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# Sex differences in active tibialis anterior stiffness evaluated using supersonic shear imaging

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## ABSTRACT

This study aimed to evaluate the sex difference in active muscle stiffness of the tibialis anterior muscle (TA) through shear modulus measurements performed using supersonic shear imaging (SSI) technique. Twenty-five women and twenty-one men participated in this study. Joint torque, electromyographic (EMG) activity and shear modulus were measured during two sets of submaximal dorsiflexions performed at 20, 30, 40, 50 and 60% of maximal voluntary contraction (MVC) in a random order. The first set was devoted to the EMG recordings and the second set was devoted to the elastographic measurements. For each set, subjects performed three 5-s trials at each level of submaximal voluntary contraction. Stiffness indexes were calculated as the slopes of the linear regressions established between shear modulus and joint torque ( $SI_{TORQUE}$ ) or estimated TA EMG levels ( $SI_{EMG}$ ). In the present study, no sex effect was reported for  $SI_{TORQUE}$ ,  $SI_{EMG}$  ( $p=0.76$  and  $p=0.86$ , respectively), and shear modulus measured at various contraction levels. The results highlight that men and women presented similar TA active stiffness indexes determined using SSI. Regardless of sex, this result suggests similar intrinsic stiffness for the contracting TA.

## 1. Introduction

Muscle-tendon complex mechanical properties are commonly described through the use of a model including a contractile component and two elastic components (parallel elastic and series elastic components) (Hill, 1938; Shorten, 1987; Zajac, 1989). Parallel elastic component is classically characterized by its passive stiffness measured during slow stretching of a relaxed muscle (Gajdosik, 2001; Morse, 2011). Conversely, active stiffness (i.e. stiffness of the series elastic component) is assessed under active conditions. Active stiffness is thought to play a major role during stretch-shortening cycles by influencing the storage-restitution of elastic energy and muscle tension transmission (Bosco et al., 1982; Komi, 1984). Hence, active stiffness may be considered as one of the potential

biomechanical parameters involved in sex differences in performance and risk of injury (Butler et al., 2003). High active stiffness may be an advantage when great rates of force development are needed (Foure et al., 2011) while it conversely may be a factor involved in injury by inducing a lack of energy absorption with loading (McNair and Stanley, 1996).

Classical experimental techniques (e.g. quick-release, sinusoidal perturbations, short range stiffness) have accordingly been previously used to report greater active stiffness in men compared to women (Blackburn et al., 2006; Blackburn et al., 2004; Foure et al., 2012; Wang et al., 2015). However, since these techniques assess the global mechanical properties of the musculo-articular complex, they do not distinguish the contribution of the various structures involved (i.e. muscles, tendon, joint). By applying the alpha method to the short range stiffness technique, Foure et al. (2012) reported higher tendon stiffness in men than women, and lower muscle active stiffness for men. The authors partly explained the latter finding by sex-related geometrical differences (i.e. cross-sectional area) (Foure et al., 2012). Alternatively, differences in intrinsic muscle properties may also be expected. Intrinsic muscle stiffness can be assessed thanks to elastographic methods by calculating the shear modulus from the speed of shear waves generated within tissue (Drakonaki et al., 2012). Since active stiffness

*Abbreviations:* EMG, Electromyographic;  $EMG_{MAX}$ , Maximal electromyographic activity; MVC, Maximal voluntary contraction; RMS, Root mean square;  $SI_{EMG}$ , Slope of the curve between TA shear modulus and EMG activity;  $SI_{TORQUE}$ , Slope of the curve between TA shear modulus and normalized torque; SSI, Supersonic shear wave imaging; TA, Tibialis anterior muscle

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at the muscle fiber level is directly related to the number of attached cross bridges (Ford et al., 1981), the first use of magnetic resonance elastography allowed to demonstrate increased tibialis anterior (TA) shear modulus during dorsiflexion contraction (Basford et al., 2002; Heers et al., 2003; Jenkyn et al., 2003). Using supersonic shear imaging (SSI), an ultrasound-based technique (Bercoff et al., 2004), biceps brachii muscle shear modulus was reported to be strongly related to individual muscle force or EMG activity level (Lapole et al., 2015; Nordez and Hug, 2010; Yoshitake et al., 2013). Hence, we previously proposed to calculate an index of muscle active stiffness as the slope of the relationship between muscle shear modulus and force or EMG activity level (Lapole et al., 2015), in a similar way than what is commonly performed with quick-release or sinusoidal perturbations (i.e. slope of the linear relationship between angular stiffness and joint torque (Grosset et al., 2010; Lambertz et al., 2001; Lapole and Perot, 2011)). This provides the advantage to obtain a normalized stiffness index, independent of the demanded muscle contraction (Lambertz et al., 2001).

Therefore, the aim of the present study was to investigate sex differences in active muscle stiffness of the TA using SSI. It was hypothesized that muscle stiffness of women would be lower compared to men.

## 2. Methods

### 2.1. Subjects

Twenty-five women (age:  $19 \pm 2$  years; height:  $164 \pm 7$  cm; body mass:  $53 \pm 8$  kg) and twenty-one men (age:  $19 \pm 1$  years; height:  $180 \pm 8$  cm; body mass:  $72 \pm 13$  kg) participated in this study. Written informed consent was obtained from all subjects prior to their participation and this study conformed to the standards from latest revision of the *Declaration of Helsinki* and was approved by the local ethics committee.

### 2.2. Elastography

Muscle shear modulus was measured using an AixPplorer ultrasonic scanner (version 6.1.1, Supersonic Imagine, Aix en Provence, France), coupled with a linear transducer array (4–15 MHz, SuperLinear 15–4, Vermon, Tours, France). The scanner was used in the general musculo-skeletal preset of the SSI mode (MSK preset). Briefly, the velocity ( $V_s$ ) of a shear wave, induced by a remote radiation force, is measured along the principal axis of the probe. The shear modulus ( $\mu$ ) was calculated using  $V_s$  as follows (Gennisson et al., 2003; Gennisson et al., 2005):

$$\mu = \rho \cdot V_s^2$$

with  $\rho$  the muscle mass density ( $1000 \text{ kg/m}^3$ ).

The ultrasound probe was placed on the tibialis anterior (TA) belly, centered at 40% of the length from the popliteal crease to the center of the lateral malleolus (Sasaki et al., 2014). The probe was carefully aligned with the shortening direction of the muscle, and perpendicular to the skin. The location of the probe was marked on the skin to allow the same placement across trials. Depth of the measurement was set to 3 cm to visualize both superficial and deep TA aponeurosis. Maps of the shear modulus (Fig. 1) were obtained at 1 Hz within a  $1 \times 1$  cm square and with a spatial resolution of  $1 \times 1$  mm. They were optimized with the penetration option, persistence removed, and no smoothing.

### 2.3. Torque and electromyographic recordings

Dorsiflexion torque was measured during voluntary contractions by a calibrated instrumented pedal (CS1060 300 Nm, FGP Sensors, Les Clayes Sous Bois, France). Subjects were seated upright in a custom-built chair with pelvis, knee and ankle angulations of 90, 120 and 90°, respectively. Electromyographic activity (EMG) of the right TA was recorded with pairs of self-adhesive surface electrodes in bipolar configuration. Signals were analog-to-digital converted at a sampling rate of 2000 Hz by PowerLab system (16/30–ML880/P, ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138, ADInstruments; common mode rejection ratio=85 dB, gain=500) with bandpass filter (5–500 Hz) and analyzed offline using Labchart 7 software (ADInstruments).

### 2.4. Protocol

Subjects first performed three 3-s maximal isometric voluntary dorsiflexion (1-min rest between contractions) to determine both maximal voluntary contraction (MVC) and TA maximal EMG activity. Since optimal locations of the ultrasound probe and surface electrodes were similar on the TA muscle belly, EMG and elastographic measurements were performed separately. Hence, two sets of sub-maximal dorsiflexion were performed. The first set was devoted to the EMG recordings. Subjects maintained 20, 30, 40, 50 and 60% of MVC for 5 s in random order (30-s interval between contractions). EMG electrodes were then removed and the second set was devoted to the elastographic measurements, after a 5-min resting period. Subjects performed three 5-s trials at each of 20, 30, 40, 50 and 60% MVC in a random order (30-s and 60-s intervals between trials and torque level, respectively). Torque and shear modulus were synchronously recorded for each trial once the torque and map of stiffness were stable.

### 2.5. Data analysis

Maximum voluntary isometric torque in dorsiflexion was considered as the highest MVC performed. TA maximal EMG activity ( $EMG_{MAX}$ ) was calculated as the EMG root mean square (RMS) calculated over a 500-ms interval centered at maximal torque.

For each submaximal contraction, shear modulus was calculated using the Aixplorer scanner software (Q-Box™). The selected circular area (approximately 1 cm in diameter) was centered on the TA shear modulus map so as to cover the greatest muscular region and avoiding aponeurosis (Fig. 1). Mean dorsiflexion torque was averaged over a 1-s period before acquisition of the shear modulus. For each subject, a linear regression analysis was then performed between TA shear modulus and dorsiflexion torque expressed as a percentage of MVC (Fig. 2), and a stiffness index taking into joint torque ( $SI_{TORQUE}$ ) was calculated as the slope of the curve (Lapole et al., 2015). To obtain a stiffness index taking into account TA EMG values ( $SI_{EMG}$ ) (Lapole et al., 2015), the slope of the linear regression analysis

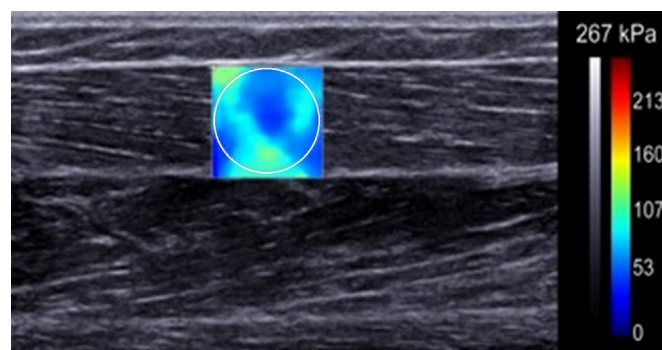


Fig. 1. Representative recording of tibialis anterior shear modulus during contraction. The colored region represents the shear elasticity map with the scale to the right of the figure. White circle shows within the region of interest the Q-Box where mean shear modulus was measured (see Section 2.5). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

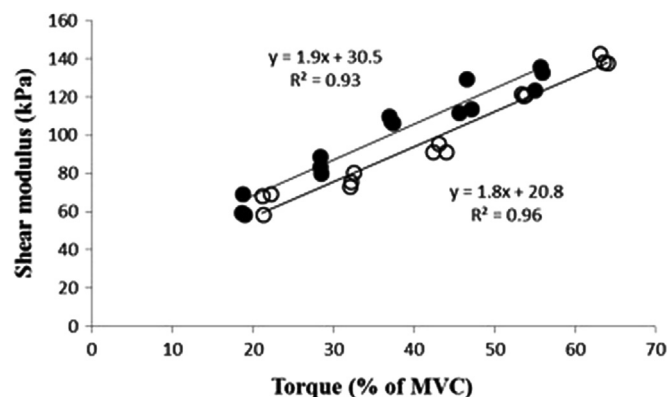
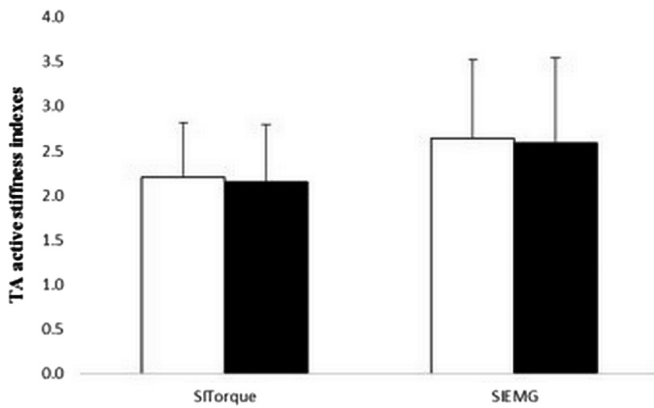


Fig. 2. Shear modulus/torque relationship for a representative woman ( $\circ$ ) and a representative man ( $\bullet$ ). Slopes of the curves were calculated as tibialis anterior active stiffness indexes taking into account dorsiflexion torque ( $SI_{TORQUE}$ ).



**Fig. 3.** Mean TA active stiffness indexes for women (in white) and men (in black).  $SI_{TORQUE}$  (expressed in  $kPa \cdot \%MVC^{-1}$ ) is calculated as the slope of the linear relationship between TA shear modulus and normalized dorsiflexion torque.  $SI_{EMG}$  (expressed in  $kPa \cdot \%EMG_{MAX}^{-1}$ ) is calculated as the slope of the linear relationship between TA shear modulus and TA EMG activity.

**Table 1**  
Shear elastic modulus (in kPa) calculated at different contraction intensity levels using the regression analysis parameters.

	Men	Women	p value
Shear modulus at 20% MVC	43.2 ± 12.8	45.8 ± 12.8	0.50
Shear modulus at 60% MVC	129.1 ± 38.9	137.0 ± 38.7	0.50
Shear modulus at 20% $EMG_{max}$	52.0 ± 19.5	56.6 ± 14.6	0.37
Shear modulus at 60% $EMG_{max}$	155.7 ± 58.9	169.3 ± 43.9	0.38

Data are expressed as mean ± standard deviation. MVC: maximal voluntary contraction;  $EMG_{max}$ : maximal EMG activity recorded during MVC.

between TA shear modulus and the estimated TA EMG was also calculated. For each trial, TA EMG (expressed as a percentage of  $EMG_{max}$ ) was estimated based on the torque value and following the equation of the linear TA EMG–torque relationship ( $R^2: 0.94 \pm 0.06$ ) (Moritani and deVries, 1979). Using the regression analyses parameters, shear modulus values were also calculated for different contraction intensity levels (20 and 60% MVC, and 20 and 60%  $EMG_{max}$ ).

## 2.6. Statistics

Coefficients of determination ( $R^2$ ) were calculated for each linear fitting. Note that shear modulus/torque and shear modulus/EMG activity relationships presented the same  $R^2$  since EMG activity was estimated based on torque values. Statistical analyses were performed using SigmaStat software (SigmaStat 3.5, Systat Software, San Jose, CA). The distribution of each variable was confirmed with the Kolmogorov-Smirnov normality test. Student *t*-tests were performed to assess the significance of sex on  $SI_{TORQUE}$ ,  $SI_{EMG}$  and absolute shear modulus at the different contraction intensity levels. Data are presented as means ± SD. Statistical significance was set at  $P < 0.05$ .

## 2. Results

Stiffness indexes presented good linear regression with mean  $R^2$  of  $0.87 \pm 0.09$ . Men presented significantly greater MVC than women ( $65.8 \pm 16.2$  versus  $43.6 \pm 10.7$  Nm, respectively;  $p < 0.001$ ). As illustrated by Fig. 3,  $SI_{TORQUE}$  and  $SI_{EMG}$ , did not differ between men and women ( $p=0.76$  and  $p=0.86$ , respectively). There was also no difference in absolute shear modulus values at 20 and 60% MVC and  $EMG_{max}$  (Table 1).

## 3. Discussion

The present study assessed and compared TA active stiffness between men and women, using SSI technique. As a result, no sex

effect was reported for  $SI_{TORQUE}$ , or  $SI_{EMG}$ , and shear modulus obtained at various percentage of contraction.

The linear relationship observed between TA shear modulus and dorsiflexion torque is in agreement with previous magnetic resonance elastography studies (Basford et al., 2002; Heers et al., 2003; Jenkyn et al., 2003). Although the precise mechanisms involved in the increased shear modulus during contraction remain to be clearly understood (Sasaki et al., 2014), this can be partly explained by the close link between stiffness at the muscle fiber level and the number of attached cross bridges (Ford et al., 1981). Our results also suggest no sex effect on the intrinsic active stiffness for the contracting TA. This contrasts with findings of Fouré et al. (2012) who suggested lower intrinsic plantar-flexors stiffness for men as a result of possible sex-related differences in fiber-type distribution, cross bridges of slow-twitch fibers being stiffer than for fast ones (Goubel and Marini, 1987). Although no biopsy was performed in the present study, our results would agree with the similar proportion of TA slow-twitch fibers reported in men and women (Holmback et al., 2003). It should be however emphasized that the influence of fiber type on shear modulus measurement remains speculative.

It should be also acknowledged that shear modulus measurement may be influenced by other structures than cross bridges (e.g. connective tissue, fatty infiltration) (Lapole et al., 2015; Sasaki et al., 2014), shear modulus being for instance increased with passive muscle force (Maisetti et al., 2012). Changes in pennation angle should be also considered because of the pinnate architecture of the TA muscle, and since pennation angle increases during dorsiflexion (Manal et al., 2006). Hence, shear modulus was not measured along the fiber direction, thus inducing a potential underestimation of the measurement (Gennisson et al., 2010). Although the precise influence of pennation angle on shear wave velocity remains to be clearly determined, its influence on TA shear modulus measurements during contractions has however recently been minored (Sasaki et al., 2014). Moreover, the influence of potential changes in muscle fascicles orientation with respect to the ultrasound probe should be considered (Chernak et al., 2013). Hence, mechanisms and anatomical structures underlying increased shear modulus during contraction remain to be further investigated.

To conclude, men and women were characterized by similar TA active stiffness indexes determined using SSI. This suggests that, at least for the contracting TA, intrinsic active stiffness may not contribute to sex differences in performance or risk of injury (Butler et al., 2003) and that other biomechanical parameters such as cross-sectional area or lever arm length should be considered.

## Conflict of Interest

None of the authors have potential conflicts of interest to be disclosed.

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