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Contracting biceps brachii elastic properties can be reliably characterized using supersonic shear imaging

Thomas Lapole · Jérémy Tindel · Robin Galy · Antoine Nordez

Abstract

Purpose Since experimental techniques classically used to investigate the mechanical behavior of muscle in vivo assess global mechanical properties of the musculo-articular complex, the aim of the present study was to assess the feasibility and reliability of localized contracting biceps brachii elastic properties' measurements using elastography.

Methods Twelve subjects participated in intra-session, inter-session and inter-observer reliability experiments. They were asked to perform a linear torque ramp of 30 s from 0 to 75 % of maximal voluntary contraction. Joint torque, electromyographic (EMG) activity and shear elastic modulus were synchronously measured in the biceps brachii. Elastic properties were determined by stiffness indexes calculated as the slopes of the linear regressions established between shear modulus and joint torque or EMG levels. Compliance indexes were also obtained by plotting logarithmic values of shear modulus versus torque or EMG.

Results Determination coefficients were high for the four relationships (0.87–0.94). In addition, reliability was significantly higher for the compliance index taking into account logarithmic values of EMG activity (mean coefficient of variation of 5.3 ± 4.1 %; $P = 0.003$). Although not statistically different from other methods ($P = 0.111$),

the mean intra-class correlation coefficient was excellent (0.98 ± 0.01) and the standard error in measurement very low (0.04 ± 0.01 for a mean index of -0.73 ± 0.28).

Conclusions Biceps brachii elastic properties can be reliably investigated during contraction via elastography using the proposed fitting method. Use of this technique could provide new insights into the understanding of localized muscle elastic properties adaptations.

Keywords Elastography · Muscle elasticity · Electromyography · Biceps brachii

Abbreviations

CI	Compliance index
CV	Coefficient of variation
EMG	Electromyographic
ICC	Intra-class correlation coefficient
MVC	Maximal voluntary contraction
RMS	Root mean square
SEC	Series elastic component
SEM	Standard error in measurement
SI	Stiffness index
SSI	Supersonic shear wave imaging

Introduction

In daily activities and sports, muscles are involved in stretch-shortening cycles. During such cycles, musculo-tendinous mechanical properties greatly influence the storage–restitution of elastic energy and muscle tension transmission (Bosco et al. 1982; Komi 1984). According to the muscle model (Hill 1938; Shorten 1987), muscle–tendon complex mechanical properties are commonly characterized by the stiffness of the series elastic

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component (SEC), known for its plasticity in response to the changing functional state (Pousson et al. 1990). The classical experimental techniques used to investigate the mechanical behavior of a functional muscle group in vivo (e.g., quick-release or sinusoidal perturbations) assess global mechanical properties of the musculo-articular complex without any differentiation of the various structures (i.e., muscles, tendons, joint) involved in the task. Recently, an in vivo adaptation of the short range stiffness method associated with the application of a mathematical model allowed the stiffness determination of both torque-dependent (active part) and torque-independent (passive part) components of the plantarflexors SEC (Foure et al. 2010, 2011). This was recently further extended to the quick-release method (Lambertz et al. 2013). However, such techniques do not differentiate the properties of muscles within a functional muscle group.

The knowledge of individual muscle mechanical characteristics would provide new insights into the understanding of neuromuscular diseases (Cornu et al. 1998), age effects (Valour and Pousson 2003), muscle performance and injuries (Pearson and McMahon 2012), fatigue (Vigreux et al. 1980), and training (Pousson et al. 1990).

More localized techniques, such as elastographic methods, have recently been implemented to quantify local muscle mechanical properties (Drakonaki et al. 2012). One of these methods is supersonic shear wave imaging (SSI) (Bercoff et al. 2004). It consists of measuring the velocity of shear waves remotely induced by focused ultrasound. The squared velocity is directly related to tissue stiffness, i.e., the shear elastic modulus (in kPa). SSI technique has been recently shown to provide reliable measurements of shear elastic modulus in a variety of resting muscles (Lacourpaille et al. 2012). During muscle contraction, it was reported that there exists a strong linear relationship between muscle shear elastic modulus and individual muscle force for the first dorsal interosseous and the abductor digiti minimi (Bouillard et al. 2011). Similar results were recently reported for the biceps brachii (Yoshitake et al. 2013).

In plantarflexors studies, the slope of the linear relationship between stiffness and joint torque is generally considered as an index of musculo-tendinous (Lambertz et al. 2001; Lapole and Pérot 2011) or musculo-articular stiffness (Grosset et al. 2010; Lambertz et al. 2001) using quick-release or sinusoidal perturbations methods, respectively. In line with previously mentioned SSI results, an index of individual muscle stiffness (i.e., not global mechanical properties) could be obtained by considering the slope of the relationship between shear elastic modulus and muscle force. However, due to muscle redundancy, individual muscle force measurement in vivo remains challenging (Bouillard et al. 2011). Thus, muscle stiffness index

determination could be more pertinent when considering electromyographic (EMG) activity level rather than global joint torque. This is confirmed by the strong linear regressions previously reported between muscle shear elastic modulus and EMG activity level of the biceps brachii (Nordez and Hug 2010; Yoshitake et al. 2013). Another way to determine muscle elastic properties would be to calculate a compliance index (i.e., the inverse of stiffness) by establishing a linear relationship between logarithmic absolute values of compliance and joint torque (or EMG activity), as classically performed in studies using the quick-release method on elbow flexors (Michaut et al. 2001; Pousson et al. 1990; Valour and Pousson 2003).

Therefore, the aim of the present study was to assess the best way to report the links between localized biceps brachii stiffness measured using elastography and muscle activation level. Thus, elastic properties were determined by the calculation of stiffness and compliance indexes (SI and CI, respectively) taking into account joint torque or EMG levels. The intra-session, inter-day and inter-observer reliabilities of these indexes were further determined.

Methods

Subjects

Twelve subjects (five females; age 38 ± 10 years; height 175 ± 9 cm; body mass 73 ± 9 kg) participated in this study. Written informed consent was obtained from all subjects prior to their participation, and this study conformed to the standards from latest revision of the *Declaration of Helsinki* and was approved by the local ethics committee.

Elastography

Muscle shear elastic modulus was measured using an Aix-Plorer ultrasonic scanner (version 6.1.1, Supersonic Imagine, Aix en Provence, France), coupled with a linear transducer array (4–15 MHz, SuperLinear 15–4, Vermon, Tours, France). The scanner was used in the musculo-skeletal preset of the SSI mode, as previously described (Bercoff et al. 2004; Tanter et al. 2008). The principle is to generate a remote radiation force through focused ultrasonic beams that induce the propagation of transient shear waves. An ultrafast ultrasound imaging sequence was then performed to determine shear wave velocity (V_s) along the principal axis of the probe using a time-of-flight estimation. The shear elastic modulus (μ) was calculated using V_s as follows (Gennisson et al. 2003, 2005):

$$\mu = \rho \cdot V_s^2$$

with ρ the muscle mass density ($1,000 \text{ kg/m}^3$).



Fig. 1 Illustration of the experimental setup

The ultrasound probe was placed on the biceps brachii belly, carefully aligned with the shortening direction of the muscle, and perpendicular to the skin (Fig. 1). The location of the probe was marked on the skin to allow the same placement across trials and days. Since the biceps is fusiform, the shear wave propagation was measured in the fiber direction (Nordez and Hug 2010). Maps of the shear elastic modulus (Fig. 2) were obtained at 1 Hz with a spatial resolution of 1×1 mm. The Aixplorer scanner may present a temporal delay in signal processing between calculation and visualization of shear elastic modulus maps (Sasaki et al. 2014). Such phase shift was avoided in the present study by removing the persistence of SSI mode.

Electromyography

Surface EMG activity was measured using a Delsys Trigno wireless EMG system, with pre-amplified bipolar (fixed 1-cm inter-electrode distance) EMG sensors (Delsys, Boston, USA). The muscle belly surface was prepared by shaving and gently abrading the skin and then cleaning it with isopropyl alcohol. To obtain a representative biceps brachii EMG activity, one EMG sensor was placed on both sides of the ultrasound probe, longitudinally with respect to the underlying muscle fiber arrangement and distal to the motor point (Nordez and Hug 2010) (Fig. 1). Signals were analog-to-digital converted at a sampling rate of 2,000 Hz

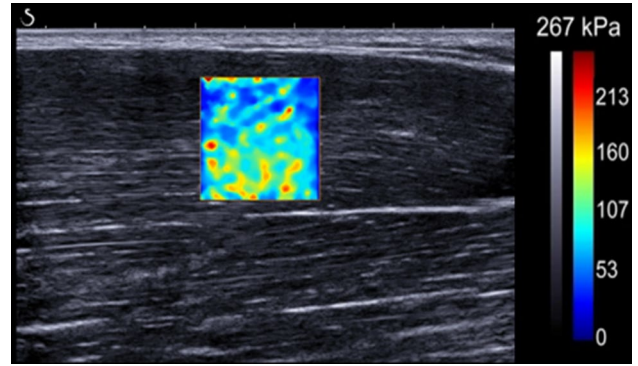


Fig. 2 Representative recording of biceps brachii shear elastic modulus during contraction. The colored region represents the shear elasticity map with the scale to the right of the figure (color figure online)

by PowerLab system (16/30-ML880/P, ADInstruments, Bella Vista, Australia) and octal bio-amplifier (ML138, ADInstruments; common mode rejection ratio = 85 dB, gain = 1,000) with bandpass filter (20–450 Hz) and analyzed offline using Labchart 7 software (ADInstruments).

Design of the study

Subjects participated in two separate testing sessions (interval between sessions of 24–72 h). Two different experimenters (A and B) performed the elastographic measurements. On the first day, the protocol was performed twice by experimenter A with a 15 min rest period between measurement sessions, allowing the assessment of intra-session reliability. In order to assess the inter-day reliability, the protocol was performed during the second testing session at the same time of the day by experimenter A. During this second testing session, the protocol was also performed by experimenter B 15 min after to assess the inter-observer reliability.

Protocol

Subjects sat in front of a handmade isometric ergometer with their right upper arm and forearm at 90° of flexion and wrist in supine position (Fig. 1). Elbow joint flexion torque signals (F2712 S-type load cell force transducer, Celians, France) were synchronously collected with EMG signals (2,000 Hz sampling rate). Subjects first performed three 3-s maximal isometric voluntary elbow flexions (1-min rest between flexions) to determine both maximal voluntary contraction (MVC) and maximal EMG activity. Then, subjects were asked to perform an incremental isometric task consisting of a 30-s smooth linear ramp from 0 to 75 % MVC. Since no hysteresis effect on the shear elastic modulus/torque relationship was previously reported (Bouillard et al. 2011), muscle elastic properties were only

investigated in the present study during loading condition rather than both loading and unloading ones. During incremental tasks, subjects had direct visual feedback of the torque signal and had to superimpose it on the target ramp (i.e., from 0 to 75 % MVC in 30 s). Preliminary experiments revealed that this incremental task did not induce any fatigue, as confirmed by unchanged MVC immediately after the ramp contraction.

Data analysis

For each incremental isometric task, 30 root mean square (RMS) values were calculated from the raw EMG data in a 1-s window. Joint torque and EMG RMS values were normalized to values from the maximal isometric contractions (calculated over a 500-ms interval centered at maximal torque). The EMG values obtained in the two recording locations were averaged (Nordez and Hug 2010). Shear modulus was averaged in the selected circular area placed on the biceps brachii (1 cm in diameter) using the Aixplorer scanner software (Q-Box™). Due to a technical limitation of the elastographic scanner, the shear elastic modulus measurements saturated at 267 kPa. If one value in the circular region reached this maximum, the mean data of this region was discarded from further analysis.

For each incremental isometric task, linear regression analyses were performed between muscle shear elastic modulus and normalized joint torque or EMG RMS values, and slopes of the curves were calculated so as to obtain stiffness indexes taking into account torque ($SI_{\%Torque}$) (Cornu and Goubel 2001; Cornu et al. 2003; Lambertz et al. 2001; Lapole and Pérot 2011 and EMG $SI_{\%EMG}$) (Lambertz et al. 2003, respectively). Linear relationships between logarithmic absolute values of compliance (i.e., inverse of shear elastic modulus) and normalized torque were also established (Michaut et al. 2001; Pousson et al. 1990; Valour and Pousson 2003), with the slope determining a compliance index taking into account torque ($CI_{\%Torque}$). Similar mathematical linearization was also applied in the present study to EMG, allowing the calculation of a compliance index taking into account EMG ($CI_{\%EMG}$).

Statistics

Coefficients of determination (R^2) of the established relationships were calculated. As recommended by Hopkins (2000), intra-session, inter-day and inter-observer reliabilities were assessed using the intra-class correlation coefficient (ICC), standard error in measurement (SEM) and coefficient of variation (CV). All variables passed the normality test (Kolmogorov–Smirnov normality test) and homogeneity of variance verification (Levene’s test). Thus, two-way ANOVAs were performed to determine the effect

of slope calculation method ($SI_{\%Torque}$, $SI_{\%EMG}$, $CI_{\%Torque}$ and $CI_{\%EMG}$) and experimental condition (intra-session, inter-session and inter-observer reliability) for R^2 , CV and ICC. Statistical analyses were performed using SigmaStat software (SigmaStat 3.5, Systat Software, San Jose, USA). When the ANOVA identified significant effects, post hoc Turkey testing was performed. Data are presented as mean \pm SD. Statistical significance was set at $P < 0.05$.

Results

Due to early saturation of some regions of the shear elastic modulus maps, one subject was excluded from the inter-session experiment ($n = 11$) and two from the inter-observer experiment ($n = 10$).

Curves obtained from a representative subject are presented in Fig. 3. The results of intra-session, inter-session, and inter-observer reliability experiments are shown in Tables 1, 2 and 3, respectively. Good linear regressions were found for the four relationships although the ANOVA showed a main slope calculation method effect ($P < 0.001$). Post hoc testing revealed that R^2 was significantly smaller for the shear elastic modulus/normalized EMG RMS relationship (Fig. 3b) (mean R^2 of 0.87 across the three experiments) than for the three other calculation methods ($P < 0.001$ for all). In addition, R^2 was higher ($P = 0.01$) for the logarithmic relation obtained with normalized torque than with EMG RMS (Fig. 3d) (mean R^2 of 0.92). No significant difference was found for the shear elastic modulus/normalized joint torque relationship (Fig. 3a) (mean R^2 of 0.94) and the logarithmic shear elastic modulus/normalized torque relationship (Fig. 3c) (mean R^2 of 0.94). Experimental condition effect tended to be significant ($P = 0.065$).

$SI_{\%Torque}$, the slope of the shear elastic modulus/normalized joint torque relationship, presented a mean value of 2.18 ± 0.62 kPa/%MVC. The mean ICC across the three reliability experiments was 0.54 ± 0.19 , and the CV was 15.1 ± 11.3 %, corresponding to a SEM of 0.30 ± 0.11 kPa/%MVC. When considering the shear elastic modulus/EMG RMS relationship ($SI_{\%EMG}$), the calculated mean slope value was 2.62 ± 1.06 kPa/%EMG. The mean ICC was 0.66 ± 0.38 , and the CV was 16.7 ± 12.1 %, corresponding to a SEM of 0.52 ± 0.25 kPa/%EMG. Mean slope of the relationship between compliance logarithm and torque logarithm ($CI_{\%Torque}$) was -0.91 ± 0.28 . The mean ICC was 0.81 ± 0.18 , and the CV was 14 ± 12.8 %, corresponding to a SEM of 0.11 ± 0.06 . Finally, $CI_{\%EMG}$, the slope of the compliance logarithm/EMG RMS logarithm relationship, presented a mean value of -0.73 ± 0.28 . The mean ICC was 0.98 ± 0.01 , and the CV was 5.3 ± 4.1 %, corresponding to a SEM of 0.04 ± 0.01 .

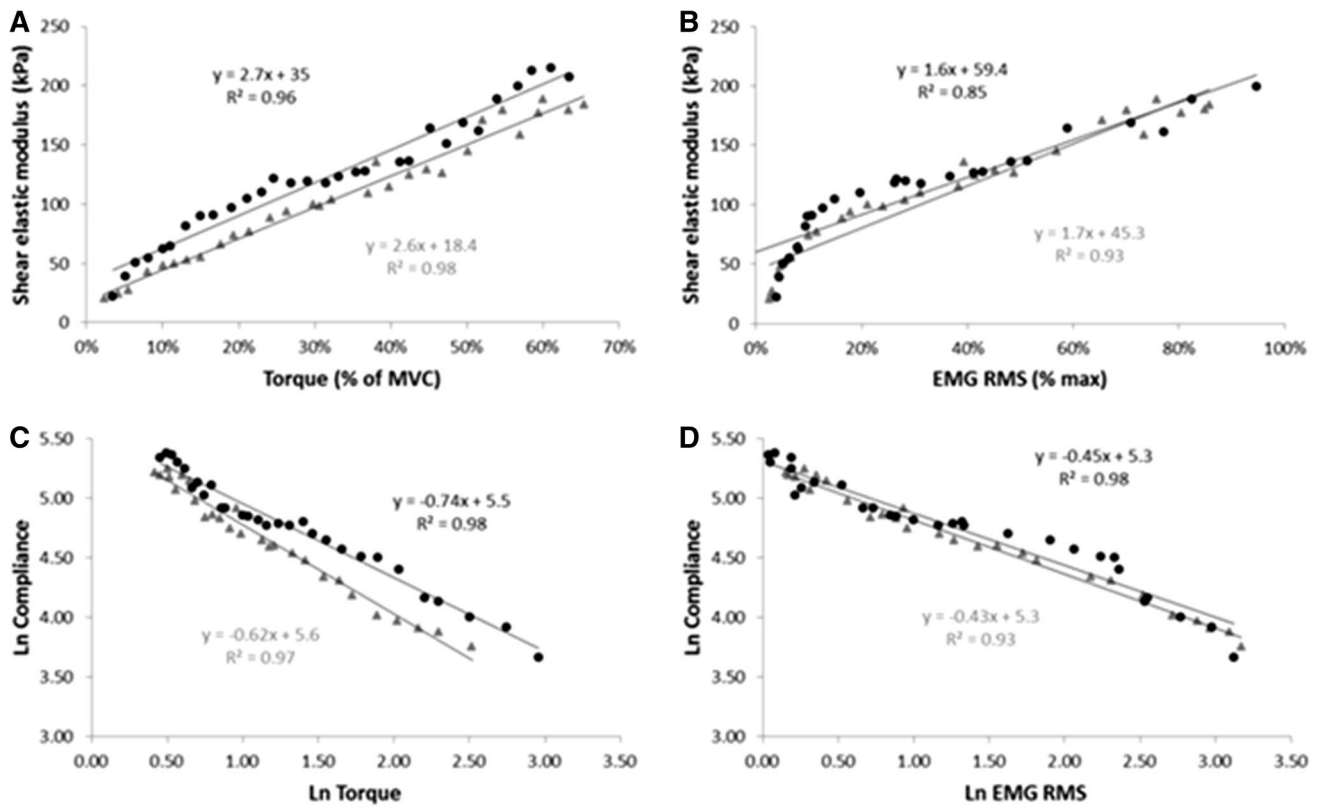


Fig. 3 Biceps brachii elastic properties determination using the four calculation methods for a representative subject in the intra-session reliability experiment. *Gray triangles* and *black circles* correspond to the first and the second incremental isometric task, respectively. Slopes of the curves were calculated as indexes of biceps brachii elastic properties. **a** Shear elastic modulus/torque relationship. **b** Shear

elastic modulus/EMG RMS relationship. Note that this relationship best fit a power curve. **c** Linearized relationship between logarithmic absolute values (Ln) of compliance and torque. **d** Linearized relationship between logarithmic absolute values (Ln) of compliance and EMG RMS

Table 1 Results for the intra-session reliability experiment

Intra-session reliability ($n = 12$)					
	Mean	R^2	SEM	CV	ICC
$SI_{\%Torque}$	2.19 ± 0.78	0.92 ± 0.06	0.40	$11.6 \pm 12.6 \%$	0.429
	2.22 ± 0.72	0.93 ± 0.05			
$SI_{\%EMG}$	2.63 ± 1.00	0.86 ± 0.09	0.24	$12.5 \pm 11.0 \%$	0.944
	2.61 ± 0.99	0.85 ± 0.09			
$CI_{\%Torque}$	-0.92 ± 0.22	0.94 ± 0.04	0.07	$9.1 \pm 6.5 \%$	0.910
	-0.89 ± 0.25	0.94 ± 0.04			
$CI_{\%EMG}$	-0.71 ± 0.29	0.92 ± 0.05	0.04	$3.6 \pm 3.7 \%$	0.982
	-0.70 ± 0.30	0.91 ± 0.05			

$SI_{\%Torque}$: slope of the shear elastic modulus/torque relationship; $SI_{\%EMG}$: slope of the shear elastic modulus/EMG relationship; $CI_{\%Torque}$: slope of the compliance logarithm/torque logarithm relationship; $CI_{\%EMG}$: slope of the compliance logarithm/EMG logarithm relationship; Mean: mean (\pm SD) slope value; R^2 : mean (\pm SD) coefficient of determination of the linear regression; SEM: standard error in measurement; CV: coefficient of variation (\pm SD); ICC: intra-class correlation coefficient. For each elastic properties index, mean and R^2 are presented for both incremental isometric tasks

The ANOVA revealed a significant main effect of calculation method on CV ($P = 0.003$). The smallest values were obtained for $CI_{\%EMG}$, ($P = 0.007$, $P = 0.003$ and $P = 0.013$ when compared with $SI_{\%Torque}$, $SI_{\%EMG}$, and $CI_{\%Torque}$, respectively). Moreover, there was a significant effect of experimental condition ($P = 0.021$). Intra-session CV values were smaller than inter-session and inter-observer ones ($P = 0.035$ and $P = 0.031$, respectively). ANOVAs performed on ICC values did not reach significance for calculation method effect ($P = 0.111$) or experimental condition ($P = 0.115$). Overall, reliability through intra-session, inter-session and inter-observer experiments was the greatest for $CI_{\%EMG}$, although not significantly so for ICC.

Discussion

The present study demonstrates that shear wave elastography can provide a reliable measurement of biceps brachii

Table 2 Results for the inter-session reliability experiment

Inter-session reliability ($n = 11$)					
	Mean	R^2	SEM	CV	ICC
$SI_{\%Torque}$	2.17 ± 0.71	0.94 ± 0.04	0.31	$14.4 \pm 9.6 \%$	0.437
	2.07 ± 0.44	0.94 ± 0.04			
$SI_{\%EMG}$	2.45 ± 0.68	0.88 ± 0.07	0.70	$20.8 \pm 15.6 \%$	0.227
	2.51 ± 0.91	0.88 ± 0.08			
$CI_{\%Torque}$	-0.90 ± 0.21	0.95 ± 0.05	0.18	$17.1 \pm 15.9 \%$	0.605
	-0.84 ± 0.34	0.94 ± 0.05			
$CI_{\%EMG}$	-0.69 ± 0.24	0.93 ± 0.05	0.04	$5.9 \pm 3.7 \%$	0.974
	-0.70 ± 0.30	0.93 ± 0.04			

$SI_{\%Torque}$: slope of the shear elastic modulus/torque relationship; $SI_{\%EMG}$: slope of the shear elastic modulus/EMG relationship; $CI_{\%Torque}$: slope of the compliance logarithm/torque logarithm relationship; $CI_{\%EMG}$: slope of the compliance logarithm/EMG logarithm relationship; Mean: mean (\pm SD) slope value; R^2 : mean (\pm SD) coefficient of determination of the linear regression; SEM: standard error in measurement; CV: coefficient of variation (\pm SD); ICC: intra-class correlation coefficient. For each elastic properties index, mean and R^2 are presented for both incremental isometric tasks

Table 3 Results for the inter-observer reliability experiment

Inter-observer reliability ($n = 10$)					
	Mean	R^2	SEM	CV	ICC
$SI_{\%Torque}$	2.37 ± 0.57	0.95 ± 0.04	0.19	$19.4 \pm 11.5 \%$	0.768
	2.06 ± 0.51	0.93 ± 0.04			
$SI_{\%EMG}$	3.05 ± 1.63	0.88 ± 0.08	0.62	$16.8 \pm 9.8 \%$	0.811
	2.47 ± 1.22	0.85 ± 0.10			
$CI_{\%Torque}$	-1.00 ± 0.36	0.95 ± 0.04	0.09	$15.9 \pm 16.0 \%$	0.923
	-0.89 ± 0.32	0.95 ± 0.05			
$CI_{\%EMG}$	-0.81 ± 0.28	0.91 ± 0.07	0.03	$6.6 \pm 4.9 \%$	0.990
	-0.78 ± 0.33	0.92 ± 0.04			

$SI_{\%Torque}$: slope of the shear elastic modulus/torque relationship; $SI_{\%EMG}$: slope of the shear elastic modulus/EMG relationship; $CI_{\%Torque}$: slope of the compliance logarithm/torque logarithm relationship; $CI_{\%EMG}$: slope of the compliance logarithm/EMG logarithm relationship; Mean: mean (\pm SD) slope value; R^2 : mean (\pm SD) coefficient of determination of the linear regression; SEM: standard error in measurement; CV: coefficient of variation (\pm SD); ICC: intra-class correlation coefficient. For each elastic properties index, mean and R^2 are presented for both incremental isometric tasks

elastic properties during contraction. The best reliability was observed for the compliance index calculated when plotting logarithmic values of compliance against logarithmic values of EMG activity level. Other calculation methods failed to present satisfactory results.

Although force and not torque was measured in the present study, we assumed a constant level arm during isometric contractions and expressed the torque in percentage of MVC. This allows clearly distinguishing the

global measurement of joint torque and individual muscle force throughout the paper. Our results demonstrated that biceps brachii shear elastic modulus linearly increased with elbow joint torque, with strong coefficients of determination. Similar results were previously reported during ramps from 0 to 60 and from 0 to 30 % MVC for the first dorsal interosseus and the abductor digiti minimi, respectively (Bouillard et al. 2011). Yoshitake et al. (2013) also found a linear relationship between biceps brachii shear modulus and elbow flexion torque over a wide range of contraction intensities (15–60 % MVC), with a mean slope (3.10 kPa/%MVC) slightly greater than that observed in the present study (2.18 kPa/%MVC). Conversely, it was reported during elbow flexion ramps from 0 to 40 % MVC that biceps brachii shear modulus only increased slightly at the lowest contraction intensities, leading to a curvilinear relationship (Bouillard et al. 2012b). In the present study, when further analyzing the relationships in the similar range of contraction intensity (0–40 % MVC), only 13 out of the 66 analyzed ramps were characterized by a curvilinear relation (i.e., greater R^2 for power curve than linear regression). Discrepancies may be explained by task specificity. While, in Bouillard et al.'s study (2012b), the tested range of contraction intensity was limited to 0–40 % MVC, the present study investigated a higher range of contraction intensities (up to 75 % MVC). The incremental task up to 40 % MVC was mainly a precision task where subjects had to precisely control their torque, while it was predominantly a force task when the target torque further increased to 75 % MVC. The shorter time spent at the lowest contraction intensities may explain the general absence of curvilinear relation. Since load sharing exists between synergist elbow flexors (Bouillard et al. 2012b), differences in motor control may account for the different relationships observed. Using quick-release methods, linear relationships between musculo-tendinous stiffness and joint torque were similarly established and slopes of the curves were considered as stiffness indexes of plantar flexors (Lambertz et al. 2001; Lapole and Pérot 2011), wrist flexors (Cornu et al. 2003) and elbow flexors (Cornu and Goubel 2001). In the same way, triceps surae musculo-articular stiffness indexes were reported using sinusoidal perturbations methods (Grosset et al. 2010; Lambertz et al. 2001). Although such methods are widely used, they assess global mechanical properties of the musculo-tendinous or musculo-articular complexes. Since the stiffness indexes reported in the present study consist of local muscle measurements, they provide interesting perspectives. However, $SI_{\%Torque}$, the slope of the biceps brachii shear elastic modulus/torque relationship, had poor reliability. $CI_{\%Torque}$ had good ICC values for intra-session and inter-observer reliability (0.910 and 0.923, respectively) with relatively high CV values (9 and 16 %, respectively). While this could

be considered as satisfactory, the inter-session reliability results ($ICC = 0.605$ and $CV = 17\%$) suggest that this compliance index could not provide reliable measurement of contracting biceps brachii elastic properties across days. The lack of reliability of $SI_{\%Torque}$ and $CI_{\%Torque}$ may be explained by the fact that the relationships are established between a local measurement (biceps brachii shear elastic modulus) and a global joint measurement (elbow flexion torque). As previously mentioned, load sharing exists between elbow flexors (Bouillard et al. 2012b) and differences in synergist recruitment may explain the high variability observed. As it is impossible to isolate in vivo biceps brachii force, it seems inappropriate to calculate a stiffness (or compliance) index based on elbow joint torque.

To account for differences in motor control, stiffness indexes can be calculated taking into account the EMG activity (Lambertz et al. 2003). Linear regressions were previously reported between biceps brachii shear elastic modulus and EMG activity level during 0–30 % MVC elbow flexion ramps (Nordez and Hug 2010) and over a wide range of contraction intensities (15–60 % MVC) (Yoshitake et al. 2013). In the present study, slope values were in the same range as in the study of Yoshitake et al. (2013); however, the linearity was relatively weak ($R^2 = 0.87$) and relationships were best fitted by a power curve. This could be explained by the known curvilinear EMG/joint torque relationship in the biceps brachii (Doheny et al. 2008; Nordez and Hug 2010), possibly due to multiple factors such as activation strategies of motor units and the effects of EMG amplitude cancelation (Keenan et al. 2005). Since shear elastic modulus is highly correlated to individual muscle force (Bouillard et al. 2011), this would suggest that not only the EMG/joint torque but also the EMG/individual biceps brachii force is curvilinear. Considering this, the use of linear regression fit may be inappropriate to characterize shear modulus/normalized EMG RMS relationships, and $SI_{\%EMG}$ may not be considered as an index of contracting biceps brachii elastic properties. This is confirmed by the poor inter-session reliability of this parameter. Therefore, a mathematical linearization of the relationships should be used. In previous elbow flexors quick-release studies, mathematical linearization was performed by calculating a compliance index defined as the slope of the linear relationship between logarithmic absolute values of compliance and joint torque (Michaut et al. 2001; Pousson et al. 1990; Valour and Pousson 2003). In the present study, we similarly used this method not only to calculate $CI_{\%Torque}$, but also to linearize shear modulus/normalized EMG RMS relationships and obtain a compliance index defined as the slope of the linear relationship between logarithmic absolute values of compliance and EMG activity level. As a result, R^2 were improved and reliability results were excellent. Across intra-session, inter-session and inter-observer experiments,

the minimum ICC value was 0.974 and maximal CV value was 6.6 %. SEM was also very low (mean SEM of 0.04 for a mean $CI_{\%EMG}$ of -0.73). It can be suggested, at least for the biceps brachii, that muscle elastic properties can be reliably investigated during contraction using elastography by quantifying a compliance index taking into account EMG activity level using the proposed fitting method. While the linear stiffness–force relationship is physiologically meaningful, this may be questioned for this compliance index, and further studies are required to better highlight its physiological significance.

When performing shear elastic modulus measurements using Aixplorer scanner, it may be difficult to synchronize the color-coded map and torque or EMG measurements, inducing a temporal delay between measurements (Sasaki et al. 2014). In the present study, both measurements were synchronized by manually triggering torque and EMG measurements on the first push of Supersonic Shear Imaging. Since the sampling rate of the shear elastic modulus maps was 1 Hz, a maximal delay of 1 s may have existed before or after the effective push (and color-coded map) and the signal of synchronization. We thus further verified how this may have impacted our results. We calculated for the intra-session experiment the stiffness and compliance indexes by shifting shear modulus values by 1 s earlier and later. As a result, CV were 3.5 ± 2.2 and $5 \pm 2.3\%$ for $SI_{\%Torque}$ and $SI_{\%EMG}$, respectively. CV for compliance indexes were 5.9 ± 3.8 and $5.1 \pm 3.7\%$ for $CI_{\%Torque}$ and $CI_{\%EMG}$, respectively. This highlights that the maximal synchronization error would have minimally impacted the results. Since the error due to the low temporal resolution is random, it would also have affected the reliability. Therefore, the good reliability reported in the present study confirms that this error is acceptable. A possible limitation of the present study is that the performed incremental contractions may have induced muscle fatigue. Although this does not influence shear elastic modulus (Bouillard et al. 2012a), it may have altered EMG activity (Enoka and Duchateau 2008), potentially affecting $SI_{\%EMG}$ and $CI_{\%EMG}$. Although fatigue cannot totally be ruled out, our preliminary experiments showed unchanged MVC immediately after the ramp contractions. Therefore, fatigue during ramps should have been minimal and probably did not influence our results.

Considering the muscle model, the muscle elastic compartment is classically decomposed into a SEC and a parallel elastic component (Hill 1938; Shorten 1987). Joint stiffness during contraction is directly related to SEC stiffness, composed of passive (i.e., mainly tendons and aponeurosis) and active (i.e., contractile structures) parts (Huxley and Simmons 1971). While ultrasound is widely used to characterize the stiffness of tendons and aponeuroses (Magnusson et al. 2008), it is much more difficult to assess the stiffness of the active part of the SEC in vivo. Interestingly, the

proposed innovative method using supersonic shear imaging may focus on the active part of the SEC of one muscle (i.e., biceps brachii). However, since shear elastic modulus is related to the stiffness of tissues included inside the ROI, influence of other structures such as connective tissues cannot be ruled out in the measurement. Thus, animal studies would be further required to better understand stiffness measurements performed using elastography in relation to classical hill-type models.

The knowledge of the individual muscle elastic properties during contraction could provide new insights into the understanding of neuromuscular diseases (Cornu et al. 1998), age effects (Valour and Pousson 2003), muscle performance and injuries (Pearson and McMahon 2012), fatigue (Vigreux et al. 1980), and training (Pousson et al. 1990). Indeed, while the interpretation of induced effects on global and complex systems (i.e., musculo-tendinous or musculo-articular complexes) is limited due to the numerous structures involved, ultrasound elastography may offer the possibility to focus on contracting muscle elastic compartment, at least for the biceps brachii as suggested by the present results. This would further provide the ability to distinguish the elastic properties of different muscles within a single muscle group, which will be useful to investigate individual muscle adaptations following training or disuse.

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