A reliable protocol for shearwave elastography of lower limb muscles at rest and during passive stretching

Guillaume Dubois[a,], Walid Kheireddine[a], Claudio Vergari[a], Dominique Bonneau[a], Patricia Thoreux[a,c], Philippe Rouch[a], Mickael Tanter[b], Jean-Luc Gennisson[b], Wafa Skalli[a]

[a] *Arts et Metiers ParisTech, LBM, 151 bd de l'hopital, 75013 Paris*
[b] *Institut Langevin, Laboratoire Ondes et Acoustique, CNRS UMR 7587, ESPCI ParisTech, PSL, INSERM ERL U979, Paris, France*
[c] *Universite Paris XIII, Paris, France*

Abstract

Accepted Manuscript. Ultrasound in Medicine and Biologyy. The original publication is available at www.sciencedirect.com.

DOI: doi:10.1016/j.ultrasmedbio.2015.04.020.

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 quantify the shear modulus of lower limb muscles in order to be used in clinical routine. Four positions were defined to evaluate shear modulus, parallel to the fibers, in the anterior and posterior aspect of the lower limb, at rest and during passive stretching, of 10 healthy subjects. Reliability was first evaluated on 2 muscles by 3 operators, measurements were repeated 6 times. Then, reliability comparison of different muscle was evaluated on 11 muscles by 2 operators, measurements repeated 3 times. Reproducibility of shear modulus was 0.48 kPa and repeatability was 0.41 kPa, with all muscles pooled. The position did not significantly influence the reliability. SWE appeared as an appropriate and reliable tool to evaluate shear modulus of lower limb muscles with the proposed protocol.

Keywords: Elastography, Muscle, Shear Modulus, Reliability, Lower Limb

---

Email address: `guillaume.dubois@ensam.eu` (Guillaume Dubois)

Introduction & Literature

In vivo assessment of muscle properties is a challenge that needs to be overcome in order to quantify neuromuscular diseases or evaluate their treatment. Quick-release method (Cornu et al., 2001) has been investigated to evaluate the elasticity of muscle-tendon unit. This technique cannot isolate the behavior of one muscle and it is difficult to apply on pathologic subjects, due technical difficulties of performing the test on pathological subjects with high joint stiffness or bone deformities. Gastrocnemius muscle-tendon unit stiffness of the calf has also been evaluated using a numerical optimization from the measurement of the passive ankle torque depending on ankle angle (Hoang et al., 2005; Nordez et al., 2010). This elegant method provided low reliability for some parameters and cannot be used to evaluate only one given muscle. Magnetic resonance elastography (MRE) was also used to evaluate muscle stiffness as shear modulus in 3D (Bensamoun et al., 2007; Ringleb et al., 2007; Basford et al., 2002). However, the measurement can only be performed in laying position and the acquisitions cost are still a limitation. Despite, the small number of subjects, stiffness in resting muscles appeared lower in the 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 fibers bundles during a tensile test (Smith et al., 2011).

The use of shear wave elastography to assess mechanical properties of soft tissue is used since about 10 years. The concept of Shear Wave Elasticity 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 so-called Supersonic Shear Imaging. Such technique allows to provide a quick measurement of muscle shear modulus using a standard ultrasonic probe (Gennisson et al., 2010). However, there is lack of consensus in the literature about the technical aspects of acquiring elastographic measurements. This produces 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 the size of the region of interest (ROI) and the probe pressure influenced elastography measurement. Moreover, subject position affects the measurement: an increase of the shear modulus was observed when muscle is passively stretched, both *in vitro* (Shinohara et al., 2010; Maïsetti et al., 2012; Koo et al., 2013) 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 in which the muscle does not produce any passive force and in which shear modulus was constant.

Clinically, SWE has been used in a recent study to evaluate subject muscles with patellofemoral pain syndrome (Botanlioglu et al., 2013). It has been shown that vastus medialis obliquus of pathologic patients appeared less stiff during contraction than healthy patients but no reliability study was performed.

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

While measurements in muscle at rest give an interesting physiological baseline, stiffness of contracted or stretched muscle are more likely to show differences between healthy and pathologic muscles (Botanlioglu et al., 2013); indeed, most clinical qualitative examinations are based on the response of the muscle to an external mechanical solicitation. Voluntary contraction, however, is difficult to reliably reproduce and maintain during the measurement. Fatigue and trembling can appear 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 the measurement reliability during passive stretching.

The aim of the present study is 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.

Materials and Methods

Subjects

Ten subjects (age: 25.5±2.8 yr, height: 176±11.0 cm, weight: 68±13.3 kg, Body Mass Index (BMI): 21.7±2.0 kg.m^{-2}) 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

Principle of shear wave elastography using SSI was previously described (Bercoff et al., 2004). Briefly, cylindrical shear wave front is generated in the tissue by successively focusing ultrasonic pushing beams at different depths. Then, using very high-frame rate imaging (up to 20000 images/s), a movie of the shear wave propagating is recorded in 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. At last, the local shear wave speed is then retrieved from a time of flight algorithm.

Measurements were performed with an Aixplorer ultrasound scanner (Supersonic Imagine, Aix-en-Provence, France, version 4.2) driving a 4-15 MHz ultrasonic probe (SL15-4, 256 elements, pitch 0.2mm). Acquisitions were performed in general mode with the following SWE settings throughout the whole study: penetration mode, tissue tuner at 1540 m.s^{-1}, 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; Wang et al., 2013; Rouze 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 (figure 1). By considering the ultrasonic probe properly aligned with muscle fibers, the shear wave speed $V_{s//}$ is directly linked to the shear modulus $\mu_{//}$ in the longitudinal direction (Eq.1) (Gennisson et al., 2003; Royer et al., 2011):

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

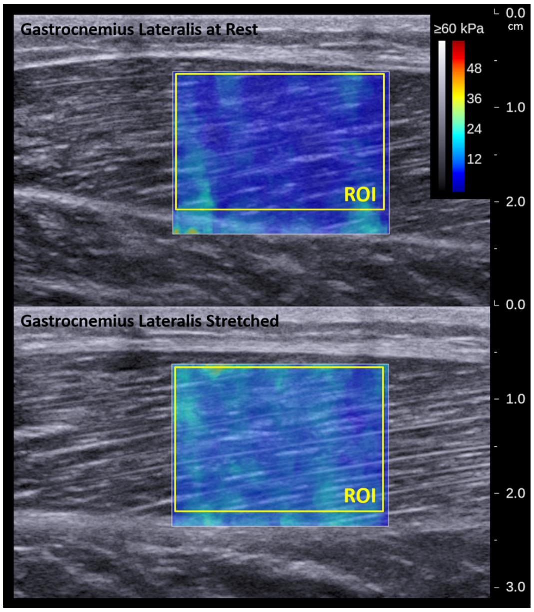


Figure 1: Shear modulus assessment using elastography in rested and stretched gastrocnemius lateralis muscle.

For muscle with several bellies, such as rectus femoris, only one belly was measured.

For each measurement, a sequence of 10 continuous images was recorded in 10 second films. Shear modulus images were then processed using custom software developed in Matlab (The Mathworks, Natick, USA) for rapid and semi-automatic processing of the all 1020 images. The region of interest ROI was defined as a rectangle (which is adapted to thin and long muscle):

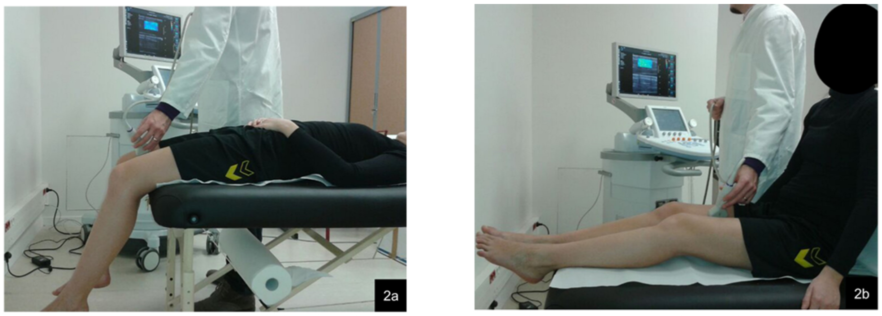
1. the operator selected a region of interest (ROI) on the first image; the ROI was then automatically tracked on the following images, using a difference-based algorithm. The ROI was defined in the square region of shear modulus measurement as the biggest square between fascia (figure 1).
2. in the ROI of each image, RGB value of each pixel color was converted in a shear modulus value, accounting for color chart opacity, according the colorbar, which is composed of 221 distinct colors.
3. muscle shear modulus was computed as the mean shear modulus of the ROI in all images.

Protocols

Measurements were performed by operators familiar with muscle elastography; the operator replaced the probe between each measurement. A large amount of gel was applied between the probe and the skin, in order to limit tissue deformation induced by the operator (Kot et al., 2012).

Positions

Shear wave elastography was performed both at rest and during passive streching in the eleven main muscles involved in the knee joint motion and in the plantarflexion of the foot: biceps femoris (BF), gracilis (GRA), rectus femoris (RF), sartorius (SAR), semimembranosus (SM), semitendinosus (ST), vastus lateralis (VL), vastus medialis (VM), gastrocnemius medialis (GM) and gastrocnemius lateralis (GL), and soleus (SOL).



(a) Measurement position for RF, VM and VL passively stretched.

(b) Measurement position for RF, VM and VL at rest.

(c) Measurement position for BF, ST, SM, GRA, SAR, GL, GM and SOL passively stretched.

(d) Measurement position for BF, ST, SM, GRA, SAR, GL, GM and SOL at rest.

Figure 2: Measurement positions of shear modulus in rested and stretched muscles of the thigh and the calf.

Four positions were defined to measure the muscles of the anterior and posterior aspect of the limb at rest and during passive stretching:

1. RF, VM and VL passively stretched: subject was laid supine on the table with the calves and the knees hanging outside the table. Subject was asked to stay in comfortable and relaxing position (figure 2(a)).

2. RF, VM and VL at rest: subject was sitting upright on the table with the heels outside, trunk and thighs had an angle of approximately 90° (figure 2(b)).
3. BF, ST, SM, GRA, SAR, GL, GM and SOL passively stretched: the subject was placed in prone position on the table with the feet outside (figure 2(c)). This position stretched the muscles of the posterior aspect of the thigh while at the same time leaving the subject in a comfortable position, which could be easily assumed by pathological patients as well.
4. BF, ST, SM, GRA, SAR, GL, GM and SOL at rest: the subject was placed in prone position on the table with an angle of approximately 90° between the legs and the thighs (figure 2(d)).

For both protocols, the same order of positions was respected (1, 2, 3 and 4).

Measurements Location

In oder to standardize the measurement location, landmarks were drawn on the subject's skin. For the anterior and posterior thigh muscles, the antero-superior iliac spine and the apex of the patella were identified by palpation and the inferior third of their distance was marked. For the posterior leg muscles, proximal and distal limits of the fibula were identified by palpation and the superior third of the distance was marked.

Complete Protocol

First, a complete protocol aimed to evaluate intra-operator measurement repeatability and inter-operator reproducibility, in particular related to the proposed subject positions. Measurements were performed by 3 operators and repeated 6 times in each of the previously defined positions.This complete protocol was only applied to one muscle of the thigh and one of the calf (VM and GL). It was assumed that these two muscles were representative of the repeatability of the measurement of the lower limb.

This protocol lasted about 75 min (3 operators times 6 measurements times 2 positions times 2 muscles times about 1 min per measurement).

For the measurement in supine position, position 1 (figure 2(a)), anterior muscles were passively stretched and the subjects reported being uncomfortable after a few minutes. This was accompanied by a measured increase in shear modulus which was observed during preliminary tests. A similar phenomenon was previously observed by Lacourpaille et al. (2012), but such increase in modulus was eliminated by asking the subject to freely strech out and walk between each series of measurement.

Reduced Protocol

Once the operator effect and the position effect on the measurement was determined, a reduced protocol aimed to compare measurement reliability between the remaining muscles and evaluate stiffnesses at rest and during passive stretching. A reduced number of operators and repetitions was used: measurements were performed by 2 operators and they were repeated 3 times.

This reduced protocol including all muscles lasted about 90 min, because of the increased number of muscles.

For the rest of the article, R or S subscripts indicate that muscle is at rest or stretched (e.g.: $\mathrm{BF_S}$: biceps femoris stretched).

Statistical Analysis

Inter-operator reproducibility and intra-operator repeatability of shear modulus measurement were determined to estimate the uncertainty of measurement as recommended by ISO 5725 Standard, which evaluate the reliability (in terms of repeatability and reproducibility) of measurement methods. This was evaluated for each muscle in both positions.

Intraclass correlation coefficient (ICC(2,1)) (Hopkins, 2000) was also computed and Bland-Altman graph was plotted for the complete protocol.

The difference between shear modulus of rested and stretched muscles was evaluated using Wilcoxon signed-rank; significance was set at 0.05.

In order to evaluate the consistency of the number of subject, a power analysis was performed: a cohort of 10 subjects gave an overall statistical power of 79% (α-level = 0.05)

Results

Complete Protocol

The shear modulus ranged from 3.9±0.6 kPa for GL to 4.0±0.7 kPa for VM at rest and from 5.6±1.3 kPa for GL to 7.6±1.4 kPa for VM under passive stretching.

The shear modulus inter-operator reproducibility (Table 1) ranged from 0.3 kPa for $\mathrm{GL_R}$ to 0.7 kPa for $\mathrm{VM_S}$ and intra-operator repeatability, ranging from 0.3 kPa for $\mathrm{GL_R}$ to 0.6 kPa for $\mathrm{GL_S}$. Inter-operator coefficient of variation (CV) ranged from 8% for $\mathrm{GL_R}$ to 11% for $\mathrm{GL_S}$ and intra-operator CV ranged from 7% for $\mathrm{GL_R}$ to 8% kPa for $\mathrm{VM_R}$.

Inter-operator ICC ranged from 0.87 for $\mathrm{GL_S}$ to 0.91 for $\mathrm{GL_R}$, while intra-operator ICC ranged from 0.91 for $\mathrm{VM_R}$ to 0.94 for $\mathrm{GL_S}$. A significant influence of the position on shear modulus was found for both muscles (VM: $p < 0.002$ and GL: $p < 0.002$).

Table 1: Inter- and intra-operator reliability of the shear modulus measurement computed (10 subjects, 3 operators, 6 measures each, rested and stretched muscles: VM and GL).

		VM$_{\mathrm{R}}$	VM$_{\mathrm{S}}$	GL$_{\mathrm{R}}$	GL$_{\mathrm{S}}$	Globality
MEAN (kPa)	(kPa)	4.0	7.6	3.9	5.6	5.3
SD (kPa)	(kPa)	0.7	1.4	0.6	1.3	1.8
Inter-operator	(kPa)	0.4	0.7	0.3	0.6	0.5
reproducibility	CV (%)	9	9	8	11	9
	ICC	0.88	0.90	0.91	0.87	
Intra-operator	(kPa)	0.3	0.6	0.3	0.5	0.4
repeatability	CV (%)	8	8	7	8	8
	ICC	0.91	0.92	0.92	0.94	

For all muscles pooled, inter-operator reproducibility was 0.5 kPa (CV 9%) and intra-operator repeatability was 0.4 kPa (CV 8%). Thus, 95% confidence interval was lower than 0.8 kPa for VM$_{\mathrm{R}}$ and GL$_{\mathrm{R}}$ and lower than 1.4 kPa for VM$_{\mathrm{S}}$ and GL$_{\mathrm{S}}$.

Bland-Altman diagrams (figure 3) show the level of agreement between the measured values and the dispersion of the measurement.

The 95% CI was lower than 0.8 kPa when muscle was at rest and lower than 1.4 kPa when muscle was stretched.

Reduced Protocol

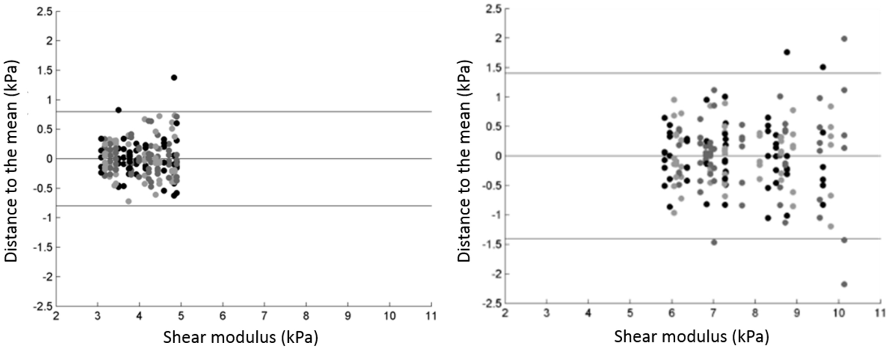
The shear modulus ranged from 3.9±0.6 kPa for VM to 6.6±1.4 kPa for SOL at rest and from 5.1±1.4 kPa for ST to 13.9±3.9 kPa for RF under passive stretching.

Inter-operator reproducibility ranged from 0.4 kPa for GM$_{\mathrm{R}}$ to 1.6 kPa for BF$_{\mathrm{S}}$, while intra-operator repeatability ranged from 0.2 kPa for VM$_{\mathrm{R}}$ to 1.4 kPa for BF$_{\mathrm{S}}$ (Table 2). Inter-operator CV ranged from 7% for VM$_{\mathrm{S}}$ to 24% for ST$_{\mathrm{S}}$ and intra-operator CV ranged from 5% for GM$_{\mathrm{S}}$ to 15% for SOL$_{\mathrm{S}}$. A significant influence of the position on shear modulus was found for all muscles ($p < 0.001$), except for SOL, which was slightly over the significance threshold ($p = 0.059$, Table 2).

For all muscles pooled, inter-operator reproducibility was 1.1 kPa (CV 17%) and intra-operator repeatability was 0.8 kPa (CV 12%).

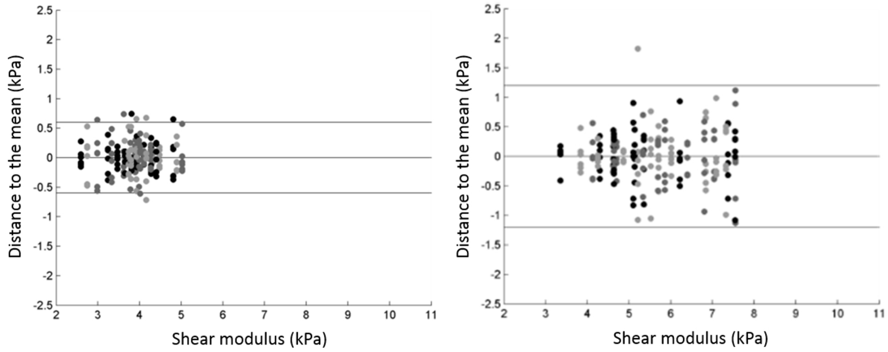
At rest, 6 muscles had a 95% CI between 0.6 and 1.4 kPa: RF, SAR, VL, VM, GL and GM. Five muscles have a 95% CI between 1.6 and 2.6 kPa: BF, GRA, SM, ST and SOL.

Measurement in stretched position was slightly less reliable, but not significantly (Wilcoxon signed-



(a) Bland-Altman diagram for VM at rest

(b) Bland-Altman diagram for VM passively stretched.



(c) Bland-Altman diagram for GL at reste

(d) Bland-Altman diagram for GL passively stretched.

Figure 3: Bland-Altman diagrams for the complete protocol applied in rested and stretched VM and GL, each operator is represented by a different grayscale point.

rank: p = 0.83). 3 muscles had a 95% CI lower than 1.4 kPa: VM, GL and GM. Six muscles had a 95% CI between 2.0 and 2.6 kPa: RF, SAR,SM, ST, VL and SOL. Two muscles had a 95% CI between 3.0 and 3.2 kPa: BF and GRA.

Table 2: Inter- and intra-operator reliability of the shear modulus measurement computed (10 subjects, 2 operators, 3 measures each) for rested and stretched muscles: BF, GRA, RF, SAR, SM, ST, VL, VM, GM, and GL and SOL..

		Thigh Muscles								Leg Muscles		
Muscle at Rest		BF_R	GRA_R	RF_R	SAR_R	SM_R	ST_R	VL_R	VM_R	GL_R	GM_R	SOL_R
MEAN	(kPa)	5.6	6.0	4.1	5.3	5.3	4.2	4.5	3.9	4.5	4.7	6.6
SD	(kPa)	1.4	1.7	0.6	1.1	1.5	1.0	1.0	0.6	0.9	0.7	1.4
Intra-operator	(kPa)	1.0	1.0	0.5	0.7	0.8	0.8	0.6	0.6	0.7	0.4	1.3
repeatability	CV (%)	18	16	12	17	15	20	12	14	15	9	20
Inter-operator	(kPa)	0.5	0.5	0.3	0.5	0.6	0.3	0.4	0.2	0.5	0.3	0.7
reproducibility	CV (%)	10	8	7	9	11	7	9	6	11	6	11
Muscle Stretched		BF_S	GRA_S	RF_S	SAR_S	SM_S	ST_S	VL_S	VM_S	GL_S	GM_S	SOL_S
MEAN	(kPa)	10.1	8.9	13.9	7.6	8.7	5.1	6.3	7.3	5.8	6.2	7.0
SD	(kPa)	2.1	1.9	3.9	1.4	1.5	1.4	1.6	0.9	0.8	1.2	1.7
Intra-operator	(kPa)	1.6	1.5	1.2	1.2	1.0	1.2	1.1	0.5	0.5	0.5	1.3
repeatability	CV (%)	16	17	8	15	12	24	18	7	9	9	19
Inter-operator	(kPa)	1.4	0.7	0.8	0.8	0.6	0.5	0.7	0.5	0.4	0.3	1.0
reproducibility	CV (%)	14	8	6	10	7	9	11	6	6	5	15
At Rest vs Stretched p-value		$<10^{-3}$	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$	0.013	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$	$<10^{-3}$	0.059

Discussion

The aim of the present study was to evaluate the inter-operator reproducibility and intra-operator repeatability of a measurement protocol to assess shear modulus of the lower limb muscles at rest and during passive stretching. Repeatability and reproducibility were determined in a two-phase study; the first one, it was ascertained on two muscles (vastus medialis and gastrocnemius lateralis) that there was no effect from the operator or from the patient position on the measurement reliability. The second one, measurement reliability was confirmed in a larger number of muscles. This second phase was necessary because muscle size and anatomical position (e.g., its depth) can affect SWE measurements.

The small differences in reproducibility between complete and reduced protocols can be explained by the lower number of operators and measurement repetitions, which decreased the degrees of freedom (from 17 to 5) for each muscle. Complete protocol is more representative of the actual reliability of the measurement due to the higher number of measures. This complete protocol could not be applied to all muscles as it was very time consuming. However, it was necessary in order to determine that there was no operator effect on the measurements (which were performed by experienced operators) and that the same reliability could be obtained in all positions (Wilcoxon signed-rank: $p = 0.83$); once that was demonstrated, the reduced protocol allowed the estimation of the muscle-dependent uncertainty in the rest of the muscle.

A choice was made not to strictly control joint angles; subjects were asked to assume the given positions and they were instructed to be comfortable. The main objective of the present study was to evaluate the reliability of a compatible protocol with clinical routine. It was therefore chosen to let the patient assume a natural posture, which would be more easily reproduced during a routine clinical exam, unlike fixed-angle postures which might need an external fixture to be reproduced. It is interesting to notice that intra-subject variability was relatively small (8%) even if joint angles were not controlled, thus confirming that small variations of joint angle produce small variation of muscle stiffness.

For the same reason, the number of measurements was kept to a minimum in the reduced protocol (i.e., 3 measurements in the longitudinal direction). Performing measurements in other direction and strict control of measurement orientation would allow a more thorough characterization of the tissue (Gennisson et al., 2010; Lee et al., 2012; Wang et al., 2013; Rouze et al., 2013), but it would significantly increase measurement time and complexity.

Shear modulus was significantly higher when muscle was stretched than at rest ($p < 0.05$) for all measured muscles, except for soleus ($p = 0.059$). This result was expected, given the mechanical non-linear behaviour of the muscle, and is in accordance with previous studies by Maïsetti et al. (2012) and Hug et al. (2013), who have found an increase of muscle shear modulus during passive stretching once the threshold of the slack length was passed. Soleus muscle is not involved in knee motion, the non-significant difference confirmed that altough the ankle joint angle was not strictly controlled, muscle stiffness varied little between positions.

On the anterior part of the thigh, shear modulus of RF increased more than VM or VL. RF is a bi-articular muscle: its origin is on the pelvis and its insertion is the quadriceps tendon. VM or VL are mono-articular muscles: their origins are on the femur and their insertion is on the quadriceps tendon. Thus, RF was more stretched in position 1 (corresponding to a supine position with an angle of approximately 90° between leg and thigh).

Reliability in SOL and BF was lower than in other muscles. The elastographic device had some difficulties in measuring SOL shear wave velocity for some subjects who had thick GL and GM. In order to improve reliability in such deep muscles (as SOL) measurements could be performed with a lower frequency probe, which suffers less from attenuation. Vastus intermedialis muscle was excluded from the study for the same reason: it is a deep muscle, and a reliable signal could not be obtained in it. This is a limitation of the technique.

Concerning BF, measurement reliability was affected by the fact that this muscle is one of the thinnest of the thigh. The small thickness makes it difficult to distinguish both heads; therefore the positioning of the probe parallel to the fibers was not always straightforward.

The intra-operator muscle reliability was less than 8% and the inter-operator muscle reliability was less than 11% for the complete protocol. These results are close to those provided by Lacourpaille et al. (2012), who found intra-operator repeatability lower than 6% and inter-operator reliability lower than 11.5%. Values measured by Lacourpaille et al. (2012) were lower of about 1 kPa compared to those presented in this study at rest (GM: 2.99 vs 4.7, VL: 3.26 vs 4.5, RF: 3.23 vs 4.1). Both studies were performed on homogeneous population healthy young subjects. The values could be explained by the difference between the practiced sports or the fat infiltration in muscles. Larger cohorts are needed to properly determine reference values between populations. An overweight or obese BMI should increase the fat infiltration and should probably increase the shear modulus as the young modulus of fat is higher than muscle (Dubuis et al., 2012).

Bensamoun et al. (2006) have evaluated muscle shear modulus with magnetic resonance elastography at rest at 3.73 ± 0.85 kPa for VL, 3.91 ± 1.15 kPa for VM, and 7.53 ± 1.63 kPa for SAR. These values are close to those measured in the present study (table 2). An intra-day coefficient of variation of two subjects was estimated at 19.4% for GL and 15.7% for biceps brachii by Ringleb et al. (2007). If intra-day variation is disregarded, SWE appeared slightly more reliable than magnetic resonance elastography, as noted by Lacourpaille et al. (2012).

No significant correlation was found between physiological parameters (age, femur and tibia length, BMI, height and weight) and shear modulus of rested or stretched muscles. This result is in accordance with several previous studies who have found no correlation of shear modulus with physiological parameters (Aubry et al., 2013; Gennisson et al., 2005; Debernard et al., 2011), altough correlations were observed between BMI and trapezius muscle stiffness (Kuo et al., 2013) or with biceps brachii and biceps femoris muscles stiffness (Berko et al., 2013). However, muscle shear modulus depends on a large number of parameters, the study of a small specific and heterogeneous sample does not suffice to evaluate correlations; a large scale study have to be considered.

A slight increase in muscle shear modulus was observed in preliminary measurements when subjects were supine with the legs hanging from the table; this phenomenon was eliminated by asking the subject to pause and walk between measurements sessions. In clinical routine, measurement can be performed in significant shorter time, so the shear modulus increase should not represent an issue. A routine clinical measurement in a given muscle in two positions (relaxed and stretched) could last about 5 minutes. This increase is in contradiction with the passive viscoelastic behavior of the muscle, so it was probably cause by slight muscle contraction due to discomfort. Viscolelastic behavior of muscle could potentially be further investigated with SWE (Gennisson et al., 2010).

The significant difference between measurement at rest and during passive stretching could be used to quantify the differences between populations (healthy, athlete, pathologic...). Work is in progress for the application of the protocol on subjects with neuromuscular diseases.

SWE appeared as an appropriate tool to evaluate shear modulus of lower limb muscles, with a good reproducibility, especially at rest, and its capability of characterizing muscle properties both at rest and during passive stretching.

Acknowledgements

The authors are grateful to the ParisTech BiomecAM chair program on subject-specific musculoskeletal modelling for funding.

Conflict of interest statement

Jean-Luc Gennisson is a scientific consultant for SuperSonic Imagine, and Mickael Tanter is cofounder and shareholder of SuperSonic Imagine (Aix-en-Provence, France). The other authors do not have any conflicting financial interests.

References

Aubry S, Risson JR, Kastler A, Barbier-Brion B, Siliman G, Runge M, Kastler B. Biomechanical properties of the calcaneal tendon in vivo assessed by transient shear wave elastography. Skeletal Radiology, 2013:1143–1150.

Basford JR, Jenkyn TR, An KN, Ehman RL, Heers G, Kaufman KR. Evaluation of healthy and diseased muscle with magnetic resonance elastography. Archives of Physical Medicine and Rehabilitation, 2002;83:1530–1536.

Bensamoun SF, Ringleb SI, Chen Q, Ehman RL, An KN, Brennan M. Thigh muscle stiffness assessed with magnetic resonance elastography in hyperthyroid patients before and after medical treatment. Journal of Magnetic Resonance Imaging, 2007;26:708–713.

Bensamoun SF, Ringleb SI, Littrell L, Chen Q, Brennan M, Ehman RL, An KN. Determination of thigh muscle stiffness using magnetic resonance elastography. Journal of Magnetic Resonance Imaging, 2006;23:242–247.

Bercoff J, Tanter M, Fink M. Supersonic shear imaging: a new technique for soft tissue elasticity mapping. IEEE Transactions on Ultrasonics Ferroelectrics and Frequency Control, 2004;51:396–409.

Berko NS, Fitzgerald EF, Amaral TD, Payares M, Levin TL. Ultrasound elastography in children: Establishing the normal range of muscle elasticity. Pediatric Radiology, 2013.

Botanlioglu H, Kantarci F, Kaynak G, Unal Y, Ertan S, Aydingoz O, Erginer R, Unlu M, Mihmanli I, Babacan M. Shear wave elastography properties of vastus lateralis and vastus medialis obliquus muscles in normal subjects and female patients with patellofemoral pain syndrome. Skeletal Radiology, 2013;42:659–666.

Cornu C, Goubel F, Fardeau M. Muscle and joint elastic properties during elbow flexion in Duchenne muscular dystrophy. The Journal of Physiology, 2001;533:605–616.

Debernard L, Robert L, Charleux F, Bensamoun SF. Characterization of muscle architecture in children and adults using magnetic resonance elastography and ultrasound techniques. Journal of Biomechanics, 2011;44:397–401.

Dubuis L, Avril S, Debayle J, Badel P. Identification of the material parameters of soft tissues in the compressed leg. Computer Methods in Biomechanics and Biomedical Engineering, 2012;15:3–11.

Eby SF, Song P, Chen S, Chen Q, Greenleaf JF, An KN. Validation of Shear Wave Elastography in Skeletal Muscle. Journal of Biomechanics, 2013;16:2381–2387.

Gennisson JL, Catheline S, Chaffaï S, Fink M. Transient elastography in anisotropic medium: application to the measurement of slow and fast shear wave speeds in muscles. Journal of the Acoustical Society of America, 2003;114:536–541.

Gennisson JL, Cornu C, Catheline S, Fink M, Portero P. Human muscle hardness assessment during incremental isometric contraction using transient elastography. Journal of Biomechanics, 2005;38:1543–1550.

Gennisson JL, Deffieux T, Macé E, Montaldo G, Fink M, Tanter M. Viscoelastic and anisotropic mechanical properties of in vivo muscle tissue assessed by supersonic shear imaging. Ultrasound in Medicine & Biology, 2010;36:789–801.

Hoang PD, Gorman RB, Todd G, Gandevia SC, Herbert RD. A new method for measuring passive length-tension properties of human gastrocnemius muscle in vivo. Journal of Biomechanics, 2005;38:1333–1341.

Hopkins WG. Measures of reliability in sports medicine and science. Sports Medicine, 2000;30:1–15.

Hug F, Lacourpaille L, Maïsetti O, Nordez A. Slack length of gastrocnemius medialis and Achilles tendon occurs at different ankle angles. Journal of Biomechanics, 2013;46:2534–2538.

Koo TK, Guo JY, Cohen JH, Parker KJ. Relationship between shear elastic modulus and passive muscle force: An ex-vivo study. Journal of Biomechanics, 2013;46:2053–2059.

Kot BCW, Zhang ZJ, Lee AWC, Leung VYF, Fu SN. Elastic Modulus of Muscle and Tendon with Shear Wave Ultrasound Elastography: Variations with Different Technical Settings. PLoS ONE, 2012;7.

Kuo WH, Jian DW, Wang TG, Wang YC. Neck Muscle Stiffness Quantified by Sonoelastography is Correlated with Body Mass Index and Chronic Neck Pain Symptoms. Ultrasound in Medicine & Biology, 2013;39:1356–1361.

Lacourpaille L, Hug F, Bouillard K, Hogrel JY, Nordez A. Supersonic shear imaging provides a reliable measurement of resting muscle shear elastic modulus. Physiological Measurement, 2012;33:19–28.

Lee WN, Larrat B, Pernot M, Tanter M. Ultrasound elastic tensor imaging: comparison with MR diffusion tensor imaging in the myocardium. Physics in Medicine and Biology, 2012;57:5075–95.

Maïsetti O, Hug F, Bouillard K, Nordez A. Characterization of passive elastic properties of the human medial gastrocnemius muscle belly using supersonic shear imaging. Journal of Biomechanics, 2012;45:978–984.

Nordez A, Fouré A, Dombroski EW, Mariot JP, Cornu C, McNair PJ. Improvements to Hoang et al.'s method for measuring passive length-tension properties of human gastrocnemius muscle in vivo. Journal of biomechanics, 2010;43:379–382.

Ringleb SI, Bensamoun SF, Chen Q, Manduca A, An KN, Ehman RL. Applications of magnetic resonance elastography to healthy and pathologic skeletal muscle. Journal of Magnetic Resonance Imaging, 2007;25:301–309.

Rouze NC, Wang MH, Palmeri ML, Nightingale KR. Finite element modeling of impulsive excitation and shear wave propagation in an incompressible, transversely isotropic medium. Journal of Biomechanics, 2013;46:2761–8.

Royer D, Gennisson JL, Deffieux T, Tanter M. On the elasticity of transverse isotropic soft tissues. Journal of the Acoustical Society of America, 2011;129:2757–60.

Sarvazyan AP, Rudenko OV, Swanson SD, Fowlkes J, Emelianov SY. Shear wave elasticity imaging: a new ultrasonic technology of medical diagnostics. Ultrasound in Medicine & Biology, 1998;24:1419–1435.

Shinohara M, Sabra K, Gennisson JL, Fink M, Tanter M. Real-time visualization of muscle stiffness distribution with ultrasound shear wave imaging during muscle contraction. Muscle Nerve, 2010;42:438–441.

Smith LR, Lee KS, Ward SR, Chambers HG, Lieber RL. Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased in vivo sarcomere length. The Journal of physiology, 2011;589:2625–2639.

Wang M, Byram B, Palmeri M, Rouze N, Nightingale K. Imaging transverse isotropic properties of muscle by monitoring acoustic radiation force induced shear waves using a 2-D matrix ultrasound array. IEEE Transactions on Medical Imaging, 2013;32:1671–84.