Published in final edited form as:

Clin Biomech (Bristol, Avon). 2015 January ; 30(1): 22–27. doi:10.1016/j.clinbiomech.2014.11.011.

Shear Wave Elastography of Passive Skeletal Muscle Stiffness: Influences of Sex and Age throughout Adulthood

Sarah F. Eby^{a,e}, Beth A. Cloud^{b,e}, Joline E. Brandenburg^c, Hugo Giambini^{d,e}, Pengfei Song^d, Shigao Chen^d, Nathan K. LeBrasseur^{c,d}, and Kai-Nan An^e

^aMayo Medical School, Mayo Graduate School, and the Medical Scientist Training Program, College of Medicine, Mayo Clinic, Rochester, MN, USA

^bMayo Graduate School and the Center for Clinical and Translational Science, College of Medicine, Mayo Clinic, Rochester, MN, USA

^cDepartment of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN, USA

^dPhysiology and Biomedical Engineering, College of Medicine, Mayo Clinic, Rochester, MN, USA

^eBiomechanics Laboratory, Division of Orthopedic Research, Mayo Clinic, Rochester, MN, USA

Abstract

Background—Numerous structural and compositional changes – related not only to age, but also activity level and sex – may affect skeletal muscle stiffness across the adult age-span. Measurement techniques available thus far have largely limited passive stiffness evaluations to those of entire joints and muscle-tendon units. Shear wave elastography is an increasingly popular ultrasound technique for evaluating the mechanical properties of skeletal muscle tissue. The purpose of this study was to quantify the passive stiffness, or shear modulus, of the biceps brachii throughout adulthood in flexed and extended elbow positions. We hypothesized that shear modulus would be higher in males relative to females, and with advanced age in both sexes.

Methods—Shear wave elastography quantified biceps brachii stiffness at 90° elbow flexion and full extension in a large sample of adults between 21–94 years old ($n=133$; 47 males).

Findings—Regression analysis found sex and age were significant parameters for older adults (>60 years) in full extension. As expected, shear modulus values increased with advancing age; however, shear modulus values for females tended to be higher than those for males.

Interpretation—This study begins to establish normative trends for skeletal muscle shear modulus throughout adulthood. Specifically, this work establishes for the first time that the higher passive joint torque often found in males relative to females likely relates to parameters other than muscle shear modulus. Indeed, perhaps increases in skeletal muscle passive stiffness, though

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Corresponding Author: Kai-Nan An, 200 First St. SW, Rochester, MN, USA 55905, Voice: 1(507)538-1717, Fax: 1(507)284-5392, an.kainan@mayo.edu.

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potentially altering the length-tension curve, serve a protective role – maintaining the tendon-muscle-tendon length-tension curve within a functional range.

Keywords

ultrasound; skeletal muscle; elastic modulus; shear wave elastography; age; sex

1. Introduction

Skeletal muscle is necessarily a very dynamic tissue; both physically: facilitating daily interactions with our environment, and biologically: continually responding and adapting to the presence or absence of mechanical and biochemical cues. While skeletal muscle function and performance is certainly related to muscle mass and strength, numerous structural and compositional changes may also modulate function throughout the lifetime, including alterations in fiber type composition and function [1–4], intramuscular fat content [5,6], capillary density [7], and intramuscular connective tissue, or collagen [6,8–10]. Though many of these characteristics can only be assessed with invasive muscle biopsy or expensive and timeconsuming MRI techniques [11,12], increased collagen content is closely associated with increases in passive skeletal muscle stiffness [8], which can be readily evaluated noninvasively. Characterizing the mechanical properties of skeletal muscle between sexes and over time may provide key strategies for preventing joint injuries, improving function and independence, and preventing falls.

In addition to the loss of muscle mass and strength, termed *sarcopenia*, aging skeletal muscle is characterized by increased collagen content and stiffness [8,13,14]. Various architectural alterations, including reduced pennation angle and fascicle length are likely related to reduced muscle bulk and loss of individual muscle fibers that are a hallmark of sarcopenia [15]. Though largely foreseeable, these architectural alterations may serve to reduce the extensibility of skeletal muscle – altering the length-tension curve and thus affecting passive muscle stiffness with aging [16]. Biochemical and histological studies of aged rat extensor digitorum longus muscles indicate both collagen and connective tissue within the muscle belly increase with aging, as well as passive and active muscle stiffness [8]. Though this study evaluated rat muscle that is composed primarily of type IIB fibers which are not present in humans [17,18], later research documents similar increases in human intramuscular connective tissue [6]. This increase in stiffness of rat muscle is in sharp contrast to the findings in aged human tendon, which tend to show reduced collagen and increased extensibility with aging [19]. Noninvasive elastography techniques are beginning to identify differences in muscle stiffness between children, young, and middle-aged subjects [20,21], with possible elevated stiffness in advanced age [22]. As altered extensibility at either the tendon or muscle level will affect their length-tension relationship and therefore joint function, especially in the elderly who are at risk of falling [23], it is imperative to monitor the mechanical properties of these tissues throughout all stages of adulthood.

Sex differences in skeletal muscle strength and speed of contraction are well-established, with females typically displaying reduced strength, cross-sectional area, and speed of

contraction relative to males [1,24–28]. Females tend to have lower resistive joint torque during passive range of motion – including about the ankle [29,30], knee [31], in the hamstrings [32], and the elbow flexors [33]. Additionally, females have reduced active joint stiffness during range of motion and functional tasks [34,35]. While many of these sex differences may be reduced or eliminated by accounting for weight, fat-free mass, or muscle mass – including differences in resistive torque [33] – the influence of sex on the stiffness of individual muscles has yet to be evaluated. The influence of sex may have implications in the tendency for increased musculoskeletal injury and resulting disability in females relative to males [36].

Shear wave elastography (SWE) is an increasingly popular ultrasound technique for noninvasively evaluating the mechanical properties of skeletal muscle tissue. SWE has been used to evaluate normal tissue at a variety of angles and tensions [37,38], finding increased passive stiffness with stretch [39]. SWE shows great promise for evaluating both passive and active muscle properties, and there is evidence of improved accuracy for estimating torque as compared to EMG, especially during fatigue [40,41]. Like typical ultrasound techniques, SWE may be susceptible to variations in individual technique – especially transducer pressure [42], though still demonstrates excellent reliability [43]. Though studies use SWE to evaluate both healthy and pathologic skeletal muscle at an increasingly rapid rate, little information is available about normative values for skeletal muscle stiffness, especially across the adult age-span. Though not a locomotive muscle, the biceps brachii is especially amenable to SWE evaluation, as it is readily accessible and has minimal pennation [44]. The biceps brachii will thus provide valuable information about baseline skeletal muscle stiffness. The purpose of this study was to quantify the passive stiffness, or shear modulus, of the biceps brachii throughout adulthood in flexed and extended elbow positions. We hypothesized that shear modulus would be higher in males relative to females, and with advanced age in both sexes.

2. Methods

2.1. Subjects

133 healthy adults (47 males) participated in this study. The mean age was 44.3 years (range: 21–94 years). We divided subjects into subgroups of younger (21–59 years; $n = 111$) and older (60–94 years; $n = 32$) adults. The majority of subjects ($n = 114$) were recruited from the employee population of Mayo Clinic, with older subjects recruited from a local retirement community ($n = 19$). Inclusion criteria were: 1) 18 years old or greater; or 2) normal function of both arms. Subjects were excluded if they reported a history of: 1) neuromuscular or musculoskeletal disease; or 2) upper extremity injury or surgery within the past six months. All subjects completed a brief questionnaire including basic demographic, exercise, and medical history information. We recorded the circumference of the dominant upper arm at the area of greatest bulk in the Mayo Clinic employee study participants. All study procedures were approved by the Mayo Clinic Institutional Review Board (Rochester, MN).

2.2 Shear Wave Elastography (SWE)

We collected SWE maps of the dominant-side biceps brachii with the elbow fixed at 90° elbow flexion and full extension. Subjects were seated comfortably with their arms resting at their sides. A custom arm rest held each subject's arm at 90° elbow flexion for the first ultrasound measurement. We removed the arm rest and asked the subject to relax their arm, extended down by their side, to obtain the second ultrasound measurement. All subjects were instructed to remain as relaxed as possible throughout all testing (approximately 30–60 seconds).

We held the ultrasound transducer over the mid-substance of the long head of the biceps brachii muscle. We coupled the ultrasound transducer with the skin surface using transmission gel, taking care to ensure minimal, even contact pressure between the two. We acquired all ultrasound measurements using the SL15-4 transducer (center frequency = 8.5 MHz) on the Aixplorer (SuperSonic Imagine, Aix-en-Provence, France) ultrasound system in SWE mode (musculoskeletal preset) as previously described [45]. Briefly, a push beam was sent into the tissue to generate shear waves within the muscle. The same SL15-4 transducer then detected the propagation of the shear waves, and the ultrasound system determined shear wave speed in each pixel of the selected SWE elastogram. We saved one image at each elbow angle (90° flexion and full extension) for post-processing. A sample set of elastograms from a representative subject are included in Figure 1.

2.3 Data Analysis

We used a plugin for the DICOM reader OsiriX (Pixmeo, Geneva, Switzerland) to determine the mean Young's modulus for the largest circular region of interest (mean diameter: 9.4 mm) within the SWE elastogram via Equations 1 and 2:

$$G = c_s^2 \rho \quad (1)$$

$$G = \frac{E}{2(1+\nu)} \quad (2)$$

where G is shear modulus, c_s is shear wave propagation velocity, and ρ is density, which can be assumed to be 1000 kg/m³ for all soft tissues, including skeletal muscle. An example 2D shear elasticity map with ROI selection and associated shear elasticity statistics given by OsiriX are included in Figure 1. The color distribution in elasticity maps of the muscle tended to be largely homogeneous, though we took care to avoid ROI selection in the few regions of poor color filling, which indicate poor shear wave measurements. The OsiriX plugin assumes tissue is isotropic and thus relates shear modulus (G) and Young's modulus (E) by Equation 2, where Poisson's ratio, ν is very close to 0.5 if assuming incompressible tissue. As muscle is anisotropic, it was necessary to reverse the automatic calculation applied by the Aixplorer software by manually converted the mean Young's modulus values back to shear modulus using Equation 2. A paired t-test compared shear modulus values at 90° flexion and full extension. We evaluated shear modulus values with multiple regression analysis using JMP software (SAS Institute, Inc., Cary, USA) evaluating age, sex, BMI, exercise frequency, and arm circumference as covariates due to their potential relationship

with skeletal muscle structure, function, and extensibility. An α -level of 0.05 determined significance for all statistical analyses.

3. Results

3.1 Subject Information

Subject demographic information is included by decade in Table 1. 133 healthy subjects volunteered for the study, seven of which reported being left-handed. 15 subjects reported race/ethnicities other than white (Hispanic/Latino: 8; Asian: 7). Subjects had no difficulty achieving the two elbow positions required for this study.

3.2 Shear Wave Elastography

As expected, shear modulus values increased from 90° flexion to full extension for all subjects, a significant difference that was confirmed using a paired t-test ($p < 0.0001$). For this reason, we separated by elbow position for all further analysis, rather than including elbow angle as a possible parameter – as increased muscle stiffness with greater joint angle was highly anticipated and already well-established. Shear modulus values by decade group for both sexes at 90° elbow flexion and full extension are included in Table 1.

We chose age, sex, BMI, exercise frequency, and arm circumference as covariates in our regression analysis, as indicated in Table 2. Using a backwards stepwise approach, we determined the most appropriate model included age and sex (coded: female = 1; male = 0). Further analysis assessed the interaction between these two variables, which was found to be significant and thus part of the final model, also shown in Table 2. The final equation for 90° elbow flexion was:

$$\text{shear modulus} = 5.46 - 0.0078 \cdot \text{age} - 1.19 \cdot \text{sex}(\text{female}) + 0.044 \cdot \text{age} \cdot \text{sex}(\text{female}) \quad (R^2 = 0.10);$$

and at full extension:

$$\text{shear modulus} = 14.47 + 0.0057 \cdot \text{age} - 1.39 \cdot \text{sex}(\text{female}) + 0.10 \cdot \text{age} \cdot \text{sex}(\text{female}) \quad (R^2 = 0.18).$$

In an effort to identify the overall contributions of age, as well as those of advanced age on skeletal muscle passive stiffness, we performed an additional multiple regression analysis on the subgroups of younger and older adults. We repeated the regression analyses using the same final parameters for both elbow angles, but divided the subjects into two age groups: 21–59 years and 60–94 years. The results are included in Figure 2. The equations for 90° elbow flexion were as follows, for the younger age group:

$$\text{shear modulus} = 4.55 + 0.022 \cdot \text{age} + 0.22 \cdot \text{sex}(\text{female}) \quad (R^2 = 0.02),$$

though no parameters were significant; and for the older age group:

$$\text{shear modulus} = 4.66 + 0.0016 \cdot \text{age} + 2.31 \cdot \text{sex}(\text{female}) \quad (R^2 = 0.16),$$

with sex as a significant parameter ($p = 0.03$). At full elbow extension, the equations were as follows, for the younger age group:

$$\text{shear modulus} = 14.43 + 0.012 * \text{age} + 2.17 * \text{sex}(\text{female}) \quad (R^2 = 0.05),$$

with sex as a significant parameter ($p = 0.03$); and for the older age group:

$$\text{shear modulus} = -6.85 + 0.27 * \text{age} + 7.69 * \text{sex}(\text{female}) \quad (R^2 = 0.44), \text{ with both age and sex as significant parameters } (p = 0.03 \text{ and } 0.0004, \text{ respectively}).$$

4. Discussion

This study begins to establish normative trends for skeletal muscle shear modulus throughout adulthood. As hypothesized, biceps brachii stiffness increases with advanced age. However, shear modulus values tended to be higher for females than for males of the same age. Though significant research indicates passive torque in males is greater than that for females in a variety of joints, including the elbow [33], it is apparent that sex differences in joint torque may be less related to muscle shear modulus, and more to other parameters, such as muscle cross-sectional area or other joint structures entirely. The present study only investigated one muscle and did not evaluate passive joint torque, but the consistent trend of lower stiffness in males is certainly worth noting.

Though aging begins to affect skeletal muscles as early as 25 years of age, muscle morphology likely remains fairly similar through the sixth decade, after which declines in fiber quantity, quality [46] and strength [28] accelerate. For this reason, we felt justified separating the subjects into younger (<60 years) and older (60+ years) groups. Gross visual inspection of the data included in Figure 2 also supports this decision. Despite notable variation in shear modulus between individuals of the same age, there is absence of any upward or downward tendencies in this measurement until approximately age 60, at which point an overall upward trend is evident. This agrees well with previous investigations which found significant reductions in strength [14,28], function [29,47], overall muscle quality [15,27], and increases in glycation [48] around this age or soon thereafter. In the group 60 years and older, age and sex account for 44% of the variation in shear modulus. We did not find significant contributions of BMI, arm circumference, or exercise frequency, not surprising given the relatively poor specificity of these measures.

This study had several limitations. All subjects were self-selected to participate, and were drawn from a generally healthy, active, well-educated population, so may not represent the general population. Aside from the shear modulus values, all data was self-reported on a brief questionnaire, and thus may have some inherent inaccuracies. In an effort to gauge overall activity level, we inquired about exercise frequency, though did not collect any information about mode, intensity or duration. It is possible for exercise that targets a specific muscle of interest to affect the shear modulus of the agonist muscle. Additionally, we did not obtain information regarding menstrual cycle for premenopausal females. There is evidence that estrogen and phase within the hormonal cycle may affect active musculotendinous stiffness in females [49], and may alter range of motion [50] so may be a factor in the large variation we observed at full elbow extension in premenopausal females. Additionally, the number of male subjects above age 30 is somewhat low: the number of men in each decade group tends to be consistently lower than that of women. This may reduce the statistical power of this study – perhaps the sex differences are slightly

exaggerated by the larger sample size and associated variability in females. Finally, we requested all subjects to remain as relaxed as possible during all shear modulus measures, and we did not obtain measures until we visually observed relaxation. As we did not confirm relaxation with electromyography, it is possible that subjects may have been unable to relax and were still minimally activating their biceps brachii, affecting the results [37].

Additional investigations evaluating locomotive muscles and those with more complex architecture than the biceps brachii will be very beneficial and may identify therapeutic targets to improve function and mobility, especially in the older population. Though the current study found no contributions of self-reported exercise frequency to muscle stiffness, previous investigations indicate baseline age-related skeletal muscle changes appear to be readily modulated with exercise training and warrants additional investigation with more sensitive questionnaires or interventions. Evaluating the connection between histologic findings from muscle biopsy with *in vivo* ultrasound elastography findings will serve to greatly improve the understanding and application of shear wave elastography in musculoskeletal pathologies.

Acknowledgments

The authors thank the Healthy Aging and Independent Living Laboratory and the ultrasound laboratory of James F. Greenleaf for their continued support, without which this work would not have been possible. This work was supported by the National Institutes of Health through grants from the National Center for Advancing Translational Sciences (NCATS) (UL1 TR000135). SFE was supported by grants from the National Institute on Aging (F30 AG044075) and the National Institute of General Medical Sciences (T32 GM65841). BAC was supported by the Foundation for Physical Therapy and a grant from the NCATS (TL1 TR000137). JEB was supported by a grant from the NCATS (KL2 TR000136).

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Highlights

- Shear wave elastography of biceps brachii in adults 21–94 years old (n=114)
- Multiple regression analysis finds sex and age significant parameters in older adults
- Female shear modulus values tended to be higher than those for males

Figure 1. Sample biceps brachii SWE elastograms for a representative subject. Green circle identifies region of interest; solid white bars indicate focusing range; scale in cm; shear wave speed color scale included for reference. A) 90° elbow flexion; B) full extension.

Figure 2.

Shear modulus with age at full elbow extension. Linear regression significant for ≥ 60 years

(lines included). Females ≥ 60 years: $shear\ modulus = 0.84 + 0.27 \cdot age$, $R^2 = 0.44$; Males

≥ 60 years: $shear\ modulus = -6.85 + 0.27 \cdot age$, $R^2 = 0.44$.

Table 1

Subject characteristics; shear modulus values given as mean (SD).

Age group (years)	n	BMI	90° shear modulus (kPa)	Full extension shear modulus (kPa)
20–29	45	23.49 (3.03)	5.23 (1.86)	16.13 (4.51)
	Male 21	23.81 (2.85)	5.32 (2.38)	15.52 (5.07)
	Female 24	23.19 (3.18)	5.16 (1.29)	16.66 (3.99)
30–39	26	25.27 (4.87)	5.44 (2.10)	15.48 (4.07)
	Male 5	26.74 (4.35)	5.65 (4.48)	13.99 (2.30)
	Female 21	24.88 (4.97)	5.41 (1.47)	15.84 (4.36)
40–49	19	25.70 (4.15)	5.75 (1.62)	17.76 (5.62)
	Male 5	26.60 (1.70)	4.97 (1.73)	13.01 (4.04)
	Female 14	25.37 (4.73)	6.04 (1.55)	19.45 (5.18)
50–59	11	27.95 (3.45)	5.78 (1.43)	16.33 (3.02)
	Male 2	23.49 (1.04)	4.31 (0.73)	13.67 (1.04)
	Female 9	28.94 (2.96)	6.14 (1.34)	16.93 (3.02)
60–69	12	28.22 (3.74)	6.15 (2.58)	15.31 (5.87)
	Male 5	27.91 (2.85)	4.88 (1.28)	11.37 (4.85)
	Female 7	28.40 (4.26)	7.21 (3.01)	18.12 (5.05)
70–79	5	29.47 (5.64)	6.15 (2.32)	18.27 (4.10)
	Male 1	24.62	3.47	17.50
	Female 4	30.67 (5.70)	6.83 (2.03)	18.47 (4.71)
80–89	10	24.28 (2.25)	6.40 (3.89)	20.98 (8.87)
	Male 4	24.14 (1.93)	4.24 (0.67)	12.81 (2.03)
	Female 6	24.39 (2.57)	7.84 (4.55)	26.42 (7.10)
90–99	5	25.98 (1.82)	5.06 (2.81)	19.82 (5.44)
	Male 4	25.79 (2.11)	5.57 (2.97)	19.55 (6.25)
	Female 1	26.57	3.03	20.90

Table 2

Multiple linear regression models for 90° elbow flexion and full extension. Exercise indicated by number of times per week.

90° Elbow Flexion		Full Extension		
	Estimate	p-value	Estimate	p-value
Initial Model Parameters				
Sex (F)	0.27	0.53	1.98	0.059
Age	0.021	0.19	0.038	0.31
BMI	0.055	0.43	0.014	0.93
Arm Circumference	−0.044	0.58	−0.29	0.13
Exercise				
1–2x	−0.26	0.70	1.93	0.23
3–4x	−0.43	0.52	1.88	0.25
5+	1.46	0.058	1.14	0.54
Final Model Parameters				
Sex (F)	−1.18	0.54	−1.39	0.49
Age	−0.0078	0.19	0.0057	0.85
Sex (F) * Age	0.044	0.015	0.10	0.011

Abbreviations: BMI, body mass index; F, female.