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Olivier Maïsetti [a,b,*], François Hug [a], Killian Bouillard [a], Antoine Nordez [a]

[a] EA 4334, Laboratoire "Motricité, Interactions, Performance", Université de Nantes, UFR STAPS, 25 bis Bd Guy Mollet, BP 72206, Nantes F-44000, France
[b] Université Paris-Est Créteil Val de Marne, 61 avenue du Général de Gaulle, 94010 Créteil Cedex, France

ABSTRACT

The passive elastic properties of a muscle–tendon complex are usually estimated from the relationship between the joint angle and the passive resistive torque, although the properties of the different structures crossing the joint cannot be easily assessed. This study aimed to determine the passive mechanical properties of the gastrocnemius medialis muscle (GM) using supersonic shear imaging (SSI) that allows the measurement of localized muscle shear modulus (μ). The SSI of the GM was taken for 7 subjects during passive ankle dorsiflexion at a range of knee positions performed on an isokinetic dynamometer. The relationship between normalized μ and the length of the gastrocnemius muscle–tendon units (GMTU) was very well fitted to an exponential model ($0.944 < R^2 < 1$) to calculate muscle stiffness (α) and slack length (l_0). This relationship was compared to the normalized force–length relationship obtained using Hoang's model. In addition, the reliability of the μ-length obtained with the knee fully extended was calculated. The μ-length relationship was highly correlated with the force–length ($0.964 < R^2 < 0.992$) although muscle force was slightly underestimated (RMSE $= 31.0 \pm 14.7$ N, range: 7.8–56.0 N). α and l_0 measured with the knee extended were similar to that reconstructed from all knee angles and displayed good intra-session reliability (for α, SEM: 9.7 m^{-1}; CV: 7.5%; ICC: 0.652; for l_0, SEM: 0.002 m; CV: 0.4%; ICC: 0.992). These findings indicate that SSI may provide an indirect estimation of passive muscle force, and highlight its clinical applicability to evaluate the passive properties of mono- and bi-articular muscles.

1. Introduction

There is substantial evidence in the literature that the assessment of muscle mechanical properties allows a better understanding of muscle function and of the mechanisms responsible for muscle adaptations following acute or chronic interventions (e.g., Goubel and Lensel-Corbeil, 2003; Caiozzo, 2002). The passive mechanical properties are an important component of muscle function because they are related to the muscle extensibility (Gajdosik, 2001). In human experiments, passive torque–angle curves are classically characterized (e.g., Gajdosik, 2001; McNair et al., 2002; Magnusson et al., 1998; Nordez et al., 2008; 2009). However, several synergistic muscles, tissues (e.g., aponeurosis, tendon), and articular structures (Riemann et al., 2001) contribute to the passive torque. Thus, assessment of individual muscle mechanical properties in vivo remains challenging.

In that framework, Hoang et al. (2005) recently proposed an elegant method to estimate the passive force–length relationship of the human gastrocnemius muscle–tendon units (GMTU). This method consists of measuring passive ankle torques at a range of knee angles. Based on the reasonable assumption that the GMTU constitute the only structure that crosses both ankle and knee joints that it produces a significant passive torque during the ankle motion, an optimization is used to identify parameters of the force-length curve from the torque–angle data (Hoang et al., 2005; Nordez et al., 2010). However, this method is valid for multi-joint muscles and necessitates passive experiments at various different knee angles, which may limit its use in clinical practice and its applicability for other muscles.

In addition, this method has led to contradictory results, where the GMTU were slack over about one-quarter of its physiological range (Hoang et al., 2007), while other studies showed that they are slack over more than half of this range (Riener and Edrich 1999; Muraoka et al., 2004, 2005). Therefore, the estimation of muscle slack length, which usually corresponds to the length beyond which the muscle begins to develop passive elastic force, remains a topic of continued interest for researchers. Thus, an alternative method of evaluating passive muscle mechanical

properties would provide fundamental information on muscle behavior in vivo.

Elastographic methods were recently used to quantify in vivo muscle mechanical properties by measuring the propagation velocity of shear waves by imaging techniques. Using transient elastography, recent experiments showed that the shear elastic modulus of gastrocnemius muscle is partly related to ankle torque during a passive stretching ($0.69 < R^2 < 0.93$), but with low reliability (Nordez et al., 2008). This lack of reliability could be explained by some limitations of transient elastography (Nordez et al., 2008), which seem to be overcome with the supersonic shear imaging (SSI) technique (Bercoff et al., 2004; Gennisson et al., 2010). For instance, using SSI, Nordez and Hug (2010) reported a reliable linear relationship between the biceps brachii shear elastic modulus and its electromyographic (EMG) activity level during incremental isometric contractions. Thus, it seems that SSI may be a valuable tool for measuring the muscle shear elastic modulus during passive stretching. Muscle–tendon structures are characterized by a non-linear stress–strain relationship. Thus, Young modulus (its first derivative) increases with lengthening. Since muscle elastic modulii are increased when the tension or the stretching levels are increased (e.g., Fung, 1993; Nordez et al., 2008), it can be hypothesized that the shear elastic modulus measured using SSI could provide an indirect estimation of passive muscle tension.

Therefore, the present study was designed to assess the relationship between muscle shear elastic modulus measured using SSI and muscle length of the gastrocnemius muscle–tendon units. This relationship was used to provide an estimation of muscle slack length and stiffness and compared to the force–length relationship obtained using the Hoang model (Hoang et al., 2005). In addition, the reliability of one simple measure of likely clinical relevance was calculated with the knee fully extended.

2. Methods

2.1. Subjects

Seven sedentary healthy men (age: 27 ± 6 yr; height: 177 ± 6 cm; weight: 74 ± 10 kg) volunteered to participate in this study. They were given detailed information about the purpose and methods used in the present experimentation, and gave written consent. This study was conducted according to the Helsinki Statement, and has been approved by the local ethics committee.

2.2. Passive torque–angle relationships

An isokinetic dynamometer (Biodex 3 medical, Shirley, NY, USA) was used to measure ankle angle, joint angular velocity, and torque during passive ankle dorsiflexions as described by Nordez et al. (2010). Briefly, subjects were seated with the hip at 120° of flexion, and their chest was firmly secured with straps. The right thigh was supported by an adjustable pad and secured with a Velcro straps (Fig. 1). The height of the dynamometer and the seat were adjusted to ensure that the shank remained in a horizontal position at each knee angle that was monitored with an electronic goniometer placed on the medial side of the right leg (Penny and Giles, Biometrics Ltd., Gwent, UK). The axis of the dynamometer was aligned with the presumed axis of rotation of the right ankle. Torque and angle (both provided by the Biodex dynamometer) were collected at 1000 Hz with an analog/digital converter (Bagnoli 16, Delsys Inc, Boston, USA).

2.3. Surface EMG activity

Dry-surface electrodes (Delsys DE 2.1, Delsys Inc., Boston, MA, USA; 1 cm interelectrode distance) were placed on the gastrocnemius medialis, gastrocnemius lateralis, soleus and tibialis anterior. EMG signals were amplified ($\times 1000$) and digitized (6–400 Hz bandwidth) at a sampling rate of 1 kHz (Bagnoli 16, Delsys, Inc. Boston, USA). A requirement was that EMG activity was less than 1% of that recorded during maximal isometric contractions performed at the end of the testing session (Nordez et al., 2008; McNair et al., 2002). This was achieved in all subjects.
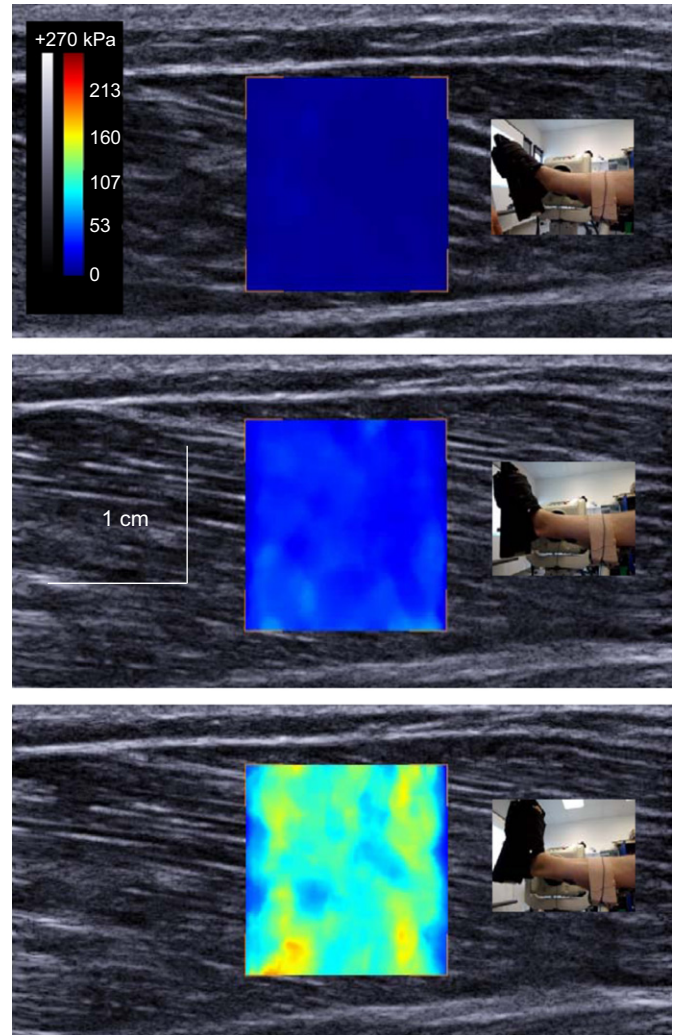

Fig. 1. Typical example of shear elastic modulus measurement with increased ankle dorsiflexion at 30° of plantarflexion, neutral position and 25° of dorsiflexion (top to bottom). The colored region represents the shear elasticity map with the scale to the left of the figure.

2.4. Muscle shear elastic modulus

An AixPlorer ultrasonic scanner (version 4.2, Supersonic Imagine, Aix en Provence, France) connected with a 60 mm 11 MHz linear transducer was used to measure the shear elastic modulus of the gastrocnemius medialis muscle. The scanner was set at the SSI (musculoskeletal preset) mode that has previously been described in detail (Bercoff et al., 2004; Gennisson et al., 2010). Briefly, it generates a remote radiation force through focused ultrasonic beams that induce the propagation of transient shear waves. An ultrafast echographic imaging sequence is then performed to acquire successive raw radiofrequency data at a very high frame rate (up to 20000 frames/s). One-dimensional cross-correlation of successive radio-frequency signals is used to calculate muscle displacements due to shear wave along the ultrasonic beam axis, and shear wave velocity is determined along the principal axis of the probe using a time-of-flight estimation. The probe was orientated parallel to 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 et al., 2006). Then, the probe was fitted into a custom made rigid cast, which restrained the probe from sliding (Fig. 1). Then, the shear elastic modulus (μ) in the main direction of the probe was calculated as follows (e.g., Bercoff et al., 2004; Gennisson et al., 2010):

$$\mu = \rho V_S^2 \tag{1}$$

where V_s is the speed of the shear wave and ρ is the muscle mass density (1000 kg/m^3).

Maps of the shear elastic modulus were obtained at 1 Hz (i.e., the maximal sampling rate of SSI measurements of the current version of the ultrasonic scanner)

with a spatial resolution of 1×1 mm^2. The averaged shear elastic modulus was calculated over each shear elastic modulus maps.

2.5. Protocol

Six different knee angles (0°, 15°, 30°, 45°, 60° and 80°, 0°=knee fully extended) were tested in a random order, and the 0° of knee angle was tested twice to assess the repeatability. The maximal range of motion (ROM) was measured for each knee angle during three slow dorsiflexions. Then, the subjects performed five slow (2°/s) passive loading/unloading cycles between 40° of plantarflexion and 80% of the maximal ROM in dorsiflexion at each selected knee angle, with a 5-min rest between each set. Since no significant changes in passive musculo-articular mechanical properties occurred between the fourth and the fifth cycle (McNair et al., 2002; Nordez et al., 2009), the latter cycle was considered for further analysis and elastographic measurements were performed at 1 Hz during the loading phase of this cycle (i.e., dorsiflexion phase). SSI measurements started before 30° of plantarflexion for all individuals. Using electromyographic feedback, the subjects and the experimenter were able to visualize any muscle activity and subjects were asked to be as relaxed as possible.

2.6. Data analysis

2.6.1. Passive force–length relationship

The analysis used to derive the passive force–length relation of the GMTU (F_G-L) has been presented in detail (Hoang et al., 2005; Nordez et al., 2010). Contributions of mono-articular structures were removed by calculating the differences in torque using the following equation (Nordez et al., 2010):

$$T_k - T_{80} = m_G(F_{Gk} - F_{G80}) \tag{2}$$

where T_k and F_{Gk} are the ankle torque and the gastrocnemius force determined at different knee angles (0°, 15°, 30°, 45° and 60°); T_{80} and F_{G80} are the ankle torque and the gastrocnemius force determined at 80° of knee angle, m_G is the gastrocnemius level arm assessed using Grieve's model (Grieve et al., 1978).

Then, the gastrocnemii were considered as the sole structure crossing both ankle and knee joints that produced a passive torque at the ankle (Hoang et al., 2005). Thus, the passive force–length relationship of the GMTU was modeled using the following exponential model (Nordez et al., 2010):

$$F_G = \frac{1}{\alpha}(e^{\alpha(l-l_0)} - 1) \quad \text{if } l > l_0$$
$$F_G = 0 \quad \text{if } l < l_0 \tag{3}$$

The muscle–tendon length (l) was estimated using the model of Grieve et al. (1978). The two unknown parameters of the Eq. (3) (i.e., α and l_0, representing muscle–tendon stiffness and slack length, respectively) were determined by minimizing the squared difference between the experimental and the modeled (Eqs. (2) and (3)) responses using Matlab (The Mathworks, Natick, USA) and the optimization toolbox (Levenberg–Marquard algorithm). For the comparison to the shear elastic modulus–length relationship, the force–length relationship was normalized with the maximal value.

2.6.2. Muscle shear elastic modulus–length relationship

First, for the comparison with the F_G-l relationship, shear elastic modulus values were divided with the maximal value and subtracted with the minimal value obtained for each subject. Then, the relationships between normalized shear elastic modulii and muscle–tendon length (calculated using the model of Grieve et al., 1978) obtained for all knee angles (Fig. 2-D) and at 0° for both trials were fitted using the previously described exponential model (3), and the determination coefficient (R^2) of each fit was calculated.

In our study, the physiological range of the GMTU was considered as the maximum in vivo length (L_{max}) minus minimum length (L_{min}) and relative slack length (%) was calculated as $100 \times (l_0 - L_{min})/(L_{max} - L_{min})$ for both relationships.

2.7. Statistical analysis

The between-trial repeatability (at 0° of knee flexion) of the parameters (α and l_0) fitted on the muscle μ-L relationship was assessed using the intra-class coefficient correlation (ICC), the standard error in measurement (SEM), and the coefficient of variation (CV) (Hopkins 2000). A one-way ANOVA with repeated measures and Scheffé post-hoc test (Statistica 8.0) were performed to compare parameters (α and l_0) obtained from: (i) experimental normalized shear elastic modulus–length relationship with the knee fully extended; (ii) experimental normalized shear elastic modulus–length relationship during all knee joint configurations; and, (iii) normalized force–length relationships estimated using Hoang's method. The root mean square error (RMSE) between normalized force–length and shear elastic modulus–length relationships was also calculated. Values are reported as means $\pm$ SD. Statistical significance was established at $P < 0.05$.

3. Results

3.1. Muscle shear elastic modulus–length relationship during passive stretches

The shear elastic modulus–length relationship obtained at the knee fully extended ($R^2 = 0.996 \pm 0.005$, ranged from 0.980 to 1.000, Fig. 3), or for all knee joint angles ($R^2 = 0.977 \pm 0.019$, ranged from 0.944 to 0.995) was very well fitted by the exponential model.

Parameters of the normalized shear elastic modulus–length relationship at 0° of knee flexion ($\alpha = 136.7 \pm 18.1$ m^{-1} and $l_0 = 0.443 \pm 0.021$ m) were not significantly different to those obtained at all knee joint configurations ($\alpha = 139.2 \pm 16.4$ m^{-1} and $l_0 = 0.444 \pm 0.022$ m).

The within-session repeatability of the shear elastic modulus–length relationship obtained at 0° of knee flexion was good for α (SEM: 9.7 m^{-1}; CV: 7.5%; ICC: 0.652), and excellent for l_0 (SEM: 0.002 m; CV: 0.4%; ICC: 0.992).

3.2. Muscle shear elastic modulus–length vs. force–length relationships

The muscle shear elastic modulus–length relationship obtained with the knee fully extended was highly correlated with the force–length curve calculated using Hoang's method ($R^2 = 0.979 \pm 0.009$, ranged from 0.964 to 0.992). However, in comparison to Hoang's model, the SSI method provides a slightly lower estimation of tension at the beginning of the range of motion (RMSE = 31.0 ± 14.7 N, range: 7.8–56.0 N, Fig. 3). Thus, the stiffness index (α) obtained for normalized shear elastic modulus–length curves was significantly higher from the value obtained for normalized force–length relationships (136.7 ± 18.1 vs. 105.0 ± 22.1 m^{-1} $P < 0.001$; Fig. 3). In addition, the slack length (l_0) when determined from the muscle shear elastic modulus–length curve was significantly longer in comparison with that derived from the force–length relationship computed using the Hoang's method (0.443 ± 0.021 vs. 0.434 ± 0.019 m; $P < 0.001$).

4. Discussion

First, the present study shows that, in comparison to the previous transient elastographic technique (Nordez et al., 2008), the Supersonic Shear Imaging method enables researchers and clinicians to greatly improve the reliability of muscle shear elastic modulus measurement during passive stretching of the plantar flexors (Fig. 2). Indeed, the repeatability of both α and l_0 was improved in the present study (CV < 7.5%), compared to the results previously reported (CV=60%; Nordez et al., 2008). In addition, the shear elastic modulus–length relationships obtained using SSI at several knee angles were very similar (Fig. 2) and very well fitted using an exponential model depicted on the Eq. (3) ($R^2 = 0.977$). This result emphasizes that the technique can be accurately used to measure muscle shear elastic modulus at a given length. Since the exponential model used is classically shown to fit passive force–length relationships very well (e.g. Hoang et al., 2005; Nordez et al., 2010), the present study indicates that changes in muscle shear elastic modulus were due to changes in muscle passive tension associated with variation in muscle length. Therefore, SSI would provide an indirect estimation of passive muscle tension at any length of the muscle–tendon complex.

In the present study, the normalized shear elastic modulus–length relationship was compared to the normalized force–length relationship obtained using the only method available in the literature for human experiments (Hoang et al., 2005). While the
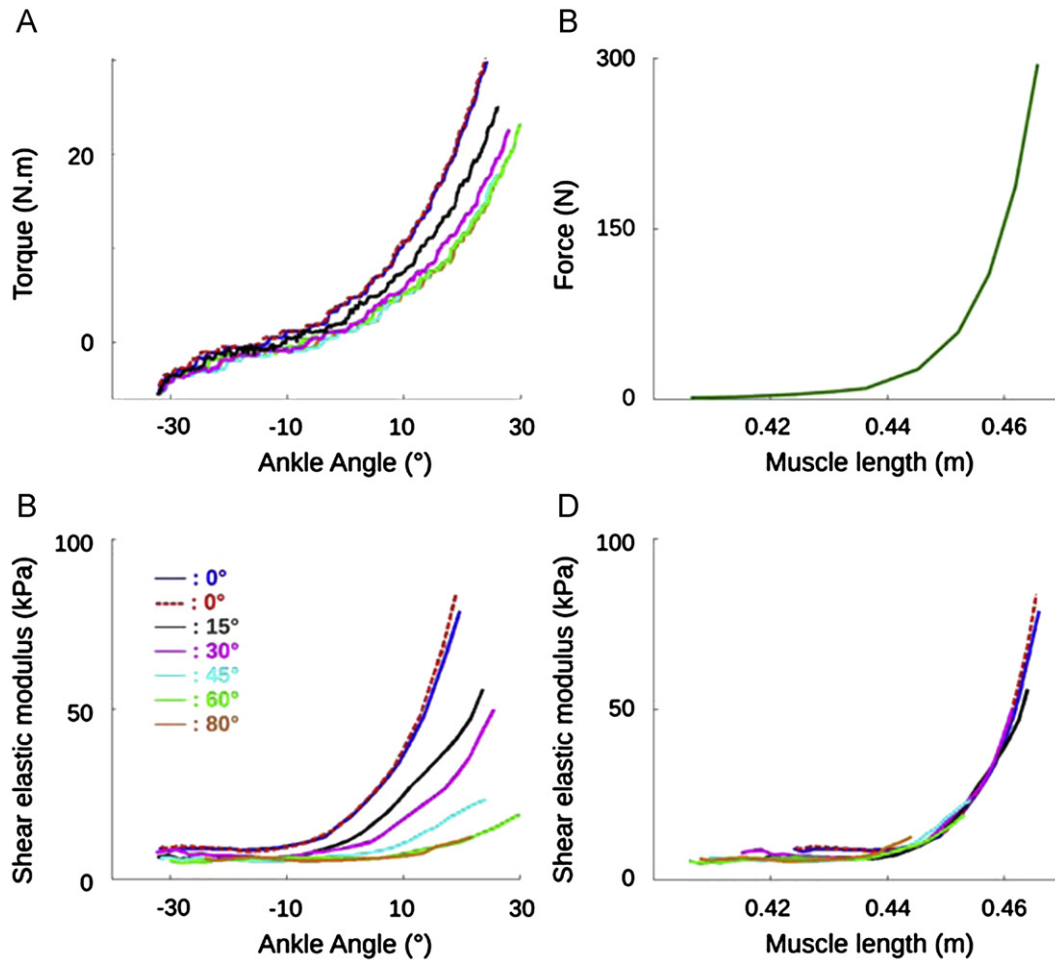
Fig. 2. (Colour online). (A) Typical torque–ankle angle curves during passive ankle dorsiflexion at a range of knee angles (0° = fully extended); (B) force–length relationship obtained using the Hoang's model; (C) shear elastic modulus–ankle angle curves and (D) shear elastic modulus–muscle length at a range of knee angles.

structures assessed were different between both methods, it was found that these relationships are similar with averaged R^2 and RMSE of 0.979 and 31 N, respectively (Fig. 3). Indeed, the shear elastic modulus of muscle belly was measured while the Hoang's method assesses the force–length relationship of the GMTU including intra- (aponeurosis) and extra-muscular (free) tendons. However, the tension is identical among the structures that are arranged in series. Therefore, since muscle belly, aponeurosis and free tendon are theoretically mainly placed in series, changes in force during passive stretching can be expected to be similar across these structures. In addition, the GMTU include both the gastrocnemius medialis and lateralis (GL) muscles. However, a previous study showed that the gastrocnemii behave similarly when passively lengthened (Maganaris et al., 1998). Therefore, it can be hypothesized that the differences in the structures assessed using both methods have few influence on the comparison performed in the present study.

A slight but significant underestimation of passive tension and a higher stiffness index (36 ± 27%) were found using the SSI technique in comparison to the Hoang model (Fig. 3). However, this model seems to underestimate the muscle slack length (34 ± 9% of its physiological range, representing a plantarflexed angle of 29 ± 5°, knee fully extended) compared to that reported in previous studies which found that the GMTU are slack over more than half of its physiological range (i.e., slack length at 18° of plantarflexion, knee fully extended, Riener and Edrich, 1999; Muraoka et al. 2004, 2005). Therefore, it is plausible that the Hoang model slightly overestimates the passive tension at the beginning of the

range of motion, and our results concerning the slack length estimation using SSI (47 ± 5% of its physiological range, corresponding to 20 ± 4° of plantarflexion, knee fully extended) are in agreement with those of these aforementioned studies. This seems to be physiologically plausible since it has been shown that, during functional activities such as gait, running, and jumping, this muscle is almost slacked before the beginning of its activity at a length around the plateau region corresponding to 50% of its physiological range (Fukunaga et al., 2002). In addition, since the slack length was lower for the force–length relationship, the curvature also needs to be lower to reach the same maximal normalized value (i.e., 1) than the shear elastic modulus–length relationship at the maximal muscle length. Therefore, the difference in the slack length between both methods could also, at least partially, explain the observed difference between stiffness indices.

In addition, the muscle fiber direction may have slightly affected our measurements. Because of the pinnate architecture of the GM, the shear elastic modulus was not measured along the fiber direction, and the direction of measurement changed during the passive lengthening. Indeed, the GM pennation angle decreases with ankle dorsiflexion (Abellaneda et al., 2009), representing a change in pennation angle of about 10° in our experiment. Due to muscle anisotropy, the shear elastic modulus is increased when it is measured along the fiber direction (Gennisson et al., 2010; Royer et al., 2011). Gennisson et al. (2010) have shown that a 10° change in the orientation of the probe with respect to the fascicle plane induces a 25% decrease in the shear elastic modulus of the biceps brachial when sustaining a 3 kg load. In contrast, they showed that the
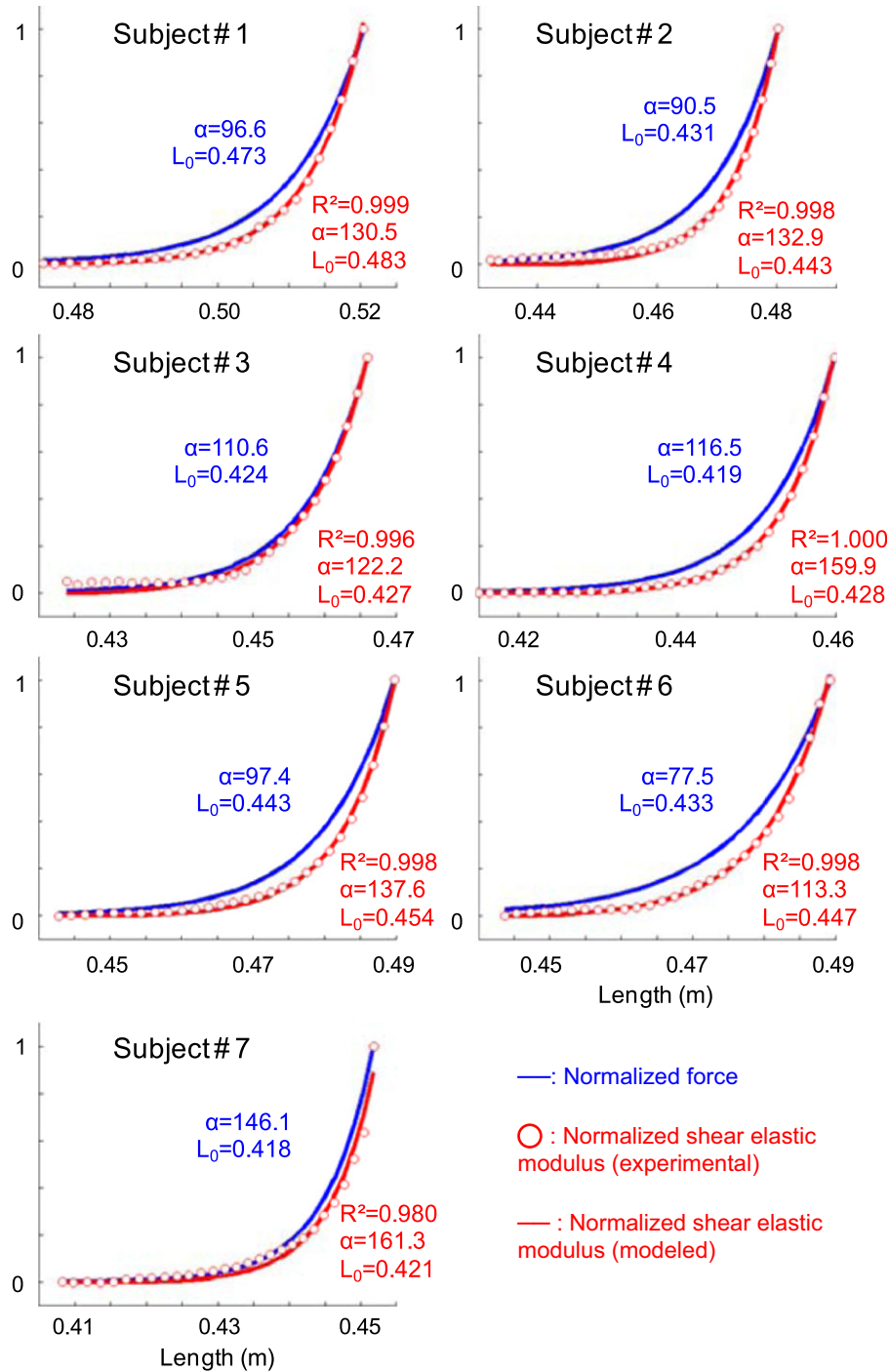
Fig. 3. Normalized shear elastic modulus–length relationship (red circles) and force–length relationship (blue line) obtained using the Hoang's model for each subject. Stiffness (α) and slack length (l_0). The exponential model (red line) fitted well the shear elastic modulus–length curve for all individuals. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

shear elastic modulus measured at rest was quite similar across probe orientations. Since the anisotropy could strongly depend on the muscle state (rest, contraction or stretching levels), the actual effect of pennation angle changes during passive stretching cannot be assessed from the available literature. Thus, an additional experiment was performed on one subject to examine the influence of the orientation of the ultrasonic probe on the μ-length relationship. After 5 cycles of conditioning, a passive dorsiflexion (knee fully extended) was performed with the probe placed in the same way than described in the methods. Then, the passive dorsiflexion was repeated three times with the probe rotated

around the axis perpendicular to the muscle surface from 10°, 20° and 30° with respect to the presumed fascicle plane. As shown on the Fig. 4A, μ-length relationship was greatly influenced by the probe orientation. However, normalized relationships were quite similar (Fig. 4B). Therefore, based on this complementary experiment, one would consider that the change in the fiber orientation had few influence on the main results of the present study. Further researches should be performed using SSI to study muscle anisotropy in vivo.

The increase in muscle shear elastic modulus with muscle elongation during passive stretches was highly reproducible, and
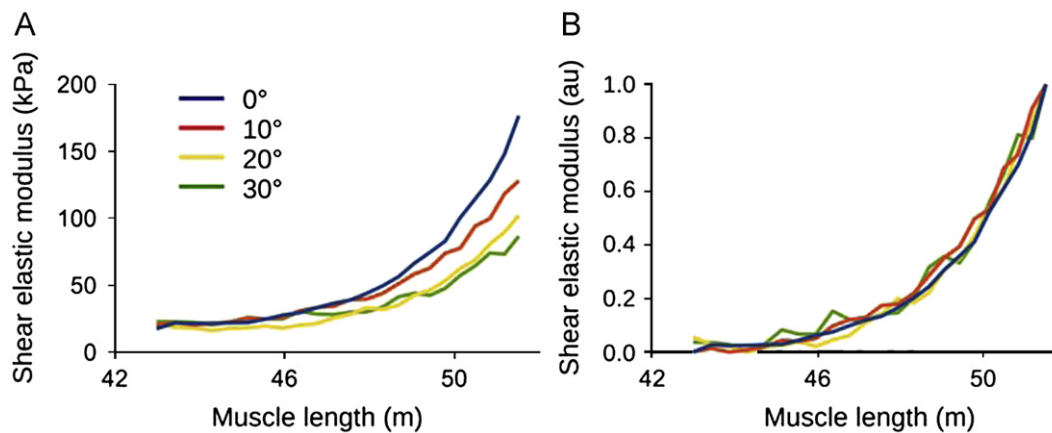
Fig. 4. (Colour online). Relationships between the shear elastic modulus and muscle–tendon length obtained for one subject with the probe placed in the same way as for the main experiment (i.e., 0°, the probe was orientated parallel to the muscle fascicles and perpendicularly to the skin. Appropriate probe alignment was achieved when several fascicles could be traced without interruption across the image), with the probe placed with angles of 10°, 20° and 30° in respect to the previous probe orientation. (A) raw relationships; (B) normalized relationships.

can be used to accurately estimate the stretching level. These results extend those recently obtained by Bouillard et al. (2011) who showed that shear elastic modulus measured using SSI is linearly related to individual muscle force during isometric contractions. Taken together, these results obtained in both active and passive conditions support the hypothesis that the SSI technique can be used to indirectly estimate muscle force and is worthwhile for determining the muscle slack length. Furthermore, the mechanical properties (α and l_0) of the gastrocnemius muscle belly can be estimated using SSI during a single passive dorsiflexion i.e. at only one knee joint configuration. Thus, this new elastographic technique might be applied to a variety of individual muscles that may be complicated to study using ergometers (e.g., shoulder muscles) or mono-articular muscles (e.g., soleus).

In summary, this study showed that the muscle shear elastic modulus measured using the SSI technique can be used to reliably and accurately to estimate the muscle stretching level. In addition, the shear elastic modulus–length relationship of the gastrocnemius muscle was highly correlated to the force–length relationship during ankle passive dorsiflexion. Thus, reliable and convenient measurements of muscle passive stiffness and slack length can be easily performed from a single passive stretch. These findings indicate that SSI may provide an indirect estimation of passive muscle force, and highlight its clinical applicability to evaluate the passive properties of mono- and bi-articular muscles as well. Further experiments are warranted to study the influence of muscle pennation angle on the shear elastic modulus–length relationship.

Conflict of interest statement

All authors disclose any financial and personal relationships with other people or organizations that could inappropriately influence this work.

Acknowledgments

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