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NOTE

Supersonic shear imaging provides a reliable measurement of resting muscle shear elastic modulus

Lilian Lacourpaille[1], **François Hug**[1], **Killian Bouillard**[1], **Jean-Yves Hogrel**[2] **and Antoine Nordez**[1]

[1] EA 4334 'Motricité, Interactions, Performance', Université de Nantes, Nantes, France
[2] Institut de Myologie, GH Pitié-Salpêtrière, Paris, France

E-mail: francois.hug@univ-nantes.fr

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Abstract

The aim of the present study was to assess the reliability of shear elastic modulus measurements performed using supersonic shear imaging (SSI) in nine resting muscles (*i.e. gastrocnemius medialis, tibialis anterior, vastus lateralis, rectus femoris, triceps brachii, biceps brachii, brachioradialis, adductor pollicis obliquus and abductor digitimi minimi*) of different architectures and typologies. Thirty healthy subjects were randomly assigned to the intra-session reliability ($n = 20$), inter-day reliability ($n = 21$) and the inter-observer reliability ($n = 16$) experiments. Muscle shear elastic modulus ranged from 2.99 (*gastrocnemius medialis*) to 4.50 kPa (*adductor digitimi minimi and tibialis anterior*). On the whole, very good reliability was observed, with a coefficient of variation (CV) ranging from 4.6% to 8%, except for the inter-operator reliability of *adductor pollicis obliquus* (CV = 11.5%). The intraclass correlation coefficients were good (0.871 $\pm$ 0.045 for the intra-session reliability, 0.815 $\pm$ 0.065 for the inter-day reliability and 0.709 $\pm$ 0.141 for the inter-observer reliability). Both the reliability and the ease of use of SSI make it a potential interesting technique that would be of benefit to fundamental, applied and clinical research projects that need an accurate assessment of muscle mechanical properties.

Keywords: elastography, elasticity, stiffness, neuromuscular disorders, follow-up

(Some figures may appear in colour only in the online journal) **Q1**

1. Introduction

Various studies have shown that muscle mechanical properties are altered in several neuromuscular diseases such as hyperthyroidism (Bensamoun *et al* 2007), Duchenne muscular dystrophy (Cornu *et al* 1998, 2001), multiple sclerosis (Rizzo *et al* 2004), spasticity (Barrett 2011) or myofascial pain syndrome (Simons 2004). These results indicate that muscle mechanical properties could provide clinically viable information to follow the effects of neuromuscular disorders or potential improvements due to various treatments. However, classical methods of the evaluation of muscle mechanical properties (e.g., sinusoidal perturbations, quick release) involve specific experimental apparatus and dynamometers designed for a given articulation. Consequently, changes in the properties of different muscles, more or less affected by pathology, cannot be easily assessed using the same experimental setup. In addition, stiffness measurements require that patients perform isometric maximal and submaximal voluntary contractions which can be painful, difficult to perform and deleterious (Agre and Sliwa 2000).

Compared to contraction, the muscle resting state is easier to standardize, painless and does not require any effort from the patients. Therefore, using methods that could involve measurements performed at rest would be beneficial in clinical practice, e.g., for the longitudinal follow-up of neuromuscular disorders. In this way, myotonometry has been shown to be a valid and reliable measurement of muscle tone (e.g., Lidstrom *et al* 2009, Gubler-Hanna *et al* 2007, Leonard *et al* 2003, Ditroilo *et al* 2011). However, this technique cannot evaluate deep muscles (Leonard *et al* 2003) and the measurement at rest can be influenced by skinfold thickness and skin stiffness. Using magnetic resonance elastography (MRE) the stiffness measurement can be focused on the muscle tissues. This technique has been used to evaluate mechanical properties of pathological muscles at rest (Basford *et al* 2002, Ringleb *et al* 2007). However, it has inherent limitations that restrict its use for clinical evaluation in a number of muscles, partly due to complex methodologies, acquisition durations, transportability, availability and cost of equipment (Shinohara *et al* 2010). Moreover, the inter-day reliability of MRE (e.g., coefficient of variation reported at 16% in Ringleb *et al* 2007) reduces its ability to detect subtle structural changes over time.

Using supersonic shear imaging (SSI; Bercoff *et al* 2004), these limitations are partly resolved. Indeed, the measurement is performed using a handheld echographic probe, and results can be obtained in real time. Consequently, an evaluation can be easily and rapidly performed on several muscles (Gennisson *et al* 2010, Shinohara *et al* 2010). The aim of the present study was to assess the intra-session reliability, the inter-day reliability and the inter-observer reliability of shear elastic modulus measurements performed using SSI in various resting muscles with different architectures and typologies. This step appears necessary before using this technique in clinical practice, particularly within the framework of follow-up studies.

2. Material and methods

2.1. Subjects

Thirty healthy subjects volunteered to participate in the present study (25 men, 5 women; aged 25 ± 7 years, height 176 ± 8 cm, weight 70.3 ± 13.1 kg). Subjects were randomly assigned to the intra-session reliability ($n = 20$), inter-day reliability ($n = 21$) and/or inter-observer reliability ($n = 16$) experiments. Participants were informed of the purpose of the study and methods used before providing written consent. The experimental design of the study was approved by the local Ethical Committee (Nantes Ouest IV—CPP-MIP-001), and was conducted in accordance with the Declaration of Helsinki.

2.2. Materials

An Aixplorer ultrasonic scanner (version 4.0; Supersonic Imagine, Aix-en-Provence, France), coupled with a linear transducer array (4–15 MHz, SL15-4, Supersonic Imagine, Aix-en-Provence, France) was used in SSI mode (musculo-skeletal preset) as previously described (Bercoff *et al* 2004, Tanter *et al* 2008). Briefly, a pushing beam occurs in order to generate shear wave within the muscle. Then, ultrafast echographic sequences are performed to catch the shear wave propagation. By applying one-dimensional cross-correlation technique of successive radio frequency signals along the ultrasound beam axis, the shear wave displacement field as a function of time is retrieved. Then, using a time of flight algorithm on the displacement movies, the shear waves speed is determined in each pixel of the image. Assuming a linear elastic behavior, a shear elastic modulus (μ) was calculated using Vs as follows:

$$\mu = \rho \mathrm{Vs}^2, \tag{1}$$

where ρ is the muscle mass density ($1000\ \mathrm{kg \cdot m^{-3}}$).

The hypothesis of linear material is classical and well accepted in muscle elastographic studies, for both transient elastography (e.g., Bercoff *et al* 2004, Catheline *et al* 2004, Deffieux *et al* 2009, Gennisson *et al* 2003, 2005, Nordez *et al* 2008, Tanter *et al* 2008) and MRE (e.g., Dresner *et al* 2001, Bensamoun *et al* 2006, 2008, Debernard *et al* 2011). In fact, the shear wave amplitude is very small, and nonlinear effects can be neglected. In addition, equation (1) considers a purely elastic material and implicitly neglects viscous effects. The influence of viscosity on shear wave velocity measurements has been previously studied (Catheline *et al* 2004, Deffieux *et al* 2009). Deffieux *et al* (2009) showed that, when measured longitudinally using SSI, the shear wave velocity is almost independent from the frequency of the mechanical shock, indicating no significant viscous effects. This result is in accordance with those of Catheline *et al* (2004) using the one-dimensional transient elastography technique. Therefore, the shear elastic modulus measured in the present study is little affected by viscous effects, and could be neglected. Maps of the shear elastic modulus were obtained at 1 Hz with a spatial resolution of 1×1 mm.

2.3. Experimental protocol

Nine muscles with different architecture (pinnate/fusiform) and fiber type compositions (slow/fast fibers) were measured in a random order: *gastrocnemius medialis* (GM), *tibialis anterior* (TA), *vastus lateralis* (VL), *rectus femoris* (RF), *triceps brachii* (TB), *biceps brachii* (BB), *brachioradialis* (BR), *adductor pollicis obliquus* (APO) and *abductor digitimi minimi* (ADM). Subject positioning and transducer placement are depicted for each muscle in figure 1. To standardize the muscle length, joint angles were set to place the muscle in a position as slack as possible. For the *gastrocnemius lateralis* measurements (figure 1(A)), subjects were lying prone, the operator positioned the leg against him involving a knee flexion (90°) and the ankle in neutral position. For *tibialis anterior* (figure 1(B)), subjects laid supine with knees fully extended and the ankle in neutral position. For *vastus lateralis* (figure 1(C)) and *rectus femoris* (figure 1(D)) subjects were seated, hips flexed (90°) and knee fully extended. For *triceps brachii* (figure 1(E)), subjects were lying prone with knees fully extended, the arms outstretched along the body with the hands in supination. For the assessment of *biceps brachii* (figure 1(F)) and *brachioradialis* (figure 1(G)), subjects were seated, elbow flexed (90°) with their forearms supported on a table and the hands in supination. For *adductor pollicis obliquus*

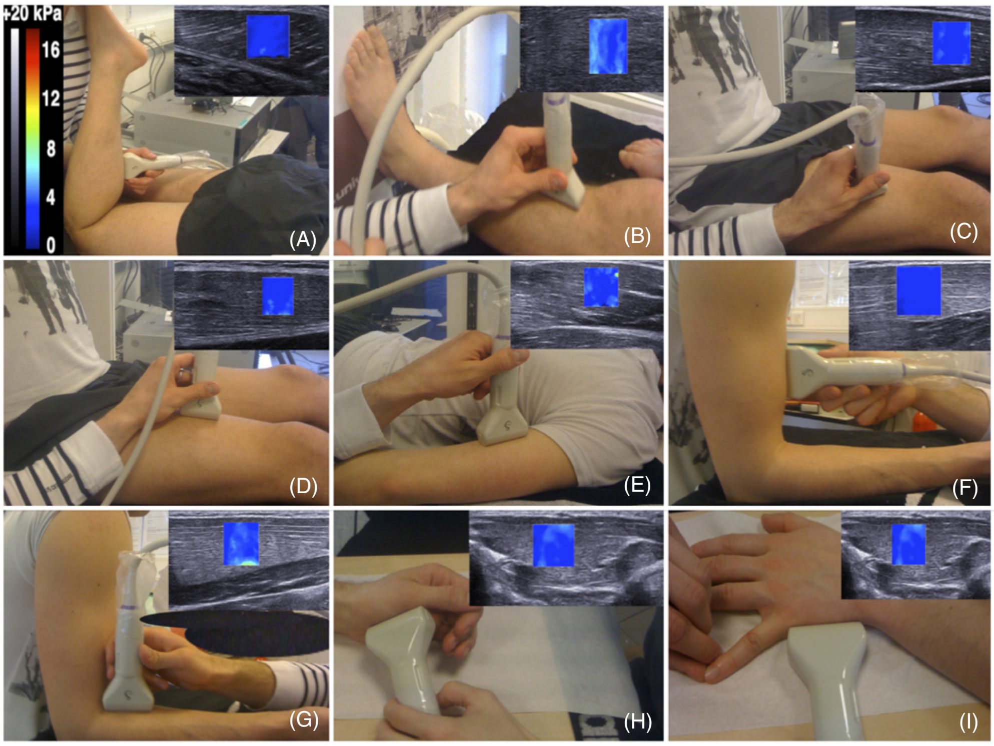

Figure 1. Subject and probe positions during measurements for (A) gastrocnemius medialis, (B) tibialis anterior, (C) vastus lateralis, (D) rectus femoris, (E) triceps brachii, (F) biceps brachii, (G) brachioradialis, (H) adductor pollicis obliquus and (I) abductor digitimi minimi. Images and shear elasticity maps obtained with the ultrasound scanner are also displayed on the upper right corner. Q2

(figure 1(H)) the hands of the subjects were placed on the lateral edge with the thumb rested on the others flexed fingers. For *abductor digitimi mini* (figure 1(I)), the hand was placed on a table in pronation and the operator manually maintained the little finger in maximal abduction.

Subjects were asked to stay as relaxed as possible. Since a very slight contraction could be observed in real time on the shear elastic modulus map, the acquisition was performed only when a stable shear modulus value was observed. The probe was orientated in the plane of the muscle fascicles and perpendicularly to the skin. Appropriate probe alignment was achieved when several fascicles could be traced without interruption across the image (Blazevich 2006). In a preliminary experiment, a temporal variability up to 5% was found for the shear elastic modulus at rest. Therefore, the muscle elastic modulus was averaged over ten measurements (i.e. 10 s). The entire protocol lasted approximately 20 min.

In order to assess the intra-session reliability, the inter-day reliability and the inter-observer reliability, the protocol was performed twice by the same experimenter (experimenter A) on the same day, with a 15 min rest period between sessions of measurement, on two different days by the same experimenter (experimenter A) at the same time of the day, and on the same day by two experimenters (experimenters A and B) with a 15 min rest between sessions of measurement, respectively.

2.4. Data processing

The data processing was blind and performed using the software of the Aixplorer scanner. Actually, the Aixplorer scanner provided Young's modulus measurement (i.e. $E = 3\rho \mathrm{Vs}^2$). Since the Young's modulus calculation requires the assumption of an isotropic material, which is obviously not true for the muscle (Royer *et al* 2001), all the measurements were divided by 3 to obtain the shear elastic modulus as in equation (1). For each measure, the shear elastic modulus was averaged over a homogeneous circular region. As recommended by Hopkins (2000), intra-session reliability, inter-day reliability and inter-observer reliability were assessed using the intraclass correlation coefficient (ICC(2,1), two way random, absolute agreement), the standard error in measurement (SEM) and the coefficient of variation (CV). Temporal variability was assessed with the standard deviation and the CV for the ten measurements. One-way ANOVAs with repeated measures were performed to determine the effect of muscle on the shear elastic modulus and the difference between conditions (intra-, inter- and inter-observer reliability) for CV, SEM and ICC. Post hoc analyses were performed using the Tukey's method. The level of significance in the present study was set at $P < 0.05$. Q3 Q4

3. Results

Depending on the muscle, the mean temporal variability over the ten measurements ranged from 0.15 to 0.18 kPa for SEM, from 2.9% to 5.0% for CV and 0.702 to 0.943 for ICC. The averaged shear elastic modulus ranged from 2.99 kPa for the GM, to 4.50 kPa for the ADM (table 1). ANOVA revealed a significant effect of muscle on the shear elastic modulus ($P < 0.001$).

The results of reliability experiments are shown in table 1. For the intra-session reliability, the mean ICC value was 0.871 $\pm$ 0.045 (ranged from 0.811 to 0.950 for TA and GM, respectively), and the CV was 5.0 $\pm$ 0.7% (ranged from 3.6% to 6.0% for ADM and APO, respectively), corresponding to a SEM of 0.18 $\pm$ 0.03 kPa. Results were similar for the inter-day reliability with ICC values slightly lower than for that observed for the intra-session reliability (0.815 $\pm$ 0.065, ranged from 0.690 to 0.922 for BR and GM, respectively), and a CV value of 6.0 $\pm$ 0.9% (ranged from 4.7% to 8.0% for RF and TB, respectively), corresponding to a SEM of 0.21 $\pm$ 0.04 kPa. Of note, the ICC values were lower than 0.8 for the VL (0.740), the TB (0.792) and the BR (0.690). Concerning the inter-observer reliability, the CV values were also lower than 8.0% except for the APO (11.5%), but the ICC values were lower than that observed for intra-session reliability and inter-day reliability (mean 0.709 $\pm$ 0.141, range 0.421–0.944). Overall, intra-session reliability was significantly higher (for both CV and ICC) than inter-observer reliability.

4. Discussion

The present study was designed to assess the reliability of muscle shear elastic modulus measurements performed in various muscles at rest using SSI. On the whole, despite a wide range of typologies and different kinds of architecture in the nine investigated muscles, an excellent reliability was observed. Satisfactory results were obtained with CV values lower than 8%, except for the inter-observer reliability of the APO muscle. For this muscle, results of the present study suggest that shear elastic modulus values should be considered with more caution. For some muscles, ICC values were lower than 0.8. Since a relatively homogeneous population was studied in the present study (healthy young subjects), this can be explained by the small inter-individual variations, and, considering CV values, these ICCs could be

Table 1. Reliability results obtained in the three different experiments (intra-session reliability, inter-day reliability and inter-operator reliability).

		GM	TA	VL	RF	TB	BB	BR	APO	ADM	Mean
Mean shear elastic modulus values ($n = 30$)	Mean (kPa)	2.99	4.50	3.26	3.23	3.05	3.11	3.46	3.80	4.50	3.54
	SD (kPa)	0.57	0.54	0.42	0.43	0.52	0.42	0.42	0.69	0.65	0.52
Intra-session reliability ($n = 20$)	SEM (kPa)	0.14	0.22	0.20	0.17	0.15	0.17	0.17	0.23	0.17	0.18
	CV (%)	4.6	4.6	5.6	4.7	5.1	5.6	5.0	6.0	3.6	5.0
	ICC	0.950	0.811	0.822	0.829	0.905	0.868	0.858	0.876	0.927	0.872
Inter-day reliability ($n = 21$)	SEM (kPa)	0.17	0.23	0.23	0.15	0.23	0.17	0.20	0.25	0.28	0.21
	CV (%)	5.5	5.3	6.3	4.7	8.0	5.6	5.6	6.3	6.5	6.0
	ICC	0.922	0.809	0.740	0.871	0.792	0.832	0.690	0.849	2.829	0.815
Inter-operator reliability ($n = 16$)	SEM (kPa)	0.19	0.29	0.21	0.21	0.21	0.11	0.15	0.41	0.10	0.21
	CV (%)	6.6	7.1	6.4	6.8	7.6	3.5	4.5	11.5	6.8	6.8
	ICC	0.728	0.616	0.665	0.679	0.699	0.944	0.878	0.421	0.748	0.709

n: number of subject involved in each experiment; SD: standard deviation; SEM: standard error in measurement; CV: coefficient of variation; ICC: intra-class correlation coefficient; GM: *gastrocnemius medialis;* TA: *tibialis anterior*; VL: *vastus lateralis*; RF: *rectus femoris*; TB: *triceps brachii*; BB: *biceps brachii*; BR: *brachioradialis*; APO: *adductor pollicis obliquus*; ADM: *abductor digitimi minimi*.

Table 2. Comparison of mean shear elastic modulus between muscles.

	ADM	APO	BB	BR	GM	RF	TA	TB	VL
ADM		$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	1.000	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$
APO	$\mathbf{P < 0.001}$		$\mathbf{P < 0.001}$	0.237	$\mathbf{P < 0.001}$	**0.001**	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	**0.003**
BB	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$		0.221	0.991	0.995	$\mathbf{P < 0.001}$	1.000	0.980
BR	$\mathbf{P < 0.001}$	0.237	0.221		**0.016**	0.763	$\mathbf{P < 0.001}$	0.063	0.873
GM	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	0.991	**0.016**		0.695	$\mathbf{P < 0.001}$	1.000	0.548
RF	$\mathbf{P < 0.001}$	**0.001**	0.995	0.763	0.695		$\mathbf{P < 0.001}$	0.916	1.000
TA	1.000	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$		$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$
TB	$\mathbf{P < 0.001}$	$\mathbf{P < 0.001}$	1.000	0.063	1.000	0.916	$\mathbf{P < 0.001}$		0.825
VL	$\mathbf{P < 0.001}$	**0.003**	0.980	0.873	0.548	1.000	$\mathbf{P < 0.001}$	0.825	

P values corresponding to between muscle differences. Significant differences ($P < 0.05$) are identified in bold. GM: *gastrocnemius medialis;* TA: *tibialis anterior*; VL: *vastus lateralis*; RF: *rectus femoris*; TB: *triceps brachii*; BB: *biceps brachii*; BR: *brachioradialis*; APO: *adductor pollicis obliquus*; ADM: *abductor digitimi minimi.*

considered as unproblematic (Hopkins 2000). Considering the CV, the SSI method shows a little bit lower reliability than myotonometry (i.e. 6% for inter-day reliability in our study versus 4.9% reported by Ditroilo *et al* (2011)). However, compared to the reliability of the MRE technique reported by Ringleb *et al* (2007) (CV of 16%), the reliability of the SSI technique seems better for muscles at rest, indicating that this technique could be used to study even small changes in muscle shear elastic modulus.

A moderate temporal variability was observed (between 0.15 and 0.18 kPa, representing CV values between 2.9% and 5.0%). This variability can be due to both methodological factors such as slight probe motion, variability inherent to the measurement or the difficulty to achieve a fully relaxed state. It would be possible to reduce the influence of potential slight probe motions by using an externally fixed bracket onto the skin (e.g., Fouré *et al* 2010). However, using these tools, it is more complicated to standardize the pressure of the probe on the skin since preliminary experiments showed that this pressure greatly influences the measured shear elastic modulus for superficial muscles. Therefore, in our experiment a water-soluble gel was applied to remove the need to contact the skin, thus eliminating the influence of the probe pressure (Blazevich 2006). The temporal variability results suggest that in these conditions, it is relevant to average shear elastic modulus on a sample (ten measurements in the present study) to improve the reliability of the measurements.

The muscle shear elastic modulus measurement performed using SSI is very sensitive to muscle contraction (Nordez and Hug 2010). In this latter study, it was shown that the shear elastic modulus was linearly related to electromyographic (EMG) activity level. The shear elastic modulus was doubled as soon as 3% of the maximal EMG was reached, compared to the resting state. Pilot studies revealed that this measurement was also highly dependent on the muscle length. As an example, the shear elastic modulus of the BB muscle was measured at rest with an elbow angle set at 50°, 90° (i.e. as described in figure 1), 135°, 180° (i.e. representing the full elbow extension) and the values were respectively 3.12, 3.23, 4.88 and 6.54 kPa. These results illustrate a high dependence on the muscle state (i.e. muscle length and muscle contraction level). Consequently, the protocol should be carefully standardized to obtain reliable measurements independent of the muscle contraction and stretching levels.

Since the muscle stiffness is linked to fiber typology (e.g., Petit *et al* 1990, Toursel *et al* 2002), it could be interesting to compare values obtained for the different muscles investigated in the present study with respect to their percentage of fast fiber that can be found in the literature (e.g., Johnson *et al* 1972). The ANOVA revealed a significant 'muscle' effect on the shear elastic modulus measurement. More precisely, table 2 depicts the results of the post-hoc analysis. However, it seems obvious that the muscle shear elastic modulus values depicted in table 1 (in ascending order: GM, TB, BB, RF, VL, BR, APO, TA, ADM) do not exactly fit to the muscle typologies (from the highest percentage of fast fibers to the highest percentage of slow fibers: TB, BR, VL, RF, BB, GM, ADM, TA, APO, Johnson *et al* 1972). Many other factors such as fat and collagen infiltrations should influence the shear elastic modulus. In addition, it is well known that, due to muscle anisotropy, muscle elastic moduli depend on the measured direction (Gennisson *et al* 2003, 2010, Royer *et al* 2001). Using the SSI technique, the modulus is measured along the main axis of the probe (Bercoff *et al* 2004, Gennisson *et al* 2010). Due to the pennation angle of some muscles, the measure of the modulus may have not been performed exactly along the muscle fiber direction. Therefore, the measured modulus also depends on the pennation angle and the comparison between muscles is complicated.

In comparison to other devices used as described in the literature for the assessment of muscle mechanical properties, two main advantages of the SSI technique are worth mentioning: (i) it can be used to rapidly investigate several individual muscles (e.g., nine muscles measured in about 20 min in our experiments); and (ii) it can be reliably used to measure the muscle at rest, indicating that the experiments are painless and do not require any constrains for the subjects. Note that the ability to measure the stiffness in the shortening direction of the muscle and thus the 'functional' stiffness is an advantage of SSI compared to myotonometry which measures stiffness perpendicularly to the shortening direction. In addition, the region of interest can be easily chosen using SSI, and therefore it is possible to focus the stiffness measurement on a given deep or superficial muscle. Thus, the depth and extra muscular adiposity do not affect the shear elastic modulus measurements. Therefore, this technique would be of benefit to fundamental and applied research projects that need an accurate assessment of muscle mechanical properties.

Some neuromuscular diseases such as dystrophies or neuropathies affect skeletal muscle structure (e.g., necroses, transition from fast to slow fibers, collagen infiltrations, etc Blake *et al* 2002) disturbing the muscle mechanical properties. These alterations can be assessed at rest (i.e. relaxed state). For instance, Bensamoun *et al* (2007) reported a significant increase in resting muscle stiffness after treatment in patients with hyperthyroidism. The speed and ease of use of SSI make it a potential interesting technique to follow the effects of neuromuscular disorders or potential improvements due to various treatments. However, before envisaging its use in clinical practice, its reliability needs to be tested in pathological populations.

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