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MEASUREMENT OF STIFFNESS CHANGES IN IMMOBILIZED MUSCLE USING MAGNETIC RESONANCE ELASTOGRAPHY

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Abstract

Background—The isolated evaluation of changes in muscle following immobilization and disuse is a challenge in living subjects. The purpose of this study was to determine whether magnetic resonance elastography is capable of detecting these changes.

Methods—An animal model was created to produce a mild joint contracture following 42 days of one forelimb immobilization in a maximally flexed position with twice-daily passive exercise. Eight pairs of dog forelimbs were harvested. Magnetic resonance elastography scans were performed on the experimental limb in an extended elbow position with a torque of 0.6 Nm. Scans of the contralateral limb were performed in two conditions, position matching and torque matching. Furthermore, wet weight, cross sectional area, resting muscle length, and range of elbow joint motion were measured.

Findings—The muscle from the experimental limb showed significant reduction in muscle mass, cross sectional area, slack length, and range of elbow motion. When comparing limbs in position matching condition, the muscle lengths were similar, and the experimental muscle had a significantly higher shear modulus (79.1 (SD 12.0) kPa) than the contralateral muscle (31.9 (SD 24.4) kPa). When comparing limbs in torque matching conditions, the muscle strains were similar, and the experimental muscle had a significantly lower shear modulus than the contralateral muscle (113.0 (SD 24.8) kPa).

Interpretation—These findings suggest that following immobilization, magnetic resonance elastography has the potential to be used as a clinical tool to guide rehabilitation and as a research tool to study the loss of passive elastic components of muscle.

Keywords

Immobilized Muscle; Magnetic Resonance Elastography; Muscle Stiffness; Muscle Contracture; Muscle Atrophy

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INTRODUCTION

Joint immobilization is commonly used for treatment after bone fracture and joint surgery. However, immobilization of the joint may lead to a clinically important contracture of the joint. At this time, the specific tissue that limits the ultimate range of the joint is not known. This contracture may be attributed to contractures of muscle-tendon units (Witzmann et al., 1982) or capsule and ligaments (Trudel and Uhthoff, 2000). Treating the primary cause of the joint contracture is ideal. However, the isolated evaluation of each tissue to detect the primary cause of the restriction is a challenge in living subjects and the primary cause of contracture is likely to change with increased immobilization time (Trudel and Uhthoff, 2000).

It seems intuitive that a muscle experiencing contracture will be stiffer than normal. Therefore, measuring a muscle's stiffness may provide insight into its contribution to joint contracture. However, muscle's passive stiffness is not a constant value; it increases with increasing strain (Ramsey and Street, 1940). In fact, it is likely that the non-linear stiffness of muscle is the reason for a muscle appearing stiffer as a result of contracture. As a result of immobilization a muscle's slack length is reduced (Williams and Goldspink, 1973), and the muscle experiences higher strain at any given joint angle and therefore will be stiffer. Otherwise, a muscle would likely be less stiff following immobilization as the two major sources of passive stiffness in a muscle, extracellular matrix (ECM) and titin, are weakened in response to disuse (Giannelli et al., 2005, Savolainen et al., 1987, Udaka et al., 2008). This also is an area worthy of additional study as the loss of titin may contribute to abnormal sarcomere organization (Udaka et al., 2008) and the ECM plays an important role in force transmission with the muscle (Purslow and Trotter, 1994) and in mechanotransduction (Maniotis et al., 1997, Purslow, 2002).

Measuring muscle stiffness in vivo is also a challenge. Muscle stiffness has been estimated with an isokinetic dynamometer by measuring passive resistance torque and joint angle (Gajdosik et al., 1999). However, this technique measures the stiffness of all structures crossing the joint. Magnetic Resonance Elastography (MRE) has the potential to be able to assess the contributions of individual muscles. MRE is a novel imaging technique that allows in vivo measurement of tissue shear modulus (Muthupillai et al., 1995). Assuming muscle is incompressible, the shear modulus is one third of the Young's modulus, the normalized stiffness of a material. The basis of the technique is the application of a small amplitude shear wave synchronized with a motion-sensitizing gradient. Phase-contrast MRI allows the shear wave to be imaged as it moves through the tissue. An inversion technique can then be performed to determine shear modulus from the displacement data (Manduca et al., 2001). This technique has been shown to be able to measure changes in modulus as a result of changes in passive tension (Dresner et al., 2001) or joint position and loading (Ringleb et al., 2007).

The purpose of this study was to assess the feasibility of MRE as a clinical tool to assess the contribution of muscles to contracture and guide management following immobilization and its feasibility as a research tool to study the effects of the disuse associated with immobilization on passive muscle structures. This will be investigated using an animal model designed to produce a mild contracture in one limb. It is hypothesized that MRE is capable of 1) detecting a higher shear modulus in the muscle on the experimental side compared to the contralateral side when measured at similar muscle lengths, primarily as a result of muscle contracture (reduced muscle slack length), and 2) detecting a lower shear modulus in the muscle on the experimental side compared to the contralateral side when measured at similar muscle strains, primarily as a result of a change in material properties.

METHODS

Animal Model

After approval by our own Institutional Animal Care and Use Committee (IACUC), eight adult mongrel dogs with mass of 20–25kg were used for this study. One forelimb was randomly selected for surgery in each dog. The selected forelimb was shaved, scrubbed with povidone-iodine, and sterilely draped. An elastic bandage was used to exsanguinate the forelimb and to act as a tourniquet for the procedure. A high radial neurectomy was performed through a lateral humeral incision in order to denervate the triceps brachii (elbow extensor muscle). Combination of this denervation and a custom vest-type sling to tightly hold the arm underneath the chest in maximally flexed position (mean 158° (SD 2°)) was utilized to produce joint contracture. These dogs were sacrificed after 42 days immobilization. To produce a relatively modest contracture, passive exercise for the immobilized forelimb was performed twice a day during the immobilization.

Preparation of Specimens

Both forelimbs of the dog were harvested immediately after sacrifice producing 8 matched pairs. The forelimbs were disarticulated from thorax, preserving as much skin as possible. After the disarticulation, the specimens were kept in a freezer at −20°C. Thawing of the specimens at room temperature began 18 hours prior to the experiment. Thawing was confirmed through preconditioned movement of the elbow and shoulder joint. The specimens were set on an experimental acrylic jig, capable of adjusting the elbow joint and immobilizing the shoulder joint, with plastic screws inserted into holes at the humeral head and epicondyles. The scapula was secured medial side up and in the position where the spine of the scapula was located perpendicular to the longitudinal axis of the humerus (Figure 1). Then, the maximum elbow extension was measured. Maximum elbow extension was defined for each limb, as the position where the joint provided a passive resistance of 0.6 Nm. This value corresponded to the end of the toe region in a torque-angle curve obtained in a preliminary trial.

MRE Measurement Procedure

The MRE measurements were conducted in 1.5T scanner (Signa, General Electric, Milwaukee, WI, USA). The specimen with the jig was placed in a birdcage style; transmit/receive head coil (1.5T Head Coil, General Electric, Milwaukee, WI, USA). An electromechanical driver was placed on the medial and most distal part of the muscle belly of the biceps (Figure 1). A gradient echo sequence was used to record a series of axial scout images of the limb. From these images a sagittal plane was selected that passed through the center of the biceps and MRE scans were performed in this plane. Data was collected with a flip angle of 45°, a 16 cm × 16 cm field of view, and a 256 × 256 resolution. The repetition time was 110 ms and the echo time corresponded to the minimum echo time allowing for motion encoding. Vibration with the electromechanical driver was applied to the biceps latero-medially at 175 Hz (Figure 1) and 8 phase offsets were obtained. In the experimental limb scans were performed in the extended elbow position with a torque of 0.6 Nm. In the contralateral limb scans were conducted at two different elbow positions. First, the position matching the extended elbow position in the experimental limb was selected to attempt to match muscle lengths. Second, a position that matched torque was selected to attempt to match muscle strains.

Before data processing, the data is phase unwrapped and band-passed directionally filtered along the direction of the muscle with filter cutoffs for wavelength of 0.008 and 0.08 m. A phase gradient algorithm was then used to calculate shear modulus (Ringleb et al., 2007). The phase gradient algorithm is a four step process. First, at each voxel the two parameters

that characterize harmonic oscillation (amplitude and phase) are calculated using a time-domain, discrete Fourier analysis. Second, around each pixel a 5 by 5 pixel window is drawn and the gradient of the phase is measured. Third, the phase gradient is then converted into spatial frequency, the inverse of the wavelength. Finally, the shear modulus at each voxel can be calculated by dividing the square of the vibration frequency by the square of the spatial frequency, assuming a density of 1.

A region of interest was selected to be representative of the muscle and the mean modulus for this region was determined. This region was selected to minimize the effects of noise near the vibration source and yet maintain high signal strength (Domire et al., 2008). The region of interest was centered in the biceps muscle in the antero-posterior direction. Two opposing criteria were considered when selecting the proximal - distal location of this region. First, it is advantageous to place the region in an area as near the tapper as possible, as the signal diminishes as the wave propagates. However, in the region near the tapper shear waves can be obscured by larger longitudinal motion. To balance these two criteria, a phase difference signal to noise ratio was calculated, by using measurements from the background to estimate noise. Therefore, the region was placed as far from the tapper as possible while remaining in an area where the signal to noise ratio was above ten (Domire et al., 2009). Previous studies demonstrated that standard deviations of MRE measurements on skeletal muscles ranged from 1.7 to 5.2 kPa and suggested that these are as repeatable as EMG data (Ringleb et al., 2006, Uffmann et al., 2004).

Geometry Measurement

Immediately following MRE scans, the biceps was exposed in order to measure its muscle belly length. A transverse marking line was made with oil-based ink at the proximal and distal border of muscle belly macroscopically defined. The lengths between these two lines at each elbow position where an MRE scan was performed were measured with digital calipers, with a resolution of 0.01mm. Strains in the biceps muscle were calculated as the change in muscle length divided by slack length. To calculate muscle belly slack length the biceps muscle was removed from the forelimb and placed into a material testing device. The muscle belly slack length was defined as the length to which the muscle belly of the biceps was stretched with a force of 1N. After the length measurement, volume of the biceps muscle was measured with a graduated cylinder (tolerance of 2.6mL). In order to measure the muscle belly volume the proximal and distal biceps tendons were cut along the marking line. Cross sectional area (CSA) of the biceps muscle was calculated by dividing muscle volume by slack length. Additionally, wet weight of the biceps muscle belly was measured using a digital scale (resolution of 1 gram).

Data Analysis

A paired t-test was used in order to determine the difference of all parameters between experimental and contralateral limbs. The alpha level was set at 0.05 for comparing geometry and ROM. For the shear modulus and muscle belly length, alpha was adjusted to 0.025 because of multiple comparisons, including experimental and contralateral limbs at matching joint positions and the experimental and contralateral limb at matching joint torque.

RESULTS

Geometry of the muscle and ROM of elbow joint along with strain are shown in Table 1. The muscles on the experimental side showed significant reduction of the wet weight ($P < 0.001$) and CSA ($P = 0.001$). Also, slack length of the muscles on the experimental side was significantly shortened ($P = 0.026$). The range of elbow extension at 0.6Nm in the muscles

on the experimental side was significantly restricted compared to that in the contralateral muscles ($P < 0.001$). The maximum elbow extension in both experimental and contralateral limbs was significantly increased by removing the biceps muscle ($P < 0.001$), but still, the maximum elbow extension in the experimental limb was significantly smaller than that of contralateral limb ($P < 0.001$). The shear modulus of the muscles from the experimental limb (79.1 (SD 12.0) kPa) was significantly higher than the contralateral muscles (31.9 (SD 24.4) kPa) when scanned at the matching elbow position ($P = 0.002$)(Table 1)(Figure 2). In this position, the muscle belly lengths were similar, whereas the muscle strain in the experimental limb was significantly higher than that of contralateral muscles ($P < 0.001$). When comparing the shear modulus in the elbow position matched joint torque, the moduli of the contralateral muscles (113.0 (SD 24.8) kPa) was significantly higher than the experimental muscles ($P = 0.017$). In this position, the muscle belly length in the experimental limb was significantly shorter than the contralateral muscles ($P = 0.002$). However, the strains were similar (2.7% and 3.3%, respectively).

DISCUSSION

The animal model used in this study simulated muscle atrophy and modest contracture successfully. The wet weight and CSA of the biceps muscle were reduced by approximately 24% and 19%, respectively. These findings were similar to previous studies reporting reductions of 24% to 54% for the muscle mass (Booth, 1977, Nordstrom et al., 1995) and 21% for muscle CSA (Veldhuizen et al., 1993). The animal model used here also showed a significant reduction of 2.6% in muscle slack length, much less than that reported in other studies of immobilized muscle (Spector et al., 1982, Witzmann et al., 1982), and is likely a result of the passive exercise during the immobilization. The model in the current study also produced less elbow extension restriction compared to studies using a complete immobilization model (Trudel and Uhthoff, 2000). However, the percentage of the contracture that can be attributed to atrogenic and myogenic contracture is similar to that reported for similar immobilization times (Trudel and Uhthoff, 2000).

As hypothesized, when MRE scans were conducted at the same position, the muscle on the experimental side had higher shear modulus compared to the contralateral muscle, and the muscle belly lengths were similar. However, because the muscle on the experimental side had reduced slack length, the strain experienced by the muscle on the experimental side was larger than the muscle strain in the contralateral limb. It seems likely that this is the cause for the increased modulus. These findings would also suggest that future studies attempting to identify the contribution of muscle to joint contracture should compare limbs by scanning in a matched joint position.

As hypothesized, when MRE scans were conducted at the same torque, the muscle on the experimental side had reduced shear modulus compared to the contralateral muscle, and the muscle strains were similar. It seems likely that this is a result of weakening of the passive elastic structures in muscle. It is known that collagen synthesis will decrease in response to joint immobilization (Savolainen et al., 1987) and that following disuse there is increased enzymatic breakdown of the extracellular matrix (ECM), particularly in the perimysium (Giannelli et al., 2005). It is also known that disuse results in loss of titin (Udaka et al., 2008). These findings would also suggest that future studies attempting to study the loss of passive elastic structures following immobilization or disuse should compare limbs by scanning in a torque matched position.

Several limitations should be taken into consideration in this study. First, it is possible that the control limb may undergo adaptations as a result of increased use during the immobilization period. However, the biceps is not a weight bearing muscle and therefore

additional demands on the muscle should not be extensive. Secondly, the animal model used in this study is rather unusual. The purpose of using this model was to produce a relatively small joint contracture and in fact the contracture produced in this study was smaller than studies using a typical immobilization model. For the purpose of determining the ability of MRE to detect muscle contracture, this was beneficial as MRE was demonstrated to detect modest muscle contractures providing additional confidence in the technique. Third, since this study utilized dog cadaver muscle, there is no active component in the muscle. Thus, only the passive state of the muscle was measured in this study. Finally, human muscle may respond differently to immobilization than dog muscle. Future studies are needed in human subjects to confirm these findings.

CONCLUSIONS

The animal model used in this study was able to replicate a mild joint contracture. Magnetic resonance elastography was shown to be able to detect even modest muscle contracture when comparing to the contralateral limb in a matched joint position. This suggests that MRE has the potential to be used as a clinical tool to identify which structures are responsible for joint contracture, providing better guidance for rehabilitation practices following immobilization. Magnetic resonance elastography was shown to be able to detect reduced muscle stiffness in the experimental limb when comparing to the contralateral limb in a torque matched position. This suggests that MRE has the potential to be used as an in vivo research tool to study the loss of passive elastic components of muscle following immobilization or disuse.

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Figure 1.

Illustration of experimental set up. The scapula and humerus were secured (a), but the forearm was movable. The forearm was held at the distal end with a plastic grip (b). A vibration bar of the electromagnetic driver was located on the medial and distal part of the biceps muscle belly. The arrow indicates the direction of the vibration.

Figure 2.

Typical MR and MRE images of the biceps muscle in the experimental limb and at each position in the contralateral limb. Arrows show the biceps muscle. A) MR image of the biceps muscle in contralateral limb when position matched, B) MRE phase image of the biceps muscle in contralateral limb when position matched, C) Phase gradient inversion image of the biceps muscle in the contralateral limb when position matched, D) MR image of the biceps muscle in the contralateral limb when torque matched, E) MRE phase image of the biceps muscle in the contralateral limb when torque matched, F) Phase gradient inversion image of the biceps muscle in the contralateral limb when torque matched. G) MR image of the biceps muscle in the experimental limb, H) MRE phase image of the biceps muscle in the experimental limb, I) Phase gradient inversion image of the biceps muscle in the experimental limb, λ : Wave length. Increased wavelengths indicate higher stiffness.

Table 1

Variables of biceps muscle size and length, maximum elbow extension and shear modulus. Values are mean and standard deviation of each variable.

	Experimental	Contralateral	
Wet weight (g) ^a	23.2 (SD 3.3)	30.7 (SD 4.3)	
Cross sectional area (mm ²) ^a	175.8 (SD 28.6)	216.9 (SD 33.5)	
Slack length (mm) ^{a, d}	123.0 (SD 3.5)	128.2 (SD 4.1)	
Ext Elbow position (°) ^a	94 (SD 16)	121 (SD 11)	
Ext Elbow position without biceps (°) ^{a, b}	133 (SD 8)	153 (SD 8)	
Scan Position	Extended to 0.6 Nm Torque	Position Matched	Torque Matched
Shear modulus (kPa)	79.1 (SD 12.0)	31.9 (SD 24.4) ^c	113.0(SD 24.8) ^c
Length (mm) ^d	126.3 (SD 4.9)	126.8 (SD 3.5)	132.3(SD 2.7) ^c
Strain ^d	0.027 (SD 0.050)	-0.010 (SD 0.035) ^c	0.033(SD 0.027)

^a Significant difference of each variable between experimental and contralateral muscles.

^b Significant difference of the elbow position with and without the biceps muscle in contralateral and experimental limbs.

^c The variable is significantly different from the experimental limb.

^d Values from seven samples.