mechanical properties observed both between different species and within individuals of the same species, there exists a strong correlation between the measure of stiffness and the maximum stress a material can withstand before failure (LaCroix et al., 2013). This suggests that failure within tendons is largely dependent on the amount of strain they undergo, with tendons becoming more prone to failure as they are subjected to higher levels of strain. Moreover, the influence of external factors such as chronic training has been recognized as stimuli capable of modulating stiffness properties (Dankel & Razzano, 2020; Finnamore et al., 2019).

Another area of focus is the impact of acute fatigue on tendon stiffness, particularly in the PT. While previous studies have found that acute fatigue reduces passive stiffness in muscle fibers (Privett et al., 2022), no study that we are aware of has looked at acute fatigue on the PT. Remarkably, despite men being more likely to injure the PT, no studies have specifically investigated sex-based disparities in this context (Hsu & Siwiec, 2024). A relationship exists between muscle stiffness, gender differences, and the influence of circulating hormones (Bell et al., 2012). It has been noted that men typically exhibit higher muscle stiffness compared to women (Maciejewska-Skrendo et al., 2020). There remains a gap in understanding regarding PT stiffness when comparing both biological sex and acute fatigue.

While previous studies have explored how maximal muscle fatigue can alter muscle stiffness, none have yet used SWE to examine the impact of acute fatigue on tendon stiffness. With these gaps in knowledge in mind, this study aimed to look at the effects of both fatigue and sex on tendon stiffness, employing advanced ultrasound-based techniques such as B-mode and newer SWE. By examining these factors, we aim to gain a greater understanding of tendon mechanics and contribute to research pertaining to preventing and managing tendon injuries.

Methods

For this study, we recruited 26 young healthy males (n=12) and females (n=14) to participate. Passive and active musculotendinous stiffness and composition of the patellar tendon (PT) were evaluated.

Volunteers

To ensure the accurate classification of each individual subject, all volunteers underwent a health screening interview prior to being integrated into the study. The screening included information regarding medical history, orthopedic limitation, endocrine disease, myopathies, dietary/smoking habits, as well as their history of resistance training. If a volunteer exhibited any impairment that resulted in the inability to perform the tasks of this study, they were excluded.

Data Collection

All participants visited the lab on multiple occasions and all measurements took place on the Biodex System 3 Dynamometer (Biodex Medical Systems, Shirley, NY). Participants were seated with their dominant leg flexed at 90 degrees to allow an extension of 180 degrees. Mechanical characteristics of the knee extensor were assessed during passive and active conditions. In addition, muscle composition was evaluated specifically during the passive conditions of this study.

Muscle Function

Torque data was extracted through a MATLAB script designated to calculate peak torque and rate of torque development (RTD) during maximum voluntary isometric contractions

(MVICs). Participants were instructed to kick as hard and fast as they can for 3-5 seconds. Their performance was continuously monitored using a real time display of force production, viewed on a television monitor positioned in front of the volunteer. Subsequently, raw torque data was put through MATLAB, allowing for the analysis of peak torque and RTD. Peak torque was determined by averaging the results of three MVIC trials, while peak RTD was computed by identifying the maximum instantaneous rate of torque change over time. Additionally, RTD values were averaged across the three MVIC trials. The rate of torque development refers to the maximal change in torque per unit of time at the onset of muscle contraction.

B-mode Ultrasound Measurements

Participants performed 3 MVICs of the knee extensors to evaluate maximal torque and RTD using an isokinetic dynamometer (Biodex Medical Systems, Shirley, NY). During ramped isometric contractions of 25%, 50% and 100% MVIC, a linear array ultrasound transducer was used to measure elongation of the PT under load. Participants were then instructed to fatigue their muscles by performing a bout of repeated maximum voluntary knee extensions with isotonic load set to 30% MVIC until task failure (inability to complete more than ~50% range of motion or maintain the pace of contractions).

SWE Measurements

In a subset of participants (n=5) shear wave velocity (SWV) measurements were observed during passive and active measurements via SWE, providing insights into the mechanical properties of the tendon under passive conditions. SWV measurements were uniformly obtained from the center of the PT for all subjects, ensuring a standardized and consistent collection of stiffness values across the board.

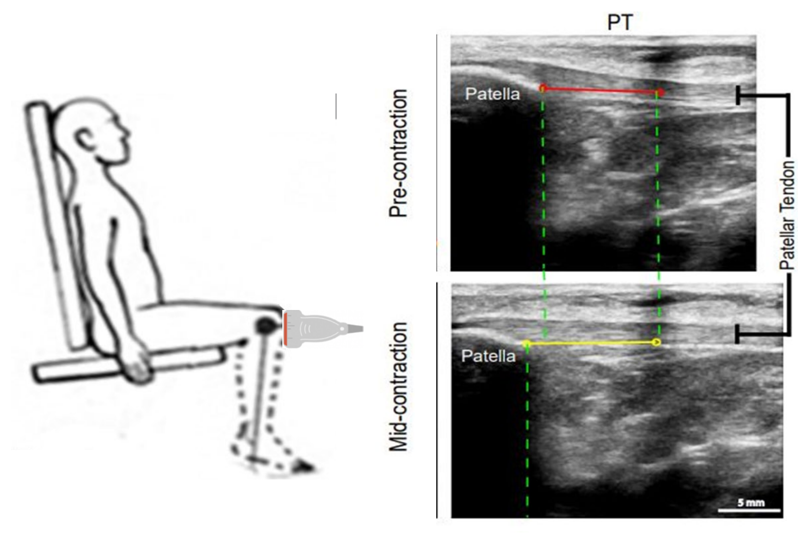

Figure 1. Image portraying the position of the volunteer and the grayscale image generated from ultrasound during MVIC.

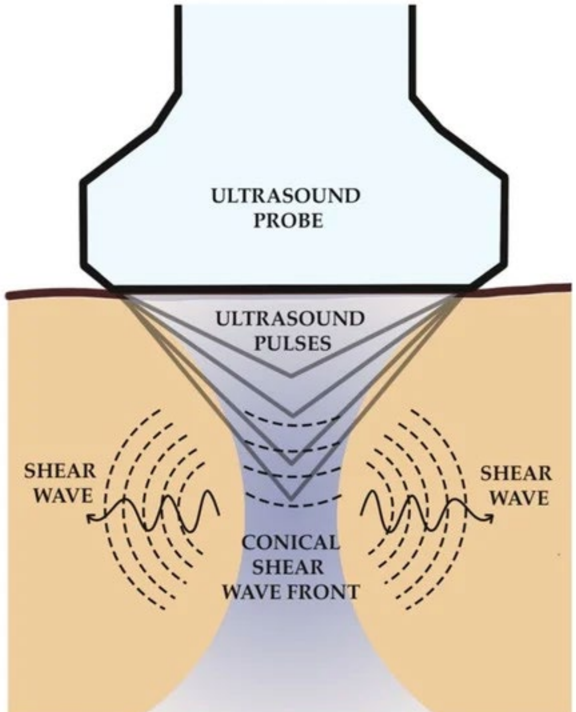

Figure 2. Image (Chimoriya et al., 2021) portraying shear waves produced from ultrasound waves propagating from the ultrasound probe.

Tissue Tracking

Using UltraTrack software, tendon elongation was analyzed by tracking the tendon frame by frame within dynamic images. To obtain these measurements, a pre-contraction segment was delineated on the ultrasound image. One end point was anchored at the distal edge of the surgical tape, while the second endpoint was positioned at the distal edge of the patella before any contraction occurred. The elongation of the PT was subsequently measured by subtracting the length of the pre-contraction segment from that measured during mid-contraction.

All data obtained from UltraTrack video analysis was exported for further analysis using a customized MATLAB program designed to take displacement measurements during the change from passive to active contractions. To find the force generated, the torque was divided by the length of the PT moment arm. Previous studies have illustrated the length of the PT moment arm to be 44.7mm. The formula for stiffness could then be calculated as follows:

$$Stiffness = \frac{\Delta Force\ (N)}{\Delta Tissue\ deformation\ (mm)}$$

Statistics

A two-way repeated measures ANOVA was used to assess differences in tendon stiffness by fatigue and sex across all contraction intensities. Where interactions or main effects were observed, comparisons between factors were made using paired T-Tests. To examine differences in anthropometric and non-repeated measures between sexes, additional analysis was conducted using t-tests.

Results

Subjects

Men and women had similar age, height, and body mass, but women had a higher relative RTD. These biometrics and other relevant information can be found in Table 1.

Variables	Men (n = 15)	Women (n= 21)	p-value
Age	21	21.4	0.55
Height (cm)	182.7	162.6	<.01
Weight (kg)	82.2	62.5	<.01
BMI (kg/m^2)	24.6	23.8	0.52
Muscle Thickness (cm)	2.5	2.1	<.01
Peak Power (W)	565.8	330.1	<.01
MVIC (N*m)	304.5	176.2	<.01
Torque rel (N*m/kg)	3.7	2.6	<.01
RTD abs (N*m/kg)	1873.4	1091.5	<.01
RTD rel (MVIC/s)	6.1	6.3	0.94

Table 1. Male and female participants characteristics for B-mode ultrasound data.

Fatigue

During the sequential isometric contractions, active and passive stiffness measurements were taken at the PT post-fatigue. Our measurements revealed no variance in stiffness values post-fatigue when assessed via B-mode ultrasound ($p = .101$). Additionally, we found no association between fatigue and sex ($p = .075$). However, as force increased, we observed a corresponding increase in tendon elongation suggesting a direct relationship between larger contractions and a heightened elongation in the PT ($p < .001$). Evaluation of PT stiffness via SWE indicated that fatigue did not influence stiffness measurements ($p = .157$). Upon further analysis by isolating males and females, fatigue did not display a significant effect for either group ($p= .053$ for males and $p= .331$ for females).

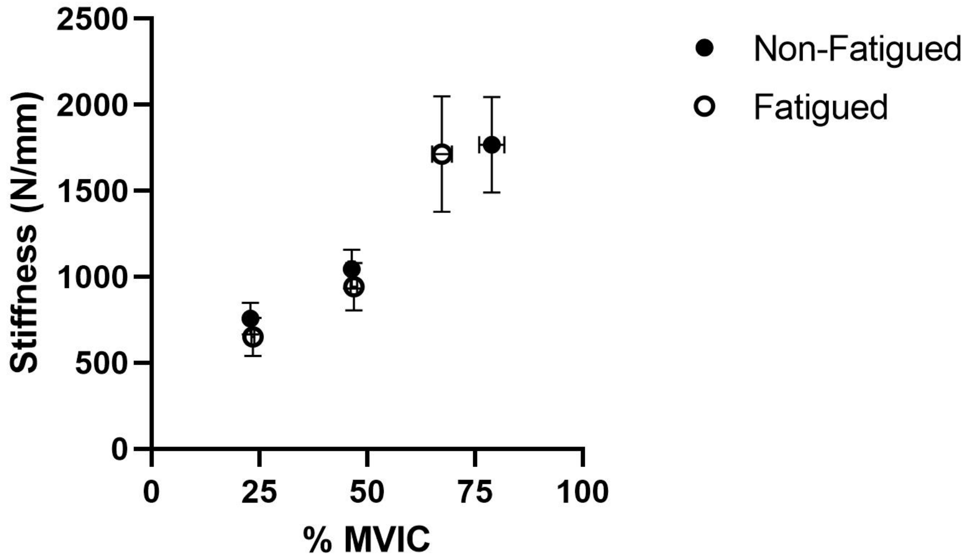

Figure 3. Visual representations portraying stiffness values (N/mm) before and after fatigue generated for both males and females via B-mode ultrasound. No significance was found between pre and post fatigue.

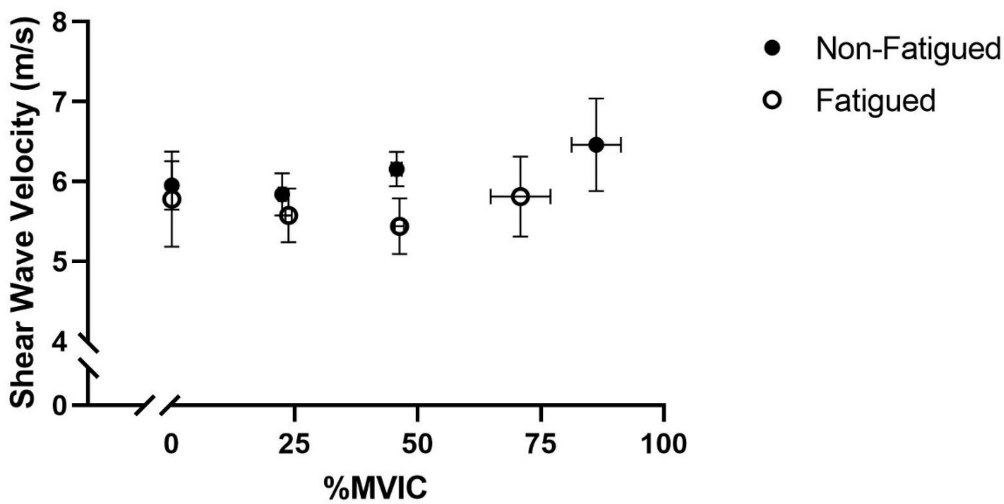

Figure 4. Visual representations portraying stiffness values (m/s) before and after fatigue generated for both male females via SWE. No significance was found between pre and post fatigue.

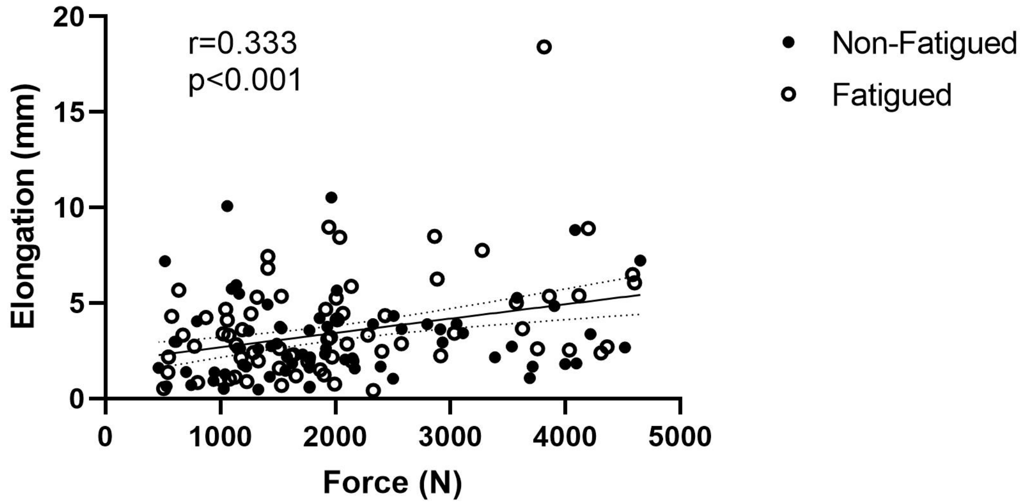

Figure 5. Elongation of the PT as force (N) increases comparing both non-fatigue to fatigue, measured from B-mode ultrasound. Significance was found between force (N) and PT elongation.

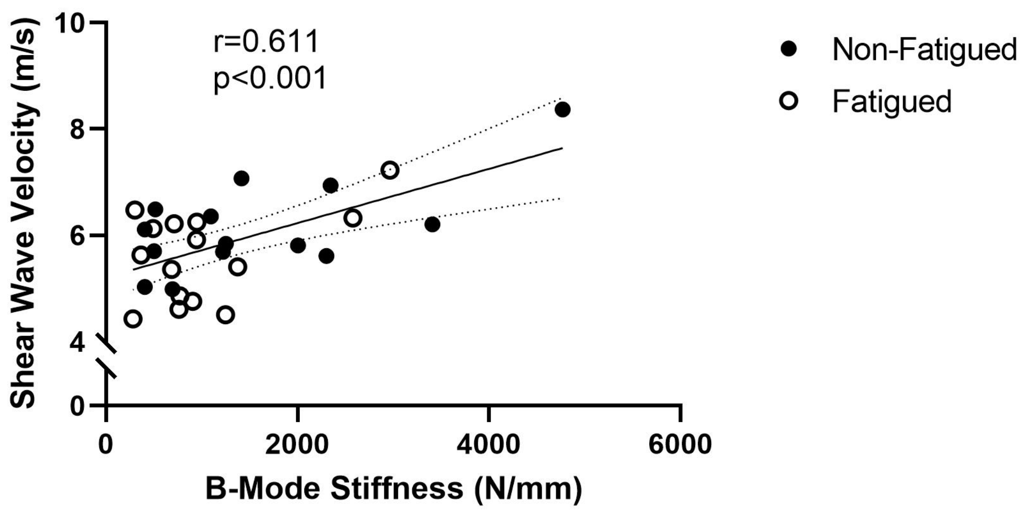

Figure 6. Visual representations comparing the B-mode ultrasound stiffness values to the that of SWE. Significance was found between ultrasound modalities ($p<.001$)

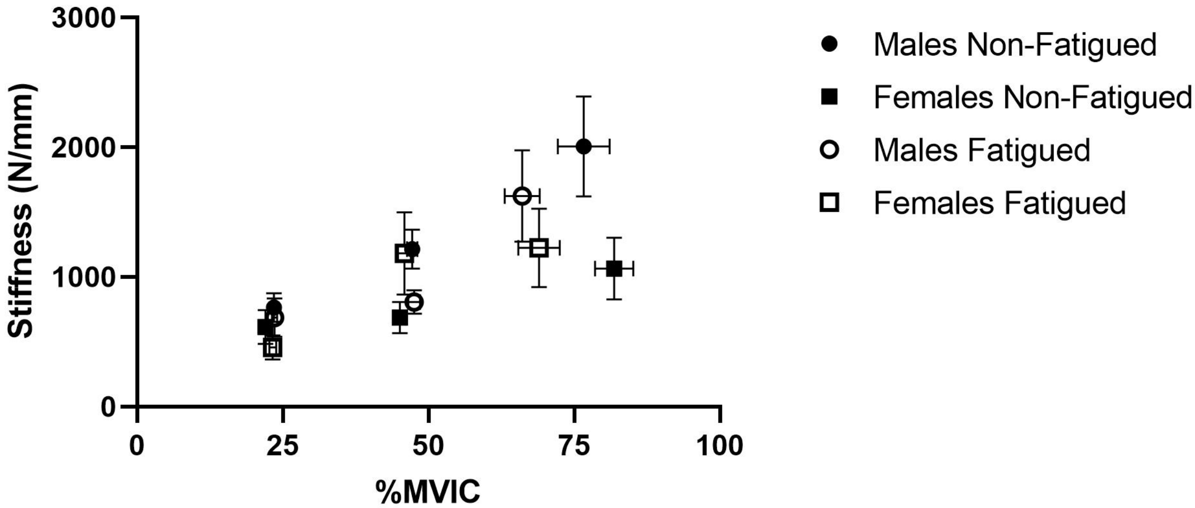

Figure 7. Effects of fatigue on sex on active PT stiffness measured via B-mode ultrasound. No significance was found between sexes pre and post fatigue.

Sex Based Differences

When using B-mode ultrasound to compare sexes, we observed no discernible effect of sex on tendon stiffness ($p = .08$). Moreover, stiffness illustrated no significance between sexes when measured via SWE ($p = .216$). A sex by fatigue response was noted at the 50% MVIC intensity level such that males demonstrated reduced PT stiffness with fatigue, but females had no change ($p < 0.05$)

Discussion

Understanding musculotendinous properties is crucial for assessing the risk of soft tissue injury. This study aimed to investigate how fatigue and sex influence tendon stiffness using two ultrasonographic techniques. Our findings revealed no significant difference in stiffness between men and women, nor did fatigue appear to effect stiffness levels. The subsequent participants who underwent SWV measurements numbered only five. Future studies would benefit from a larger participant pool to draw more definitive conclusions. With that being said, our analysis validated the utility of SWE in predicting muscle stiffness compared to traditional B-mode imaging. Detecting alterations in musculotendinous structure holds immense value not only in research environments but also in clinical practice, where tendinopathies can lead to pain, strength loss, and other debilitating effects (Xu & Murrell, 2008). Our study reveals notable findings not only between men and women but also in response to acute fatigue. Additionally, it suggests a degree of reliability of SWE when compared to traditional B-mode ultrasound for assessing tendon properties.

Results in this study indicate that fatigue did not exert an effect on PT stiffness, which is not congruent with existing literature (Breda et al., 2022). This group noted a reduction in PT stiffness among athletes undergoing progressive tendon-loading exercises therapy over 12 weeks (about 3 months) and observed no change from eccentric training. Additionally, a separate study examining professional athletes reported an average PT stiffness of 3.165m/s (Römer et al., 2023), notably lower than our pre-fatigue passive stiffness mean of 5.95 m/s. The athletes observed in that study ranged from various sports such as handball, volleyball, soccer, hammer throwing, and sprinting. It is intriguing to note that our sample population exhibited a higher stiffness than these professional athletes, underscoring the potential influence of various factors such as exercise regimen and athlete specialization.

In a 2005 study, researchers observed that females were found to be 2 to 9.7 times more likely to injure their anterior cruciate ligament (ACL) than their male counterparts. Moreover, they observed women to have lower levels of active muscle stiffness compared to men (Padua et al., 2005). Evaluating the stiffness of lower extremities, specifically that of the knee joint, is crucial to better understanding the risk of soft tissue injuries between sexes. Despite previous studies suggesting that hormones like estrogen and testosterone might influence tendon stiffness (Hansen & Kjaer, 2016), no significant differences in stiffness values were observed between sexes. Future research could consider exploring the separate effects of testosterone and estrogen levels in males and females, potentially uncovering significant variations in stiffness values.

As emphasized earlier, stiffness serves as an important indicator in assessing the risk of soft-tissue injuries, with stiffer tendons being associated with a higher likelihood of tendon failure (LaCroix et al., 2013). SWE offers distinct advantages in this regard, providing a real time and direct measurement of tissue, such as muscle to help with the diagnosis of acute musculoskeletal injuries (Brandenburg et al., 2014). While biopsies offer detailed insights into the microscopic structure of tissue, they fall short in characterizing the mechanical properties of muscles and are invasive and time consuming. SWE utilizes ultrasound technology to precisely measure mechanical properties and effectively distinguish between normal and abnormal tissue properties. As a result, SWE holds promise as a clinical tool for diagnosing tissue abnormalities with previous studies suggesting it is useful for various diagnoses (Cai et al., 2023; Youk et al., 2017). In this study, we confirmed that SWE can prove to be accurate in assessing stiffness when compared to B-mode ultrasound. However, SWE did not illustrate an increase in stiffness as

%MVIC increased; conversely B-mode ultrasound did. Although previous literature states that muscle stiffness increases with force (Rassier & Herzog, 2005), we did not observe that in tendon measurements obtained via SWE. While SWE can be an important tool for identifying soft tissue abnormalities (Zhang et al., 2015), there is a pressing need for future studies to establish a standardized protocol for measuring stiffness values consistently at specific anatomical locations.

When comparing sexes at 25%, 50%, and 100% MVIC we observed no significant difference in stiffness post fatigue between men and women at 25% and 100% MVIC. However, there is a notable distinction at the 50% contraction level: men exhibit a decrease in stiffness post-fatigue, whereas women show no change in stiffness. There may be underlying factors within the knee capsule that cause variations in stiffness levels between men and women. Future research could focus on this 50% contraction level, employing a larger sample size to better understand the differences in post-fatigue stiffness between men and women.

To gain a greater appreciation for the mechanical properties inherent in musculotendinous structures, the stiffness and composition data gained from this study can help future studies. By comparing the dataset with existing literature on sex-based stiffness differences, we can deepen our understanding of soft tissue injuries. Furthermore, the findings of this study, coupled with the comparison between traditional B-mode ultrasound and SWE offer a starting point for deeper investigations into the validity and capabilities of these imaging modalities in accurately assessing stiffness.

Conclusion

Overall, this study investigated the mechanical properties of the PT, utilizing advanced ultrasound techniques. By assessing PT stiffness under acute fatigue across sexes, we aimed to shed light on its dynamic response to physiological stressors. Our findings revealed consistency in tendon stiffness between sexes as both males and females exhibited similar responses to acute fatigue. Moreover, the comparison between B-mode ultrasound and SWE yielded intriguing results. While B-mode ultrasound demonstrated an increase in stiffness relative to the %MVIC, SWE showed no difference in stiffness as %MVIC increased. While collectively, both modalities illustrated an unaltered stiffness response to acute fatigue, highlighting the reliability between both ultrasound methods. However, when isolating individual %MVICs, there is a notable difference at the 50% contraction level: men exhibit a decrease in stiffness post-fatigue, whereas women show no significant change. From these observations, one can extrapolate that the musculotendinous properties are multifaceted and rely on various factors such as sex, age, and training status. Moving forward, future research endeavors could consider additional variables, like estrogen and testosterone levels. Moreover, refining protocols for stiffness measurements via SWE would enhance the consistency and reliability of our findings. By further researching these complexities we can gain a greater understanding of musculotendinous health and performance optimization.

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