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● *Original Contribution*

RELIABLE PROTOCOL FOR SHEAR WAVE ELASTOGRAPHY OF LOWER LIMB MUSCLES AT REST AND DURING PASSIVE STRETCHING

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Abstract—Development of shear wave elastography gave access to non-invasive muscle stiffness assessment *in vivo*. The aim of the present study was to define a measurement protocol to be used in clinical routine for quantifying the shear modulus of lower limb muscles. Four positions were defined to evaluate shear modulus in 10 healthy subjects: parallel to the fibers, in the anterior and posterior aspects of the lower limb, at rest and during passive stretching. Reliability was first evaluated on two muscles by three operators; these measurements were repeated six times. Then, measurement reliability was compared in 11 muscles by two operators; these measurements were repeated three times. Reproducibility of shear modulus was 0.48 kPa and repeatability was 0.41 kPa, with all muscles pooled. Position did not significantly influence reliability. Shear wave elastography appeared to be an appropriate and reliable tool to evaluate the shear modulus of lower limb muscles with the proposed protocol. (E-mail: guillaume.dubois@ensam.eu) © 2015 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Elastography, Muscle, Shear modulus, Reliability, Lower limb.**

INTRODUCTION

In vivo assessment of muscle properties is a challenge that needs to be met to quantify neuromuscular diseases or evaluate their treatment. A quick-release method for evaluation of the elasticity of the muscle–tendon unit was investigated by Cornu et al. (2001), but this technique cannot isolate the behavior of one muscle. In addition, it is technically difficult to use in subjects with high joint stiffness or bone deformities. Stiffness of the gastrocnemius muscle–tendon unit of the calf has been evaluated by numerical optimization of the measurement of passive ankle torque depending on ankle angle (Hoang et al. 2005; Nordez et al. 2010). This elegant method had low reliability for some parameters and cannot be used to evaluate only one muscle. Magnetic resonance elastography (MRE) has also been used to evaluate muscle stiffness as shear modulus in three dimensions (Basford et al. 2002; Bensamoun et al. 2007; Ringleb et al. 2007). However, the measurement can only be performed with the patient lying down, and the acquisition cost remains a limitation. Despite the small number of subjects, stiffness in resting muscles appeared lower in control subjects than in patients with neuromuscular dysfunction (Basford et al. 2002). This difference was highlighted *in vitro* by comparing the stiffness of stretched muscle fiber bundles during a tensile test (Smith et al. 2011).

Shear wave elastography (SWE) has been used to assess the mechanical properties of soft tissue for about 10 years. The concept of SWE imaging (SWEI) using acoustic radiation force was proposed by Sarvazyan et al. (1998). More recently, Bercoff et al. (2004) coupled this concept with ultrafast ultrasound imaging to provide a specific technique called supersonic shear imaging. This technique provides a quick measurement of the muscle shear modulus using a standard ultrasonic probe (Gennisson et al. 2010). However, there is lack of consensus in the literature on the technical aspects of acquiring elastographic measurements. This has resulted

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in large variability between studies depending on the measurement technique (subject position, measurement position, at rest or during contraction, *etc*.).

Kot et al. (2012) found that size of the region of interest (ROI) and probe pressure influence elastography measurement. Moreover, subject position affects the measurement: an increase in shear modulus was observed when muscle was passively stretched, both *in vitro* (Koo et al. 2013; Maïsetti et al. 2012; Shinohara et al. 2010) and *ex vivo* (Eby et al. 2013). Maïsetti et al. (2012) and Hug et al. (2013) determined *in vivo* the slack length of the muscle, corresponding to a range of motion within which the muscle does not produce any passive force and shear modulus is constant.

Clinically, SWE was recently used to evaluate muscles in a subject with patellofemoral pain syndrome by Botanlioglu et al. (2013), who reported that the vastus medialis obliquus was less stiff during contraction in patients with pathology than in healthy patients, but they did not perform a reliability study.

Lacourpaille et al. (2012) evaluated the reliability of this technique in nine muscles and proposed standardizing the muscle length by controlling joint angles, which were chosen to leave the muscle as slack as possible. These positions seemed to improve reliability because of the range of muscle slack length, which allowed the shear modulus to remain constant under small angle variations between joints.

Although measurements in muscle at rest provide an interesting physiologic baseline, the stiffness of contracted or stretched muscle is more likely to differ between healthy and pathologic muscles (Botanlioglu et al. 2013); indeed, most clinical qualitative examinations are based on the response of the muscle to external mechanical solicitation. Voluntary contraction, however, is difficult to reliably reproduce and maintain during the measurement. Fatigue and trembling can occur rapidly, even in isometric contraction, thus negatively affecting measurement reliability. Passive stretching could represent an interesting alternative, but to our knowledge no study has characterized measurement reliability during passive stretching.

The aim of the present study was to define a measurement protocol to quantify the shear modulus of 11 muscles of the lower limb, both at rest and during passive stretching, and to quantify inter-operator reproducibility and intra-operator repeatability.

METHODS

Subjects

Ten subjects (age: 25.5 ± 2.8 y, height: 176 ± 11.0 cm, weight: 68 ± 13.3 kg, body mass index [BMI]: 21.7 ± 2.0 kg/m) with no documented muscular pathology gave their written consent to participate in this protocol (approved by the Institutional Ethics Committee, CPP 06036, Paris).

Shear wave elastography measurement

The principle underlying SWE using supersonic shear wave imaging (SSI) was previously described (Bercoff et al. 2004). Briefly, a cylindrical shear wave front is generated in the tissue by successively focusing ultrasonic pushing beams at different depths. Then, with very high frame rate imaging (up to 20,000 images), a movie of the shear wave propagating is recorded in an imaging plane parallel to the main axis of the cylinder, which is parallel to the ultrasound axis. In this plane, B-mode images and shear wave velocity movies are acquired. Last, the local shear wave speed is retrieved from a time-of-flight algorithm.

Measurements were performed with an Aixplorer ultrasound scanner (Version 4.2, Supersonic Imagine, Aix-en-Provence, France) driving a 4- to 15-MHz ultrasonic probe (SL15-4, 256 elements, pitch 0.2 mm). Acquisitions were performed in general mode with the following SWE settings throughout the whole study: penetration mode, tissue tuner at 1540 m/s, middle persistence, smoothing 5, SuperRes 2.

Muscle is highly anisotropic; therefore, the shear wave velocity depends on the probe orientation relative to the muscle fibers (Gennisson et al. 2010; Lee et al. 2012; Rouze et al. 2013; Wang et al. 2013). Thus, acquisitions were performed with the probe in a plane parallel to the muscle fibers and perpendicular to the skin. This orientation was determined when several fibers were continuously visible on the B-mode image (Fig. 1). When the ultrasonic probe is properly aligned with the muscle fibers, shear wave speed $V_{s//}$ is directly linked to the shear modulus $\mu_{//}$ in the longitudinal direction by (Gennisson et al. 2003; Royer et al. 2011)

$$\mu_{//} = \rho \cdot V_{s//}^2 \quad \text{with } \rho = 1000\ \text{kg/m}^3 \qquad (1)$$

For muscles with several bellies, such as the rectus femoris, only one belly was measured. For each measurement, a sequence of 10 continuous images was recorded in 10 s films. Shear modulus images were then processed using custom software developed in MATLAB (The MathWorks, Natick, MA, USA) for rapid and semi-automatic processing of all 1020 images. The ROI was defined as a rectangle (which is adapted to thin and long muscle):

1. The operator selected an ROI on the first image; the ROI was then automatically tracked on subsequent images using a difference-based algorithm. The ROI was defined in the square region of shear modulus measurement as the largest square between fasciae (Fig. 1).