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Quantifying changes in material properties of stroke-impaired muscle

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Abstract

Background: Material properties of muscles are clinically important parameters for evaluating altered muscle function. Stroke survivors display motor impairments almost immediately after the vascular event, and then gradually develop altered muscle properties. Little is known about the magnitude of these changes in muscle material properties, specifically stiffness. Previous measures of stiffness are limited to estimates of joint stiffness or groups of muscles. Thus, our aim was to determine changes in passive muscle stiffness and composition by measuring: (1) shear wave speed using shear wave ultrasound elastography and (2) echo intensity of the B-mode ultrasound images of the biceps brachii muscle in individuals who have had a stroke.

Methods: Shear wave ultrasound elastography and B-mode ultrasound images of the biceps brachii muscle of the paretic and non-paretic limbs of sixteen stroke survivors were captured at rest.

Findings: Our main results show that shear wave speed and echo intensity of the paretic side were on average 69.5% and 15.5% significantly greater than those of the non-paretic side, respectively. Differences in shear wave speed between the non-paretic and the paretic muscles were strongly correlated with differences in echo intensity, time since stroke, and with Fugl–Meyer scores.

Interpretation: Muscle stiffness and muscle composition, as indicated by SW speed and echo intensity, may be altered in stroke-impaired muscle at rest. These findings highlight the potential for SW elastography as a tool for both investigating the fundamental mechanisms behind changes in stroke-impaired muscle, and for evaluation of muscle mechanical properties as part of clinical examination.

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Conflict of interest

The authors have nothing to disclose.

Keywords

Stroke; Muscle; Ultrasound; Stiffness; Echo intensity

1. Introduction

Stroke is one of the leading causes of long-term disability in the United States, with an annual incidence of approximately 800,000 persons (Lloyd-Jones et al., 2010). Stroke survivors routinely experience long-term motor and sensory impairments, especially in the upper extremity (Gray et al., 1990; Nakayama et al., 1994). These motor impairments include weakness for voluntary movement, spasticity, and impaired coordination, and they emerge almost immediately after the vascular event. Over time, material properties of muscles in the impaired limbs can also change gradually, further disrupting motor function, and adversely impacting the stroke survivor's quality of life. These material changes appear to be associated with the increasing accumulation of collagenous connective tissue (Lieber and Ward, 2013) and are accompanied by a progressive loss of skeletal muscle fibers (McLachlan and Chua, 1983; Tabary et al., 1972) and ultimately, with contractures, that potentially limit range of joint motion. Currently, the origins of these changes in material properties remain unclear. It is our objective in this study to compare material properties in spastic-paretic muscles with contralateral muscles of stroke survivors, using shear wave (SW) speed measurements as a surrogate measure of stiffness changes.

There have been a number of prior descriptions of muscle material properties in stroke survivors. Muscle stiffness has been shown to be different in individuals after a stroke (Katz and Rymer, 1989) in both lower (de Vlught et al., 2010; Roy et al., 2011; Sinkjær and Magnussen, 1994) and upper extremity musculature (Chardon et al., 2010). However, these earlier stiffness estimates were made indirectly, such as by using kinematic protocols while recording muscle electrical activity, by calculating limb dynamics (Sinkjær and Magnussen, 1994), or by measuring the force generated during tendon indentation (Chardon et al., 2010).

Historically, the accurate quantification of passive muscle stiffness has only been possible through such approaches as cadaveric biomechanical studies, animal *in situ* experiments, muscle biopsies, and intra-operative force and torque measures. Obtaining non-invasive measurements that can quickly quantify changes in muscle stiffness of specific muscles in a clinical setting remains a challenge.

Recently, ultrasound imaging techniques appear to offer promising alternative approaches. Building upon traditional elastography (Brandenburg et al., 2014; Ophir et al., 1991), shear wave (SW) elastography, allows measurement of SW speed using either magnetic resonance imaging or ultrasound imaging, which is related to stiffness, specifically the shear modulus:

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

where μ is the elastic shear modulus, ρ is the muscle mass density ($\rho \approx 1000 \text{ kg m}^{-3}$), and V_s is the SW speed (Bercoff et al., 2004; Brandenburg et al., 2014; Muthupillai et al., 1995).

The stiffer the tissue, the faster the SWs will travel. Here we use supersonic shear imaging (SSI) (Bercoff et al., 2004), a method that uses acoustic radiation forces to induce the SWs and subsequently measure the SW speed in muscle.

Several studies have investigated the stiffness of muscles using SSI in muscles of intact subjects, including the biceps brachii (Bouillard et al., 2012; Lacourpaille et al., 2012; Nordez and Hug, 2010; Yoshitake et al., 2014), gastrocnemius (Chernak et al., 2013; Lacourpaille et al., 2012; Maïsetti et al., 2012), and vastus lateralis muscles (Lacourpaille et al., 2012). SW speed has been reported to be higher in spastic muscles of children with cerebral palsy and in individuals with Duchenne muscular dystrophy compared to muscles of typically developing children (Kwon et al., 2012; Park and Kwon, 2012) and individuals without muscle disorders (Lacourpaille et al., 2015), respectively. By measuring the SW speed in muscle, we can indirectly estimate stiffness of muscle.

Accordingly, we sought to determine whether there are differences in SW speed in the biceps brachii muscles by comparing the paretic and contralateral limbs in stroke survivors. We also assessed the echogenicity of these muscles (measured as echo intensity from the B-mode image) and correlated our estimates of material properties with major clinical assessments, including the Fugl–Meyer scale (Gladstone et al., 2002), the modified Ashworth (Pandyan et al., 1999), and the modified Tardieu tests (Singh et al., 2011). (The Ashworth and Tardieu each provide a clinical measure of the severity of spasticity.) Conveniently, the quantification of muscle echo intensity from ultrasound images can also provide information about the tissue composition of muscle (Strasser et al., 2013) in that increased amounts of fibrous tissue can result in higher ultrasound echo intensity (Pillen et al., 2009a). Higher echo intensity has been associated with aging (Arts et al., 2010; Fukumoto et al., 2012; Strasser et al., 2013) and arise in children with myopathic and neuromuscular disorders (Lamminen et al., 1988; Pillen et al., 2007).

2. Methods

2.1. Subjects

Sixteen subjects participated in this study (10 females and 6 males, mean (SD) age: 60.7 (8.0) years; height: 1.71 (0.15) m; body mass: 85.5 (18.2) kg; time post-stroke: range: 1.9–42.2, mean (SD) 11.6 (11.4) years; Fugl–Meyer: range: 4–48, mean (SD): 19 (15); modified Ashworth: range: 0–3; modified Tardieu: 1–3 muscle quality, 62°–145° catch angle for three speeds). All subjects gave informed consent prior to testing and Northwestern University's Institutional Review Board approved all procedures.

2.2. General setup

Participants were instructed to sit upright in a Biodex (Biodex Medical Systems, Inc., Shirley, NY) chair with their upper arm resting on a plastic support. To standardize the hand position and to minimize activity of unrecorded muscles, the forearm was secured in a fiberglass cast, with the wrist and forearm held in a neutral position (flexion–extension and supination–pronation) and placed in a ring-mount interface that was mounted to the table.

The shoulder was positioned so that the humerus was abducted 45°, and the elbow positioned at 90°.

Surface EMG was recorded from the long and short heads of the biceps brachii and the triceps muscles using bipolar electrodes with the signal amplified by 1000 and band-pass filter of 20–450 Hz (Bagnoli, Delsys, Inc., Boston, MA). Visual inspection of the EMG signal during data collection was conducted to ensure there were no bursts of activity when the ultrasound image was captured.

2.3. Ultrasound

Ultrasound images were captured using an ultrasonography system (Aixplorer SuperSonic Imagine, Aix en Provence, France) with a linear transducer array (4–15 MHz, SuperLinear 15–4, Vermon, France) (Bercoff et al., 2004). We collected images of the paretic side and non-paretic side sequentially (five images per side, Fig. 1). Technical details of this technology have been described previously (Bercoff et al., 2004). The transducer was positioned at the mid-belly region of the biceps brachii and aligned with the fascicles as viewed from the B-mode image. A customized neoprene sleeve held the transducer in place to minimize undesired translation of the transducer. The SW speed map region of interest (12 mm by 12 mm, Fig. 1) was manually placed in middle section between the superficial and deep aponeuroses.

2.4. Data analysis

A custom software, written in Matlab (Mathworks, Natick, USA), was used to extract the SW speed and “quality factor” values from the region of interest (12 mm by 12 mm region) as given by the Supersonic Imaging software, Q Box. Each SW speed value is accompanied by a “quality factor” that indicates the reliability of the SW speed value. The software, Q Box, requires the experimenter to choose a circular region with a desired diameter from which the mean SW speed is calculated. Our custom software required the experimenter to verify that the region of interest did not include the superficial or deep aponeuroses and calculated the mean SW speed from the SW speed values in those boxes that had a quality factor greater than 0.8. Thus, the custom software ensured this step was systematic and objective.

The thickness of the muscle and adipose tissue was measured from the B-mode ultrasound image using a custom-written software in Matlab. To confirm that no contraction was generated while images were captured, EMG data were scanned, and signals with potential EMG activity were discarded. EMG data were fully-wave rectified and then low-pass filtered with a first-order Butterworth filter at 5 Hz.

To quantify the echo intensity of the B-mode images (Fig. 2), the parameters of the ultrasound system, gain and power, were kept the same for imaging the paretic and non-paretic muscles for all subjects. The mean echo intensity of the region of interest within the muscle, using the same region as for SW velocities, was calculated using the “impixel” function in Matlab, which determines the intensity values of each pixel. Only eleven subjects, out of the total sixteen subjects, were used for this analysis, as the ultrasound

parameters (gain and power) were not kept the same when imaging the paretic and non-paretic for five subjects.

2.5. Repeatability and reliability

We have previously tested the repeatability and reliability of the SW speed and echo intensity measurements. In these analyses, three unimpaired subjects were tested twice on separate days; the results obtained were highly consistent. SW speed and echo intensity measurements were reliable and repeatable (SW speed: ICC = 0.932, CV = 4.5%; echo intensity: ICC = 0.882, CV = 4.2%). Inter-day reliability of the SSI technique has been previously reported (Lacourpaille et al., 2012; Yoshitake et al., 2014).

2.6. Clinical tests

The clinical tests and physical measurements were conducted by a licensed physical therapist. The modified Ashworth (Pandyan et al., 1999) and the modified Tardieu tests (Singh et al., 2011) were used for evaluating spasticity. The Fugl–Meyer assessment of motor recovering after stroke was used as a test for motor impairment as it measures recovery in post-stroke hemiplegic patients (Gladstone et al., 2002). Physical examination measurements included passive and active elbow range of motion. The passive range of motion measurements involved the physical therapist moving the limb to maximum elbow flexion and extension while the muscles were at rest, whereas for active range of motion measurements, subjects were instructed to actively move the limb to maximum elbow flexion and extension. Elbow angles were defined as the angle between the humerus and the ulna (flexion 0° to extension 180°).

2.7. Statistics

Student's paired *t*-tests were conducted to compare the SW speed, echo intensity, and thickness of muscle and fat between the paretic and non-paretic muscles. Analysis of covariance (ANCOVA) was also conducted to determine if fat and muscle thickness influenced the SW speed and echo intensity values between the two sides. The coefficient of determination, r^2 , was calculated to determine the relationship between the difference in SW speed and echo intensity of the paretic and non-paretic side, and the clinical tests.

3. Results

Our key findings are that in our cohort of individuals who have sustained a chronic stroke, measures of SW speed and echo intensity in the resting biceps brachii were systematically greater on the paretic side than on the contralateral non-paretic side. We also found a strong correlation between SW speed and echo intensity, especially when considering the differences between the two sides of the same person. Finally, consistent with observations of function, we observed a linear relationship between the SW speed and the elapsed time since the individuals had the stroke as well as with their Fugl–Meyer scores, a measure of functional impairment. Details of each result are described in the following sections.

3.1. Ultrasound measurements

As demonstrated in Fig. 3, in 15 of 16 subjects, the SW speed was systematically greater in the resting paretic biceps brachii than on the contralateral non-paretic side. In all but one subject (S14), SW speed was higher in the paretic biceps muscle, and in many subjects (S6, S8, S11, S12), SW speed was 2–3 times greater. On average across all the subjects, SW speed was 69.5 % greater in paretic muscle (3.67 (1.28) m/s) than in the non-paretic muscle (2.23 (0.40) m/s) on the contralateral side ($p < 0.001$ (Fig. 3). There is some variability in the non-paretic SW speed, but as Fig. 3 demonstrates, the variability in the paretic muscles is substantially greater.

Fig. 4 shows a comparison of echo intensity values between the paretic and non-paretic muscle in individual subjects and demonstrates that echo intensity values of the paretic side were again systematically greater in the spastic-paretic limbs of our stroke cohort, except in S3 and S7, where no difference was detected between the muscles of the two sides. The scale of the figure refers to the echo intensity in that pixels that are black have a value of 0, whereas pixels that are very bright, or white, have a value of 255. The higher the echo intensity, the brighter or “whiter” the pixel appears. On average, across all the subjects, echo intensity values were 15.5% greater in paretic muscle (109.48 (21.90)) than in the non-paretic muscle on the contralateral side (83.03 (28.96), $p = 0.004$, Fig. 4).

In addition to stiffness estimates provided by SW speed measurements, B-mode ultrasound imaging was used to measure muscle thickness and adipose tissue thickness, as calculated between the superficial and deep aponeuroses and between skin and the superficial aponeurosis, respectively. The biceps brachii thickness of the paretic side (21.52 (8.12) mm) was on average 3.4% less than that of the non-paretic side (22.04 (5.46) mm), but this difference was not significant ($p = 0.67$). The superficial adipose tissue thickness was on average 3.8 % greater on the paretic side (6.35 (3.29) mm) than on the contralateral side (6.75 (3.13) mm), but this also was not significant ($p = 0.508$). Consequently, the results from the ANCOVA revealed that adipose tissue and muscle thickness probably did not influence SW speed and echo intensity ($p = 0.25$ and $p = 0.31$, respectively).

In addition, we examined the relation between SW speed and echo intensity differences within each subject. Here, we found that differences in SW speed between the non-paretic and paretic muscles were strongly correlated, with the differences in echo intensity of the two sides ($r^2 = 0.703$, $p = 0.002$) as shown in the Fig. 5. The subjects showed a wide range of SW speed as well as echo intensity between the two sides. Individuals with large differences in SW speed between the two sides also had large differences in echo intensity. The individual whose SW speed was not higher on the paretic side also did not have higher echo intensity on the same side.

3.2. Clinical assessments

We found that the subjects had greater passive and active elbow range of motion on the non-paretic arm than on the contralateral paretic side. The non-paretic side of individuals had significantly greater maximum active and passive range of motion (active: 180 (5)° to 44

(11)°; passive: 181 (4)° to 37 (6)°) than the paretic side (active: 145 (27)° to 59 (10)°, passive: 175 (8)° to 45 (7)°; both $p < 0.001$).

As shown in Fig. 6, we observed that individuals with a longer duration since the stroke onset also had greater differences in SW speed between their non-paretic and paretic side. A coefficient of determination analysis revealed a significant linear relationship between the difference of SW of the two sides and time from stroke ($r^2 = 0.301$, $p = 0.03$, Fig. 6). There was a range of elapsed times since the stroke, with majority of subjects having had a stroke within the past ten years. Furthermore, this difference in SW speed between the two sides was also significantly correlated with the degree of functional impairment, as measured using the Fugl–Meyer score ($r^2 = 0.327$, $p = 0.02$, Fig. 7).

In contrast, the difference in echo intensity between the two sides was not correlated with either the Fugl–Meyer score ($r^2 = 0.325$, $p = 0.06$) or with time since the stroke ($r^2 = 0.062$, $p = 0.45$). Other clinical tests, such as the modified Ashworth and Tardieu tests (which measures muscle resistance and also angle of muscle reflex activation), also did not show any correlations with SW speed or echo intensity.

4. Discussion

Using SW ultrasound elastography, we demonstrate that SWs travel faster and that the echo intensity is greater in the biceps brachii muscle of the paretic limb than in the contralateral non-paretic limb of stroke survivors, when the muscle is at rest. In addition, the reported strong correlation between the differences in SWS and echo intensity between the non-paretic and paretic muscle further suggests that the paretic muscles have potentially altered muscle composition and material properties. In particular, there is likely to be a greater passive stiffness, which can be potentially quantified with SW elastography. These changes in material properties are also correlated with the time since the stroke, and broadly with the degree of functional impairment, as indicated by the Fugl–Meyer score.

Changes to muscle material properties are often viewed as secondary to the neurologic injury (Burke et al., 2013). Stiffness of spastic muscle initially at rest depends on both muscle reflex mechanisms (in that hyperexcitable reflexes may be activated by even small movements) and on changes in the passive mechanical properties of muscle. These two features may together lead to contracture, which manifests as a reduction in joint range of motion (Gracies, 2005). Currently, standard clinical examinations cannot readily distinguish between these active reflex and passive tissue components (Burke et al., 2013; Lorentzen et al., 2010; Vattanasilp et al., 2000) as both changes may oppose stretch with an abnormal increase in torque for a given length (Broberg and Grimby, 1982). Furthermore, as stiffness increases progressively, this loss of range of motion further contributes to impaired limb and motor function (Dietz et al., 1991).

Our findings of increased SW speed in the paretic biceps brachii potentially provide a quantitative measure related to muscle stiffness in stroke survivors. Sources of increased passive stiffness in muscle include structures containing collagen such as the perimysium, the myofibril itself (Magid and Law, 1985), intracellular proteins such as titin (Lieber et al.,

2003), and the extracellular matrix (ECM) (Lieber and Ward, 2013). Through muscle biopsies, spastic muscles associated with cerebral palsy has been shown to have increased collagen (Booth et al., 2001; Castle et al., 1979; Lieber et al., 2003; Smith et al., 2011), a protein that primarily comprises the ECM (Lieber and Ward, 2013; Lieber et al., 2004). It has been suggested that muscle fibrosis, a related pathological descriptor, associated with an abnormal accumulation of ECM, may also occur frequently in stroke skeletal muscle (Lieber and Ward, 2013). Although there are some data on altered connective tissue and collagen content related to carotid vasculature in stroke survivors (Bot et al., 2009; Leeuwis et al., 2010; Lopez-Andres et al., 2012), there are very limited data on collagen content in stroke skeletal muscle.

We further observed higher echo intensity values of the B-mode ultrasound images in the biceps brachii of the paretic limb compared to the contralateral limb, which again suggests differences in muscle tissue composition. Enhanced echo intensity is often associated with increased intramuscular connective and adipose tissues (Arts et al., 2010; Fukumoto et al., 2012; Pillen et al., 2009a,2009b). Chronic hemiplegic patients often also display increased fat content in paretic upper arm muscles (Booth et al., 2001; Castle et al., 1979; Lieber et al., 2003; Smith et al., 2011). Our findings are supported by previous work showing higher Heckmatt values, a non-quantitative visual evaluation of echo intensities, in the gastrocnemius muscle of the paretic limb compared to that of the non-paretic limb (Heckmatt et al., 1982). Other relevant echo intensity observations include higher echo intensities in various lower extremity muscles of older adults compared to younger adults (Strasser et al., 2013), as well as in muscles of children with myopathic and neuromuscular disorders compared to muscles of typically developing children (Lamminen et al., 1988; Pillen et al., 2007). As there is variation in ultrasound system parameters, it is difficult to compare absolute values of echo intensity across different studies. Thus, we have focused our analyses on the difference in echo intensity between the muscles paretic and non-paretic side (Figs. 5 and 6). Our results do suggest that echo intensity measurements can be used non-invasively to assess changes in muscle composition in stroke survivors.

Increased passive stiffness may be related to increased collagen content in the muscles, but the origins of the proposed muscle connective tissue accumulation in our stroke survivor cohort remain unclear. Paretic muscles are routinely immobilized immediately after a stroke, which can lead to reduced longitudinal tension (Gracies, 2005). Animal and human studies have demonstrated that contractures that develop due to unloading conditions can involve muscle atrophy (McLachlan and Chua, 1983; Tabary et al., 1972), loss or shortening of sarcomeres (Baker and Hall-Craggs, 1980; McLachlan and Chua, 1983), and accumulation of connective tissue and fat (Gracies, 2005; Williams and Goldspink, 1984). Immobilized rat muscles show substantial increases in the endo- and perimysial connective tissue, with an increase in the number of collagen fibers (Järvinen et al., 2002) and an increase in the ratio of collagen to muscle-fiber tissue with associated changes in gene expression (Goldspink et al., 1991; Loughna and Brownson, 1996).

Much of our knowledge of the process of muscle contracture in the acute phase is from animal experiments where atrophy can be initiated within hours of immobilizing the limb (Booth, 1982) and increased amounts of connective tissue in the perimysium are observed

within two days of immobilization (Goldspink and Williams, 1990). It remains to be seen whether these animal models are applicable to human stroke. Moreover, the relationship between SW speed and time since stroke found in this current study suggests that in addition to the acute changes associated with the stroke, changes in material properties continue for many years.

In this study, we compared the muscles of the paretic side to the contralateral non-paretic side in order to determine the effects of stroke on muscle material properties and composition. Using the non-paretic side as the control is an acceptable comparison (in our view), as there can be much variability of limb segment length and muscle size between subjects. We report SW speed values ranging from 1.65 to 3.04 m/s for the non-paretic muscle and 2.39–6.02 m/s for the paretic muscle across 16 subjects. Although, the SW speed values for the non-paretic side measured here are comparable to those reported in the literature (derived from reported shear modulus) for the biceps brachii at similar muscle lengths (Bouillard et al., 2012; Lacourpaille et al., 2012, 2015; Nordez and Hug, 2010; Yoshitake et al., 2014), we focused largely on the difference between the two sides to reflect individual differences (Figs. 5–7). We acknowledge that future work comparing the muscles of the paretic and non-paretic side of individuals who have had a stroke with age-matched healthy controls is also needed.

SW ultrasound elastography has been previously used to determine changes in muscle stiffness in individuals with muscular disorders such as Duchenne muscular dystrophy (Lacourpaille et al., 2015) and cerebral palsy (Kwon et al., 2012; Park and Kwon, 2012). Lacourpaille et al. reported mean shear modulus values of the biceps brachii, as measured using SSI, of 3.9 kPa and 5.8 kPa (1.97 m/s and 2.4 m/s) in healthy individuals and those with Duchenne muscular dystrophy, respectively. In addition, children with cerebral palsy were found to have greater SW velocities in the gastrocnemius muscles compared to typically developing children, with the ankle at 90° in each group (Kwon et al., 2012). Thus, these other studies provide supporting evidence for our findings that increased SW speed may occur in individuals with altered muscle properties (Cornu et al., 2001; Lieber and Ward, 2013). Although we saw a correlation between the difference in SW speed between the paretic and the non-paretic side and the Fugl–Meyer, the lack of correlation with the modified Ashworth scores further emphasizes that SW elastography and echo intensity measures at rest are likely measuring independent variables and can potentially be used to evaluate muscle material properties and composition.

Limitations of this work include the constraint that measurements were only obtained at 90° of elbow flexion for both the paretic and non-paretic side, as resting or slack length of the muscles may differ. Recently, SSI was used to measure the shear modulus, calculated as the SW speed squared, of the gastrocnemius at different lengths during passive conditions and the authors observed a similar relationship to that of the normalized force-length relationship (Chernak et al., 2013; Maïsetti et al., 2012). Thus, measurements at different lengths of stroke-impaired muscle may vary, and this warrants further investigation. We further recognize that the correlation results of the paretic and non-paretic differences in SW speed and echo intensity, Fugl–Meyer scores, and time since stroke would benefit with more subjects with a wider range of elapsed time since stroke onset. For example, the majority of

the subjects in this study had a stroke within 10 years and there were few between 10 and 40 years.

5. Conclusions

We demonstrate that SW speed, as measured using SSI SW ultrasound elastography, and echo intensity are both greater in the biceps brachii muscle of the paretic limb than in the non-paretic limb of stroke survivors, when the muscle is at rest. These results suggest that the paretic muscles of stroke survivors have potentially altered muscle composition and material properties, such as increased stiffness. These changes in material properties are also strongly correlated with the time since the stroke, as well as with functional impairment, as indicated by the Fugl–Meyer score.

Since many components of rehabilitation therapy attempt to increase both passive and active range of motion, both of which are likely influenced by muscle stiffness, this US technology can become an important clinical tool. Knowing the changes in muscle material properties in stroke muscle and understanding their potential causes are important, may be valuable, both for determining fundamental mechanisms of impairment, for aiding clinical decisions, and for guiding rehabilitation interventions.

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References

- Arts IMP, Pillen S, Schelhaas HJ, Overeem S, Zwarts MJ, 2010 Normal values for quantitative muscle ultrasonography in adults. *Muscle Nerve* 41, 32–41. [PubMed: 19722256]
- Baker JH, Hall-Craggs E, 1980 Changes in sarcomere length following tenotomy in the rat. *Muscle Nerve* 3, 413–416. [PubMed: 6448348]
- Bercoff J, Tanter M, Fink M, 2004 Supersonic shear imaging: a new technique for soft tissue elasticity mapping. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control* 51, 396–409. [PubMed: 15139541]
- Booth F, 1982 Effect of limb immobilization on skeletal muscle. *J. Appl. Physiol* 52, 1113–1118. [PubMed: 7047468]
- Booth CM, Cortina-Borja MJ, Theologis TN, 2001 Collagen accumulation in muscles of children with cerebral palsy and correlation with severity of spasticity. *Dev. Med. Child Neurol* 43, 314–320. [PubMed: 11368484]
- Bot PT, Hoefer IE, Sluijter JP, van Vliet P, Smits AM, Lebrin F, Moll F, de Vries JP, Doevendans P, Piek JJ, Pasterkamp G, Goumans MJ, 2009 Increased expression of the transforming growth factor-beta signaling pathway, endoglin, and early growth response-1 in stable plaques. *Stroke* 40, 439–447. [PubMed: 19074480]
- Bouillard K, Nordez A, Hodges PW, Cornu C, Hug F, 2012 Evidence of changes in load sharing during isometric elbow flexion with ramped torque. *J. Biomech* 45, 1424–1429. [PubMed: 22406469]
- Brandenburg JE, Eby SF, Song P, Zhao H, Brault JS, Chen S, An KN, 2014 Ultrasound elastography: the new frontier in direct measurement of muscle stiffness. *Arch. Phys. Med. Rehabil* 95, 2207–2219. [PubMed: 25064780]
- Broberg C, Grimby G, 1982 Measurement of torque during passive and active ankle movements in patients with muscle hypertonia. A methodological study. *Scand. J. Rehabil. Med. Suppl* 9, 108–117.
- Burke D, Wissel J, Donnan GA, 2013 Pathophysiology of spasticity in stroke. *Neurology* 80, S20–S26.

- Castle M, Reyman TA, Schneider M, 1979 Pathology of spastic muscle in cerebral palsy. *Clin. Orthop. Relat. Res* 142, 223–233.
- Chardon MK, Suresh NL, Rymer WZ, 2010 An evaluation of passive properties of spastic muscles in hemiparetic stroke survivors, Engineering in Medicine and Biology Society (EMBC). 2010 Annual International Conference of the IEEE. IEEE, pp. 2993–2996.
- Chernak LA, DeWall RJ, Lee KS, Thelen DG, 2013 Length and activation dependent variations in muscle shear wave speed. *Physiol. Meas* 34, 713–721. [PubMed: 23719230]
- Cornu C, Goubel F, Fardeau M, 2001 Muscle and joint elastic properties during elbow flexion in Duchenne muscular dystrophy. *J. Physiol* 533, 605–616. [PubMed: 11389216]
- de Vlugt E, de Groot JH, Schenkeveld KE, Arendzen JH, van der Helm FC, Meskers CG, 2010 The relation between neuromechanical parameters and Ashworth score in stroke patients. *J. Neuroeng. Rehabil* 7, 35. [PubMed: 20663189]
- Dietz V, Trippel M, Berger W, 1991 Reflex activity and muscle tone during elbow movements in patients with spastic paresis. *Ann. Neurol* 30, 767–779. [PubMed: 1789693]
- Fukumoto Y, Ikezoe T, Yamada Y, Tsukagoshi R, Nakamura M, Mori N, Kimura M, Ichihashi N, 2012 Skeletal muscle quality assessed from echo intensity is associated with muscle strength of middle-aged and elderly persons. *Eur. J. Appl. Physiol* 112, 1519–1525.
- Gladstone DJ, Danells CJ, Black SE, 2002 The Fugl–Meyer assessment of motor recovery after stroke: a critical review of its measurement properties. *Neurorehabil. Neural Repair* 16, 232–240. [PubMed: 12234086]
- Goldspink G, Williams P, 1990 Muscle fibre and connective tissue changes associated with use and disuse. *issues in neurological physiotherapy Butterworth-Heinemann, Key Oxford*, pp. 197–217.
- Goldspink G, Scutt A, Martindale J, Jaenicke T, Turay L, Gerlach G, 1991 Stretch and force generation induce rapid hypertrophy and myosin isoform gene switching in adult skeletal muscle. *Biochem. Soc. Trans* 19, 368–373. [PubMed: 1716229]
- Gracies JM, 2005 Pathophysiology of spastic paresis. I: Paresis and soft tissue changes. *Muscle Nerve* 31, 535–551. [PubMed: 15714510]
- Gray CS, French JM, Bates D, Cartlidge NE, James OF, Venables G, 1990 Motor recovery following acute stroke. *Age Ageing* 19, 179–184. [PubMed: 2363378]
- Heckmatt J, Leeman S, Dubowitz V, 1982 Ultrasound imaging in the diagnosis of muscle disease. *J. Pediatr* 101, 656–660. [PubMed: 7131136]
- Järvinen TA, Józsa L, Kannus P, Järvinen TL, Järvinen M, 2002 Organization and distribution of intramuscular connective tissue in normal and immobilized skeletal muscles. *J. Muscle Res. Cell Motil* 23, 245–254. [PubMed: 12500904]
- Katz RT, Rymer WZ, 1989 Spastic hypertonia: mechanisms and measurement. *Arch. Phys. Med. Rehabil* 70, 144–155. [PubMed: 2644919]
- Kwon DP, Young Gi, Lee Sung Uk, Chung Inbum, 2012 Spastic cerebral palsy in children: dynamic sonoelastographic findings of medial gastrocnemius. *Radiology* 263, 794–801. [PubMed: 22495685]
- Lacourpaille L, Hug F, Bouillard K, Hogrel JY, Nordez A, 2012 Supersonic shear imaging provides a reliable measurement of resting muscle shear elastic modulus. *Physiol. Meas* 33, N19–N28. [PubMed: 22370174]
- Lacourpaille L, Hug F, Guevel A, Pereon Y, Magot A, Hogrel JY, Nordez A, 2015 Non-invasive assessment of muscle stiffness in patients with duchenne muscular dystrophy. *Muscle Nerve* 51, 284–286. [PubMed: 25187068]
- Lamminen A, Jääskeläinen J, Rapola J, Suramo I, 1988 High-frequency ultrasonography of skeletal muscle in children with neuromuscular disease. *J. Ultrasound Med* 7, 505–509. [PubMed: 3054144]
- Leeuwis JW, Nguyen TQ, Theunissen MG, Peeters W, Goldschmeding R, Pasterkamp G, Vink A, 2010 Connective tissue growth factor is associated with a stable atherosclerotic plaque phenotype and is involved in plaque stabilization after stroke. *Stroke* 41, 2979–2981. [PubMed: 20966418]
- Lieber RL, Ward SR, 2013 Cellular Mechanisms of Tissue Fibrosis. 4. Structural and functional consequences of skeletal muscle fibrosis. *Am. J. Physiol. Cell Physiol* 305, C241–C252. [PubMed: 23761627]

- Lieber RL, Runesson E, Einarsson F, Fridén J, 2003 Inferior mechanical properties of spastic muscle bundles due to hypertrophic but compromised extracellular matrix material. *Muscle Nerve* 28, 464–471. [PubMed: 14506719]
- Lieber RL, Steinman S, Barash IA, Chambers H, 2004 Structural and functional changes in spastic skeletal muscle. *Muscle Nerve* 29, 615–627. [PubMed: 15116365]
- Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, 2010 Heart disease and stroke statistics—2010 update A report from the American Heart Association. *Circulation* 121, e46–e215. [PubMed: 20019324]
- Lopez-Andres N, Rousseau A, Akhtar R, Calvier L, Inigo C, Labat C, Zhao X, Cruickshank K, Diez J, Zannad F, Lacolley P, Rossignol P, 2012 Cardiotrophin 1 is involved in cardiac, vascular, and renal fibrosis and dysfunction. *Hypertension* 60, 563–573. [PubMed: 22733458]
- Lorentzen J, Grey MJ, Crone C, Mazevet D, Biering-Sørensen F, Nielsen JB, 2010 Distinguishing active from passive components of ankle plantar flexor stiffness in stroke, spinal cord injury and multiple sclerosis. *Clin. Neurophysiol* 121, 1939–1951. [PubMed: 20457538]
- Loughna PT, Brownson C, 1996 Two myogenic regulatory factor transcripts exhibit muscle-specific responses to disuse and passive stretch in adult rats. *FEBS Lett.* 390, 304–306. [PubMed: 8706882]
- Magid A, Law DJ, 1985 Myofibrils bear most of the resting tension in frog skeletal muscle. *Science* 230, 1280–1282. [PubMed: 4071053]
- Maïsetti O, Hug F, Bouillard K, Nordez A, 2012 Characterization of passive elastic properties of the human medial gastrocnemius muscle belly using supersonic shear imaging. *J. Biomech* 45, 978–984. [PubMed: 22326058]
- McLachlan EM, Chua M, 1983 Rapid adjustment of sarcomere length in tenotomized muscles depends on an intact innervation. *Neurosci. Lett* 35, 127–133. [PubMed: 6856191]
- Muthupillai R, Lomas D, Rossman P, Greenleaf J, Manduca A, Ehman R, 1995 Magnetic resonance elastography by direct visualization of propagating acoustic strain waves. *Science* 269, 1854–1857. [PubMed: 7569924]
- Nakayama H, Jørgensen H, Raaschou H, Olsen T, 1994 The influence of age on stroke outcome. The Copenhagen Stroke Study. *Stroke* 25, 808–813. [PubMed: 8160225]
- Nordez A, Hug F, 2010 Muscle shear elastic modulus measured using supersonic shear imaging is highly related to muscle activity level. *J. Appl. Physiol* 108, 1389–1394. [PubMed: 20167669]
- Ophir J, Cespedes I, Ponnekanti H, Yazdi Y, Li X, 1991 Elastography: a quantitative method for imaging the elasticity of biological tissues. *Ultrason. Imaging* 13, 111–134. [PubMed: 1858217]
- Pandyan A, Johnson G, Price C, Curless R, Barnes M, Rodgers H, 1999 A review of the properties and limitations of the Ashworth and modified Ashworth scales as measures of spasticity. *Clin. Rehabil* 13, 373–383. [PubMed: 10498344]
- Park G-Y, Kwon DR, 2012 Sonoelastographic evaluation of medial gastrocnemius muscles intrinsic stiffness after rehabilitation therapy with botulinum toxin A injection in spastic cerebral palsy. *Arch. Phys. Med. Rehabil* 93, 2085–2089. [PubMed: 22776155]
- Pillen S, Verrips A, Van Alfen N, Arts I, Sie L, Zwarts M, 2007 Quantitative skeletal muscle ultrasound: diagnostic value in childhood neuromuscular disease. *Neuromuscul. Disord* 17, 509–516. [PubMed: 17537635]
- Pillen S, Tak RO, Zwarts MJ, Lammens MM, Verrijp KN, Arts IM, van der Laak JA, Hoogerbrugge PM, van Engelen BG, Verrips A, 2009a Skeletal muscle ultrasound: correlation between fibrous tissue and echo intensity. *Ultrasound Med. Biol* 35, 443–446. [PubMed: 19081667]
- Pillen S, van Dijk JP, Weijers G, Raijmann W, de Korte CL, Zwarts MJ, 2009b Quantitative gray-scale analysis in skeletal muscle ultrasound: a comparison study of two ultrasound devices. *Muscle Nerve* 39, 781–786. [PubMed: 19301363]
- Roy A, Krebs HI, Bever CT, Forrester LW, Macko RF, Hogan N, 2011 Measurement of passive ankle stiffness in subjects with chronic hemiparesis using a novel ankle robot. *J. Neurophysiol* 105, 2132–2149. [PubMed: 21346215]
- Singh P, Joshua AM, Ganeshan S, Suresh S, 2011 Intra-rater reliability of the modified Tardieu scale to quantify spasticity in elbow flexors and ankle plantar flexors in adult stroke subjects. *Ann. Indian Acad. Dent. Neurol* 14, 23.

- Sinkjær T, Magnussen I, 1994 Passive, intrinsic and reflex-mediated stiffness in the ankle extensors of hemiparetic patients. *Brain* 117, 355–363. [PubMed: 8186961]
- Smith LR, Lee KS, Ward SR, Chambers HG, Lieber RL, 2011 Hamstring contractures in children with spastic cerebral palsy result from a stiffer extracellular matrix and increased in vivo sarcomere length. *J. Physiol* 589, 2625–2639. [PubMed: 21486759]
- Strasser EM, Draskovits T, Praschak M, Quittan M, Graf A, 2013 Association between ultrasound measurements of muscle thickness, pennation angle, echogenicity and skeletal muscle strength in the elderly. *AGE* 35, 2377–2388. [PubMed: 23456136]
- Tabary J, Tabary C, Tardieu C, Tardieu G, Goldspink G, 1972 Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J. Physiol* 224, 231–244. [PubMed: 5039983]
- Vattanasilp W, Ada L, Crosbie J, 2000 Contribution of thixotropy, spasticity, and contracture to ankle stiffness after stroke. *J. Neurol. Neurosurg. Psychiatry* 69, 34–39. [PubMed: 10864601]
- Williams P, Goldspink G, 1984 Connective tissue changes in immobilised muscle. *J. Anat* 138, 343. [PubMed: 6715254]
- Yoshitake Y, Takai Y, Kanehisa H, Shinohara M, 2014 Muscle shear modulus measured with ultrasound shear-wave elastography across a wide range of contraction intensity. *Muscle Nerve* 50, 103–113. [PubMed: 24155045]

Fig. 1.

Shear wave ultrasound (SW) elastography images of the non-paretic and paretic biceps brachii muscles of a representative subject. The squares represent the region of interest from which SW speed was measured. In these images, the non-paretic muscle has a mean SW speed of 2.5 m/s, whereas the paretic muscle has a mean SW speed of 4.7 m/s.

Fig. 2.

B-mode ultrasound images of the non-paretic and paretic biceps brachii muscles of a representative subject. The scale of the figure refers to the echo intensity in that pixels that are black have a value of 0, whereas pixels that are very bright, or white, have a value of 255. The squares represent the region of interest from which echo intensity was calculated. The higher the echo intensity, the brighter or “whiter” the pixel appears. In these images, the non-paretic muscle has a mean echo intensity of 75 whereas the paretic muscle has a mean echo intensity of 91.

Fig. 3. Mean shear wave speeds and standard deviation of the non-paretic (black bars) and paretic (gray bars) biceps brachii muscle of individual subjects. All but one subject (S14) has greater SW speed in the paretic muscle.

Fig. 4.

Mean echo intensity values and standard deviations of the non-paretic (black bars) and paretic (gray bars) biceps brachii muscle of individual subjects. All but two subjects (S3 and S7) have greater echo intensity in the paretic muscle. Echo intensity values range from 0 to 255, indicating black to white, respectively.

Fig. 5. Relationship between the differences in shear wave speed and echo intensity of the non-paretic and paretic biceps brachii muscle.

Fig. 6. Relationship between the difference in shear wave speed of the non-paretic and paretic biceps brachii muscle and time since stroke.

Fig. 7. Relationship between the difference in shear wave speed of the non-paretic and paretic biceps brachii muscle and Fugl-Meyer test score. Lower Fugl-Meyer scores indicate more severe functional impairments.