Quantification of *in vivo* muscle elastic anisotropy factor by steered push beams

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Abstract — Through the past few years, ultrasound (US) elastography has been widely applied to quantify muscle anisotropy. Generally, it is performed with an acoustic radiation force push beam that generates shear waves followed by US imaging. Recently, Ngo *et al*. (2021) proposed to use a steering push beam to comprehensively assess the mechanical properties of transverse isotropic skeletal muscle tissue. Here, we integrate the equation of shear vertical wave mode to the steering push beam method which allows to retrieve the mechanical parameters of anisotropic muscle tissue. *Ex vivo* experiments showed a good agreement between the tensile anisotropy χ_E found by our method and by the mechanical tensile tests. *In vivo* experiments were conducted to evaluate the anisotropy ratio measured by steering push beam method during different isometric contraction intensities. We observed a growing trend of this ratio with the contraction intensity in both fusiform (*Biceps brachii*) and pennate muscles (*Medial gastrocnemius*) of two healthy volunteers. Despite this trend was different between the two types of muscle architecture across contractions intensities, the overall difference had about the same magnitude for both volunteers.

Keywords — shear wave elastography, ultrasound, anisotropy, muscle

I. Introduction

US elastography has recently emerged as a noninvasive modality to quantify soft tissue stiffness. Classically, it is performed by using push beams that generate shear waves (SW) which are then followed by ultrafast US imaging. In isotropic tissues, the SW velocity (SWV) is directly related to stiffness (shear modulus μ) by the relationship: $\mu = \rho.V^2$, where ρ is the density of tissues. However, in transverse isotropic (TI) tissues such as skeletal muscle, two shear moduli $\mu_{//}$ and $\mu_{\perp}$ can be defined parallel or perpendicularly to the fibers respectively. Theoretically, also two SW modes can be defined [1, 2]: a shear horizontal (SH) and a shear vertical (SV) wave mode. In this case, SW propagation direction and polarization are not either parallel or perpendicular to the tissue symmetry axis, so the SWV is not directly related to a single μ. This scenario typically occurs in pennate muscles. Recently, a novel method that uses steered push beams (SPB) was suggested by *Ngo et al. (2021)* to tackle this limitation [3]. Here, we combined SPB with SV mode of SW propagation to fully characterize the mechanical properties of TI tissue, such as muscle. This allowed us to estimate the tensile anisotropy χ_E , which is the ratio of the two Young's moduli along and across the muscle fibers, and to assess its behavior during submaximal contractions in fusiform and pennate muscles (*tibialis anterior, gastrocnemii, ...*). We hypothesized that this parameter can help to characterize muscle structure and function properties more comprehensively.

II. MATERIALS AND METHOD

A. Shear wave propagation in transverse isotropic tissue

Shear wave modes, SH or SV are quantified differently depending on the type of muscle investigated. In the case of fusiform muscle, such as *biceps brachii* (BB), the classical way to estimate shear moduli is to rotate the US probe at the surface of the muscle. In this configuration the SH mode is quantified and described by equation 1.

$$\boldsymbol{\mu_{SH}(\psi) = \rho v_{SH}^2(\psi) = \mu_{\parallel} \cos\psi^2 + \mu_{\perp} \sin\psi^2 \quad (1)}$$

In the case of pennate muscle, such as *medial gastrocnemius* (MG), the SV mode is generated by an angled US pushing beam with the US probe parallel the fibers. The shear modulus is then described by the following equation:

$$\boldsymbol{\mu_{SV}(\psi) = \rho v_{SV}^2(\psi) = \mu_{\parallel} + 4(\chi_E.\mu_{\perp} - \mu_{\parallel})\sin(\psi)^2\cos(\psi)^2 \quad (2)}$$

With $\chi_E = E_{//}/E_{\perp}$, $E_{//}$ and $E_{\perp}$ the Young's moduli parallel and perpendicular to fibers axis, ψ is the angle between the push beam and the fiber's direction.

B. Ultrasound sequence

To investigate these two SW modes, two shear wave elastography sequences were programmed on an ultrafast ultrasound scanner (Mach 30, Hologic, France) driving a 7.5 MHz centre frequency 256-element linear probe (SL18-5, Hologic, France). The first sequence (sequence 1) was a classical supersonic shear imaging (SSI) sequence constituted of an acoustics radiation force beam along the axis of ultrasound followed by an ultrafast acquisition sequence [4]. The second sequence (sequence 2) was constituted of inclined pushing beams (from 20° to -20° with 5° step) each followed by an ultrafast imaging mode inclined on the same axis of the pushing beam [3].

For fusiform muscle the first sequence is used quantifying the shear wave phase velocity. The SH mode is quantified and the equation 1 is used to retrieved $\mu_{//SSI}$ and $\mu_{\perp SSI}$. For pennate muscle, the second sequence is used. From the movies of the propagation of SW for each push beam angles, the SW dispersion curve was calculated and the phase velocity was quantified the central frequency. This velocity describing the SV mode is dependent on the angle of shear wave direction regarding the fiber's direction according to the equation (2) [1]. That allowed us to calculate the longitudinal shear modulus $\mu_{//}$ and the factor $\chi_E.\mu_{\perp}$. In practice the fiber angle is measure from the ultrasound image (B-mode) and added to the angle of the pushing beams to fit equation 2 to the data following the workflow diagram presented in figure 1.

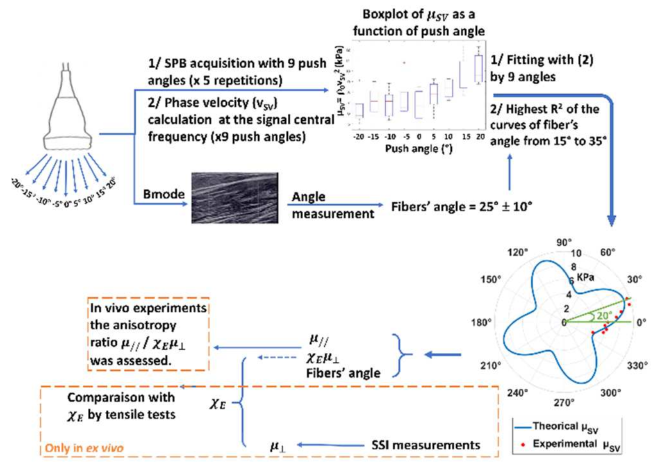


Figure 1: Acquisition method for pennate muscles and fitting procedure

C. Mechanical testing

In *ex vivo* experiment, tensile tests were conducted on a mechanical testing device (Instron 5944 Norwood, MA, USA) to quantify χ_E . The two values of χ_E were compared to the one obtained in ultrasound to validate the method.

D. Ex vivo experiments

Experiments were conducted with the use of *ex vivo* porcine muscles, which allowed us to control perfectly the alignment of muscle's fibers. Two *iliopsoas* muscles of a same pig (left side and right side) were surgically extracted. After extraction, each muscle was cut into 6 equal rectangular parts (5 x 1 cm^2): two longitudinal (A and B) and four perpendicular to the fibers (C5, C7, D5, D7) (figure 2).

Figure 2: Extracted muscle and positioning of the of the excised muscle samples

For A and B, SPB (US sequence 2 with SV mode) was performed to deduce μ_L and the factor $\chi_E.\mu_{\perp}$. For transverse samples classical SSI (sequence 1 with SH mode) measurements were performed to obtain $\mu_{//SSI}$ and $\mu_{\perp SSI}$, allowing to retrieve χ_E by combining results with is obtained with sequence 2 on A and B.

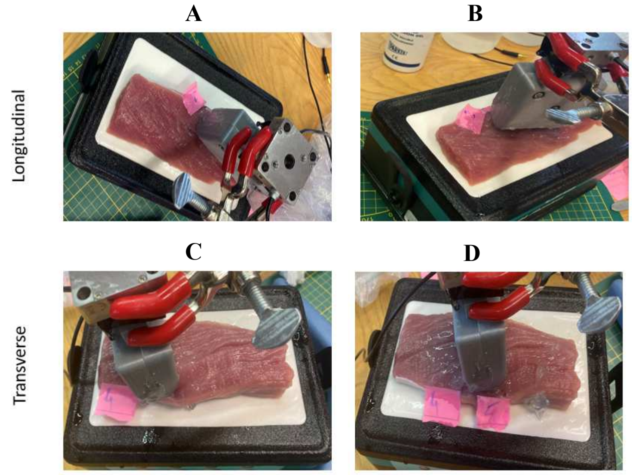


Figure 3: Ultrasound SPB performed on different muscles

Alignment of the fibers was previously checked with ultrasound B-mode. After that, each muscle was cut into different samples (Figure 2, yellow rectangles correspond to probe position) to measure the Young's moduli with traction tests. Each sample was attached to the testing device on the two ends by a system of jaws. The mechanical device allowed to apply an axial defined stress along the direction of the cut sample. For each stress step the ultrasound probe was applied on the muscle sample to quantify SWV with elastography sequences (figure 4). The sample's size was measured, the stretch was programmed to stop at a strain of 14%. Using Hooke's law, the Young's modulus (E) could be calculated as the slope of the stress-strain curve (3).

$$\sigma = E.\epsilon \quad (3)$$

Thus, the theoretical ratio χ_E could be found by the traction tests on both longitudinal ($E_{//}$) and transverse ($E_{\perp}$) samples.

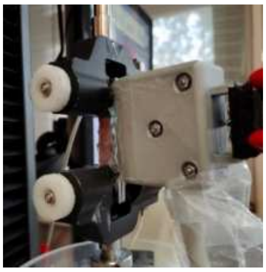


Figure 4: Ultrasound acquisition during tensile test on each sample

E. In vivo experiments

In vivo experiments were also carried out on 2 healthy volunteers with a dedicated setup to control muscle contraction and force (figure 5). Two types of muscles were selected: fusiform BB and pennate MG. The targeted muscle was positioned on an optimal length to be imaged, using an ergometer to measure the produced force.

After an initial warm-up, the participant was asked to perform three maximum voluntary contractions of approximately three seconds in order to quantify the maximum force torque (MVC). This type of physical effort is similar to that carried out during sports practice. The measurements were then performed firstly at rest, then during four distinct muscle contractions at low intensity: 5%, 10%, 15% and 20% of the MVC. The participants had a visual feedback displayed on a screen so that they can maintain the intensity of the targeted muscle contraction during acquisition.

Figure 5: A setup for acquisitions in vivo on the MG

III. RESULTS

A. Validation of χ_E estimation in ex vivo muscle

In *ex vivo* experiments, we obtained firstly the B-mode image of extracted muscle, which shows a parallel muscle bundle. After applying SPB method, the results were displayed with the fitted curve of μ_{SV} as a function of the push angle taking into account the fiber's angle of 0° since this muscle is fusiform.

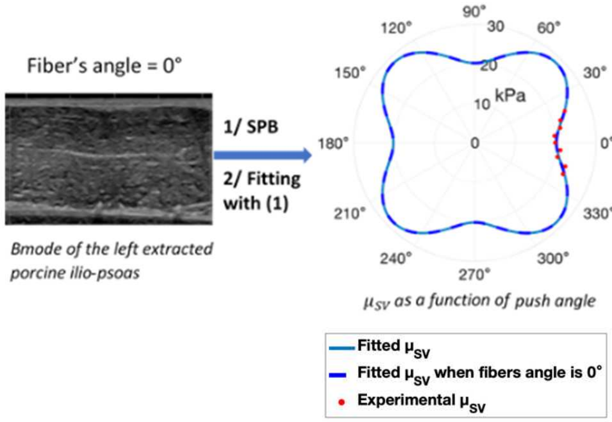


Figure 6: Results of SPB acquisition on ex vivo left-side iliopsoas muscle

Experiments were performed on left-side and right-side muscles.

Table 1 Shear moduli measured by SPB and SSI methods

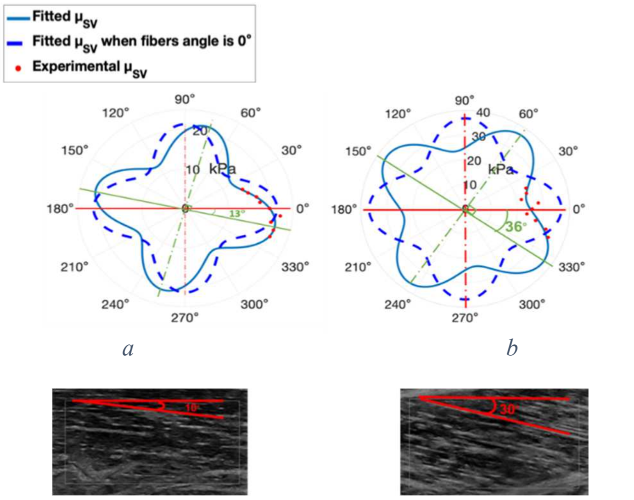
		Left	Right
SPB	$\mu_{//}$ **(kPa)**	20.05 ± 0.16	9.12± 1.35
	$\chi_E.\mu_\perp$ **(kPa)**	28.64 ± 0.23	21.91± 1.8
SSI	$\mu_{//}$ **(kPa)**	21.65 ± 0.08	8.00 ± 0.05
	$\mu_\perp$ **(kPa)**	9.33 ± 0.36	7.59 ± 0.29

Table 2 χ_E retrieved by ultrasound and tensile test

Method	χ_E	
	Left	Right
Ultrasound $\chi_E = \chi_E.\mu_\perp/\mu_{\perp SSI}$	**3.07 ± 0.13**	**2.88 ± 0.23**
Tensile test $\chi_E = E_{//}/E_\perp$	**2.90**	**2.93**

B. In vivo experiments

For *in vivo* experiments, we show here the result of a BB and a MG of a same volunteer at relaxation (figure 7). Firstly the muscles's B-mode image was obtained to estimate the fiber's angle. Taking the BB as an example, by manual measurement, this angle is 10° ± 5°. Next, PSB was performed following with a fitting considering the angle range of the fibers bundle from 5° to 15°. We found that the experimental data fit the best with the μ_{SV} curve as a fonction of the push angle taking into account the fiber's angle of 13° (R^2 = 0.9). The results were displayed with fitted curves of μ_{SV} as a function with the fiber's angle of 0° and 13° (figure 7a.1 and 7b.1).

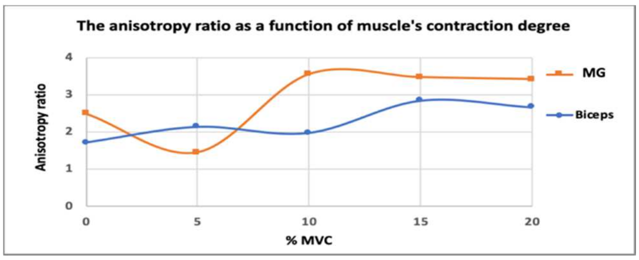


a

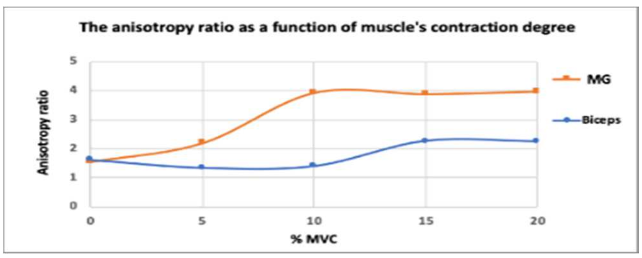
b

Figure 7: μSV as a function of push angle during a SPB acquisition in vivo on a human BB a) and MG b) with the corresponding Bmode below.

The ratio $\mu_{//}/\chi_E.\mu_{\perp}$ was then calculated to assess the effect of anisotropy on muscle stiffness during contraction, knowing that fiber's orientation changes with the muscle's contraction degrees.



a



b

Figure 8:Evolution of the anisotropy ratio as a function of % MVC of two healthy volunteers (a and b) during different contraction states

IV. DISCUSSION

For *ex vivo* experiments, there is a difference of shear moduli ($\mu_{//}$ and $\mu_{\perp}$) between two *iliopsoas* muscles. This is may be due to the delay in the acquisitions on the right-side muscle since it was performed a time after being immersed in saline (several hours). The first sample tested was the left one directly after excision. A better method of muscle preservation during experiments should be considered. Despite this difference, the tensile anisotropy χ_E does not vary significantly in the two muscles and remains consistent with the value found by mechanical test (table 2). We can thus hypothesize that χ_E is independent of muscle shear moduli. Overall, the measurement of χ_E was preliminarily validated by tensile test. Therefore, it can be said that SPB is a reliable and promising method to quantify muscular elastic anisotropy. That paves the development of a faster assessment of the shear anisotropy factor by the SPB method, which is carried out within a single acquisition.

For *in vivo* experiments, first, we observed that the fiber's angles of a BB and a MG, determined by SPB method (13° and 36°), are different from the angles measured from the B-mode images (10° and 30°) . That can be explained by the fact that the ROIs selected in our method are small, thus, fiber's angle found is only a local value. Second, the ratio $\mu_{//}/\chi_E.\mu_{\perp}$ was calculated to assess the muscular elastic anisotropy on two types of muscle. One can notice that generally, this ratio tends to increase as the contraction increases. That was observed in both muscles. However, this tendency is different between two muscles. For two different healthy volunteers, the MG trends to soar higher and stabilize from 10% of MVC. Further studies are required to confirm this result and better understand this tendency.

ACKNOWLEDGMENT

This work was funded by: ANR INNOVAN (ANR-19-CE19-0017), PEPS CNRS MUSCLOR 2022, PEDECIBA and ANII (FMV_1_2019_1_155527), and supported by the IRIS platform of ICube.

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