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Skeletal muscle stiffness as measured by magnetic resonance elastography after chronic spinal cord injury: a cross-sectional pilot study

Mina P. Ghatas[1], M. Rehan Khan[2], Ashraf S. Gorgey[1, 3, *]

Abstract

Skeletal muscle stiffness is altered after spinal cord injury (SCI). Assessing muscle stiffness is essential for rehabilitation and pharmaceutical interventions design after SCI. The study used magnetic resonance elastography to assess the changes in stiffness after chronic SCI compared to matched able-bodied controls and determine its association with muscle size, spasticity, and peak torque in persons with SCI. Previous studies examined the association between muscle stiffness and spasticity, however, we are unaware of other studies that examined the effects of muscle composition on stiffness after SCI. Ten participants (one female) with chronic SCI and eight (one female) matched able-bodied controls participated in this cross-sectional study. Magnetic resonance elastography was utilized to monitor stiffness derived from shear waves propagation. Modified Ashworth scale was used to evaluate spasticity scores in a blinded fashion. Peak isometric and isokinetic torques were measured using a biodex dynamometer. Stiffness values were non-significantly lower (12.5%; $P = 0.3$) in the SCI group compared to able-bodied controls. Moreover, stiffness was positively related to vastus lateralis whole muscle cross-sectional area (CSA) ($r^2 = 0.64$, $P < 0.005$) and vastus lateralis absolute muscle CSA after accounting for intramuscular fat ($r^2 = 0.78$, $P < 0.0007$). Stiffness was also positively correlated to both isometric ($r^2 = 0.55$–0.57, $P < 0.05$) and isokinetic peak ($r^2 = 0.46$–0.48, $P < 0.05$) torques. Our results suggest that larger clinical trial is warranted to confirm the preliminary findings that muscle stiffness is altered after SCI compared to healthy controls. Stiffness appeared to be influenced by infiltration of intramuscular fat and modestly by the spasticity of the paralyzed muscles. The preliminary data indicated that the relationship between muscle stiffness and peak torque is not altered with changing the frequency of pulses or angular velocities. All study procedures were approved by the Institutional Review Board at the Hunter Holmes McGuire VA Medical Center, USA (IRB #: 02314) on May 3, 2017.

Key Words: chronic spinal cord injury; isometric and isokinetic torques; magnetic resonance elastography; muscle size; rehabilitation; shear modulus; spasticity; stiffness

Introduction

Spinal cord injury (SCI) is a devastating medical condition generally known to affect the neuromuscular system and is characterized by motor, sensory and autonomic sequela (National Spinal Cord Injury Statistical Center). Extreme muscle atrophy is triggered within few weeks post-injury and reaches its nadir within the first year of injury (Castro et al., 1999; Gorgey and Dudley, 2007). Muscle atrophy is accompanied with changes in muscle composition as characterized by a remarkable increase in intramuscular fat (IMF) infiltration; a depot of ectopic adipose tissue with negative metabolic consequences (Elder et al., 2004). Density of fat mass (0.94 kg/m^3) is lower than that of lean muscle mass (1.1 kg/m^3) (Modlesky et al., 2004; Gibbs et al., 2015; Lester et al., 2019); moreover, IMF percentage is almost four folds greater in persons with SCI compared to able-bodied (AB) controls (Gorgey and Dudley, 2007). Those changes in muscle composition may affect tissue mechanical properties (such as density and tensile properties) of the paralyzed muscles after SCI and may be linked to neuromuscular disorders such as spasticity (Adams and Hicks, 2005).

Spasticity is a highly prevalent disorder which commonly impacts 70% of persons with SCI (Skold et al., 1999). Several reports have clearly indicated that spastic persons with SCI may experience dramatic changes in their quality of life as manifested by disruptive involuntary spasms, pressure injuries, and reduced social activities (Skold et al., 1999; Adams and Hicks, 2005). Moreover, spasticity negatively impacts soft tissue structures, disrupts the myofibril integrity, leads to contractures and limited range of motion (Adams and Hicks, 2005). However, spasticity may maintain muscle mass, decrease accumulation of IMF, improve circulation and

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help to maintain lean mass in persons with SCI (Gorgey and Dudley, 2008; Gorgey et al., 2010). Previous work showed that increased muscle spasticity is associated with higher stiffness (Cho and Nam, 2015). Stiffness is a neuromuscular property defined as the resistance of muscles to lengthen when a force is applied to produce required movements (Cho and Nam, 2015); which may be due to transformation in muscle fibers types after SCI (Cho and Nam, 2015). However, previous measurements of muscle stiffness did not take into consideration the changes in muscle tissue composition after SCI (Gorgey and Dudley, 2007; Bilston and Tan, 2015), Therefore, our current understanding of muscle stiffness after SCI is still incomplete and fragmented. Providing a clear understanding of muscle stiffness may further impact the efficacy of pharmaceutical and/or rehabilitation interventions to manage spasticity (Jiang et al., 2017).

Low muscle density, a marker of IMF accumulation, may reduce the muscle force that is applied on the bone and may further result in bone loss and deterioration (Rutherford and Jones, 1992; Goodpaster et al., 2001; Gorgey et al., 2013). It is well established that osteoporosis is commonly impacted distal femur and proximal tibia in persons with SCI (Bauman and Cardozo, 2015). However, mechanistic understanding of muscle-bone interplay in the process of osteoporosis is not fully understood after SCI (Qin et al., 2013). It is likely to assume that impaired muscle stiffness will result in diminished the force-generating capacity and according to Wolff's law may incur bone loss in person with SCI (Rutherford and Jones, 1992). However, the relationship between torque and muscle stiffness has not been established after SCI. This relationship may provide credence to our recent findings that indicated that 16 weeks of surface neuromuscular electrical stimulation in combination with testosterone replacement therapy resulted in increased both peak isometric and isokinetic torques compared to testosterone replacement therapy only in men with SCI (Holman and Gorgey, 2019b). We can indirectly hypothesize that rehabilitation interventions targeted towards increasing force-generating capacity may enhance muscle stiffness and indirectly prevents bone loss in persons with SCI.

Skeletal muscle stiffness has been previously measured via different techniques including "*in vitro*" tensile testing, isokinetic dynamometers or manual muscle palpation (Diong et al., 2012; Cvjetkovic et al., 2015; Kang et al., 2018). These techniques may require extensive training, cannot provide accurate measurements of specific muscle groups, subjected to human errors and do not account for the changes in muscle tissue composition after SCI. Imaging elastography is a non-invasive technique that can be used to measure changes in mechanical properties of human tissues. Different modalities are available for imaging elastography including ultrasound and magnetic resonance imaging (MRI) which rely on three main consecutive steps: a) mechanical excitation of the target tissue, b) imaging the wave propagation through the tissue, and c) post processing of acquired images and creating an elastogram (i.e. stiffness map) (Basford et al., 2002; Drakonaki et al., 2012; Chakouch et al., 2014). Magnetic resonance elastography (MRE) is a noninvasive MRI based technology that measures tissues stiffness. MRE has been primarily used for liver to detect hepatic fibrosis by measuring liver tissue stiffness (Venkatesh et al., 2013, 2018). MRE has also been used to assess skeletal muscle stiffness (Dresner et al., 2001; Bensamoun et al., 2006; Chakouch et al., 2015) after applying mechanical shear waves to the body surface and calculating the speed of wave propagation inside the tissue, where shear waves propagate faster in stiffer tissues (Dao et al., 2014).

The current work had a major objective to compare stiffness of the paralyzed muscles in individuals with SCI to matched able-bodied (AB) controls. Moreover, we were interested to investigate the impact of spasticity on muscle stiffness in persons with SCI, and the relationships between stiffness and both anatomical and functional muscle properties such as muscle composition, cross-sectional area (CSA) and peak torque (PT) during isometric and isokinetic actions. The primary hypothesis was that muscle stiffness would be altered in persons with SCI compared to healthy AB controls.

Participants and Methods

Participants
In this cross-sectional study, participants with chronic SCI were included from both genders aged 18–65 years, with > 1-year post SCI, body mass index ≤ 30 kg/m^2 with traumatic motor complete or incomplete C5-L2 level of injury, American Spinal Injury Association (ASIA) impairment scale (AIS) A, B, or C. Participants with any pre-existing medical conditions such as: cardiovascular disease; uncontrolled type 2 diabetes mellitus; uncontrolled hypertension; insulin dependence; pressures sores stage 3 or greater; hematocrit > 50%; symptomatic urinary tract infection; or participants with neck of femur or total body osteoporosis (T-score ≤ –2.5) were excluded. Period of data collection: November 2017 to June 2019. All participants provided written informed consent before enrollment (**Additional file 1**). All study procedures were approved by the Institutional Review Board at the Hunter Holmes McGuire VA Medical Center, USA (IRB #: 02314, approval date: May 3, 2017) (**Additional file 2**) and conducted in accordance with the *Declaration of Helsinki*. For the SCI group, twelve participants were recruited from a convenient pool as a part of a parent clinical trial registered at ClinicalTrials.gov (NCT01652040). This study was reported in accordance with the STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) statement (**Additional file 3**).

The study was originally approved for twenty participants. Two SCI participants withdrew from the study because of failure to commit to the schedule of the proposed trial. We have intentionally recruited additional two SCI participants to account for those who dropped off because the primary goal was to study muscle stiffness after SCI. The remaining ten SCI participants completed all aspects of the study. This limited recruitment to only eight AB controls who were matched to the SCI participants based on age, gender and body mass index. The AB controls, without any prior history of neuromuscular disorders, were recruited from the general population of the McGuire VA Medical Center such as visitors, caregivers, and employees who were interested to participate in the study.

System description
An external source of mechanical vibrations (drive) was used to introduce mechanical shear waves within audible frequency range (10–1000 Hz) to the surface of the skin above the region of interest (ROI). The shear wave's propagation was then imaged by the MRI and measured by tissue displacement. Computer algorithms then calculated the shear modulus based on tissue density and displacement. An elastogram, stiffness map, was then created for the area of interest based on shear wave velocity. Shear waves travel faster through denser (stiffer) tissues which are represented by the red regions in an elastogram (Dao et al., 2014).

Set up and drive placement
Participants were placed in a supine position. Both lower limbs were strapped together using an elastic theraband to prevent movement inside the magnet. An external hard plastic circular (diameter = 18 cm) passive drive (A1806105, Resoundant, Rochester, MN, USA; **Figure 1A**) was placed with

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its center above the belly of the vastus lateralis (VL) muscle and its inferior end was approximately two fingers above the superior border of the patella. The drive was attached to the thigh via elastic straps and oriented in a position which covers the maximum volume of the muscle. The drive was connected to an air-compressor through a tube where shear waves were introduced to the thigh muscle at a frequency of 120 Hz.

MRE acquisition

T1 weighted MR sequence was obtained from a total body General Electric Signa 1.5-T (GE, Waukesha, WI, USA) MRI; (Fast Spin Echo; Repetition Time (TR) 850–1000 ms; Echo time (TE) 6.7 ms, Number of excitation (NEX) 1, TE length 3, flip angle 90°, field of view (FOV) 20 cm; matrix size 256 × 256). Five trans-axial images; slice thickness 8 mm with 16 mm interspace gap, were captured to cover the region of the mid-thigh for the right leg. Scan time was ~5 minutes to complete the entire scan. For reliability purposes, the MRE sequence (5 images) was repeated twice, coefficient of variability was calculated between two repeated sequences (see results). Only one sequence was selected for data analysis. The participants were not removed from the bore of the magnet between measurements due to difficulty in positioning participants with SCI on the MRI scanning table.

Shear waves and stiffness calculations

Stiffness values for the muscle tissues were calculated based on the shear waves modulus derived from the following equation (Dresner et al., 2001; Basford et al., 2002).

$$G = \rho \, (f \cdot \lambda)^2$$

Where G is the shear modulus, representative of tissue stiffness; measured in Pascal (Pa); ρ is the tissue density measured in (kg/m^3); for muscle tissue ~1000 kg/m^3, f is the frequency of the shear waves propagation measured in Hz; which is the frequency of the external drive set at 120 Hz, and λ is the length of the shear wave in the direction of the wave propagation through the tissue; which is the distance between the wave peak and trough and measured in (m).

Figure 1 shows views from the MRE software used in the study; McKesson Radiology Station v 12.2.3 (McKesson Technology Solution, Alpharetta, GA, USA). **Figure 1B** shows the T1-MRI of the right for an AB participant thigh, with a representation of the position of the mechanical drive. **Figure 1C** shows the T1-MRI of the right thigh for an SCI participant, **Figure 1D** shows the shear wave propagations through the muscle tissues with red and blue regions representing the shear waves peaks and troughs, respectively. **Figure 1E** shows the mapped stiffness image after the software algorithm computed the stiffness values based on the shear wavelength measurements and tissue displacement. **Figure 1F** shows the final color map elastogram for the thigh. An elliptical ROI, in proportion to the size of the VL muscle, was drawn based on visual identification of its anatomical borders on the MRI view (**Figure 1B** and **C**) to capture as much as possible from the VL muscle; it was then automatically replicated on the stiffness map (**Figure 1E**). The built-in algorithm then computed the average value of the pixel intensity as a proxy for the average stiffness value of the entire VL ROI. For each participant, only one MRE sequence (five images) was selected for data analysis. The average of the three stiffness readings with the least standard deviation was calculated and this average was then used as a representative of the VL stiffness. This was done to reduce variability and to account for any movement artifact that may have occurred from a non-observed spasm.

Modified Ashworth Scale for the SCI participants

Modified Ashworth scale (MAS) was used to evaluate spasticity of the knee extensor muscle group as previously performed (Gorgey and Dudley, 2008; Gorgey et al., 2010). **Table 1** shows different MAS scores assigned and their definitions. Participants were instructed to lay flat in a supine position for 30 minutes at a room temperature kept between 21°C and 24°C. A specialized physician (blinded to the study design) evaluated muscle spasticity three times before assigning a score. The doses of anti-spastic medications are listed in **Table 2**.

Table 1 | Modified Ashworth scale score definitions

Score	Definition
0	No increase in skeletal muscle tone.
1	Slight increase in skeletal muscle tone manifested by catch and release at end of range of motion.
2	Slight increase in skeletal muscle tone, manifested by catch, followed by minimal resistance throughout range of motion.
3	Considerable increase in skeletal muscle tone.
4	More marked increase in skeletal muscle tone throughout range of motion.
5	Rigid in flexion or extension.

Table 2 | Spasticity scores and corresponding anti-spastic dose and medications for participants with chronic spinal cord injury

Subject ID	Modified Ashworth scale score	Anti-spastic dose and medication
1	3	20 mg baclofen
2	4	4 mg diazepam
3	1	20 mg baclofen
4	1	Intrathecal baclofen
5	0	20 mg baclofen
6	4	None reported
7	2	None reported
8	2	5 mg diazepam
9	2	10 mg diazepam
10	3	None reported

MRI segmentation for SCI participants

Thigh muscle CSA for the SCI group was performed as previously discussed (Gorgey and Dudley, 2007; Holman and Gorgey, 2019b). Manual tracing segmentation was conducted using commercially available software Win Vessel (Version 2.011; written by Ronald Meyer at Michigan State University, Lansing, MI, USA). Vastus lateralis (VL) muscle CSA was quantified by manually tracing along anatomical borders. After establishing a bimodal histogram, IMF CSA was quantified using the midpoint between the two peaks of muscle and fat voxels' signal intensity. Both whole and absolute muscle CSAs were used for data analysis. Whole muscle CSA refers to the CSA of the VL including IMF and absolute muscle refers to the VL CSA after excluding IMF. The sum of voxels within a bounded region was multiplied by the voxel area to calculate the CSA (cm^2).

PT measurements for the SCI group

To measure peak isometric and isokinetic torque of the knee extensors muscle group, participants were transferred to isokinetic dynamometer chair (Biodex Medical Systems Inc., Shirley, NY, USA) using a ceiling lift (Holman and Gorgey, 2019b). Participants were securely strapped to the dynamometer chair with two-crossover shoulder harnesses and a belt across the hip joint. The dynamometer's rotation axis was aligned to the anatomical axis of the knee. The lever arm was placed ~2 inches (1 inch = 2.54 cm) above the lateral malleolus. Participants were seated with the hip and knee flexion angles of 90° (0 is full extension).

Surface neuromuscular electrical stimulation was applied through two surface electrodes that were placed on the skin ~3 cm above the superior aspect of the patella over the vastus medialis muscle and 30 cm above the patella over the vastus lateralis muscle. A biphasic wave form (100 mA, 450-µs pulse width; Theratouch 4.7; Richmar, Inola, OK, USA) was applied to induce isometric and isokinetic PTs. For isometric torques, isometric actions were maintained for 2–3 seconds and the current was introduced at varying frequencies (10, 20, 30, 40, 50, 60, 80, and 100 Hz) to establish a force-frequency curve. The frequency order was randomly assigned to all participants. For the isokinetic torque, two complete concentric actions were obtained at three different angular velocities of 60° (PT_{60}), 90° (PT_{90}), and 180° (PT_{180}) per second (Holman and Gorgey, 2019b).

Toque signal was sampled at a frequency of 1000 Hz, the isometric PT (PT_0) was calculated as the average of a 500 ms window during the plateau after the rise of the torque, the rise and cessation of the signal were visually identified, signal processing was performed by a custom code in Matlab (MathWorks, Inc., Natick, MA, USA). For the purpose of the current work, we chose to only analyze torques at 30 and 100 Hz. The two frequencies were selected because they represent the rise (30 Hz) and the plateau (100 Hz) sections of the force-frequency curve as has been previously executed. Moreover, PTs at 30 Hz were adjusted to account for the effects of potentiation in participants who had the frequency of 30 Hz randomly applied at the beginning prior to the 100 Hz. Isokinetic PT was calculated as the absolute maximum torque, then averaged over two consecutive actions. Isokinetic torque data were analyzed using LabChart (version 7.3.8; AD Instruments, Colorado Springs, CO, USA) (Holman and Gorgey, 2019b).

Statistical analysis
As this is a pilot study,no sample size calculations were performed prior to the study. All data points were tested for normality using the Shapiro-Wilk tests and if necessary ($P <$ 0.05), non-parametric Mann-Whitney U tests were conducted. Outliers were detected using normal Q-Q plots. Independent-samples t-tests were conducted to determine the difference in MRE stiffness between SCI and AB control group. Paired samples t-test was used to test for differences in isometric PT values at 30 and 100 Hz. Repeated measure analysis of variance (ANOVA) was used to test for differences in PT for the three angular velocities (PT_{60}, PT_{90}, and PT_{180}). A follow-up Bonferroni's pairwise adjustment test was conducted after repeated measure ANOVA. Simple linear regression analyses were conducted to determine the relationships between stiffness and PT values. Because of the ordinal nature of MAS, spearman correlations were used to test for the relationships between MRE-stiffness and MAS spasticity scores. Statistical analyses were performed using IBM-SPSS version 26.0 (IBM, Armonk, NY, USA) and all values are presented as mean ± SD. $P < 0.05$ was considered statistically significant.

Results
The physical characteristics of the subjects are listed in **Table 3**. The physical characteristics, persons with SCI ($n = 10$) were not statistically different from the matched AB-control ($n = 8$) groups (**Table 3**).

Stiffness values
Stiffness values were checked for normality, all values were normally distributed in both SCI and AB groups and no outliers were detected. Inter-rater reliability between the two repeated MRE sequences of the same thigh region (5 MRI slices per sequence); linear regression suggested that measurement of stiffness was highly reliable ($n = 18$; $r^2 = 0.97$,

$P < 0.05$). Stiffness values were non-significantly 12.5% lower ($P = 0.3$) in the SCI group compared to AB controls (SCI: 2747 ± 624.1 vs. AB: 3085.52 ± 818.74 Pa). **Table 4** shows the stiffness values and the CSA for the elliptical ROI for each participant in both SCI and AB groups.

Linear regression between stiffness and muscle CSA
For the SCI participants ($n = 10$); stiffness values were positively related to the VL muscle CSA. **Figure 2** shows linear relationship between the average stiffness values and a) VL-whole muscle CSA ($r^2 = 0.64$, $P = 0.0057$) and b) VL-absolute muscle CSA ($r^2 = 0.78$, $P = 0.0007$).

Spasticity measurements for the SCI group
Table 2 shows the MAS for each participant with SCI and their reported anti-spastic medications. MAS scores for persons with SCI ($n = 10$) were classified into three groups. One participant had no spasticity (MAS score = 0, $n = 1$), second group with intermediate level of spasticity (MAS scores = 1–2, $n = 5$) and the third group with the highest level of spasticity (MAS scores = 3–4, $n = 4$). **Figure 3A** shows a bar plot of the average stiffness (Pa) across different scores of MAS. First participant had an average stiffness of 1773.4 Pa, second group had an average stiffness of 2699.9 ± 431.4 Pa, and the third group had an average stiffness of 3049.2 ± 640.4 Pa. There was no statistical difference between the second and the third groups and the person with the lowest stiffness score was not identified as an outlier. Furthermore, Spearman rho analysis did not reveal significant relationship between MAS and stiffness ($n = 10$; $r_s = 0.30$, $P = 0.43$).

Figure 3B shows a bar plot of the average VL-whole muscle CSA (cm^2) for the three groups with different spasticity scores (0, 1–2, and 3–4). The first group (MAS score 0) had an average CSA of 4.6 cm^2, the second group (MAS score 1–2) had an average CSA of 13.2 ± 5.2 cm^2, and the third group (MAS score 3–4) had an average CSA of 16 ± 1.3 cm^2. There was no statistical difference between the second and the third groups. **Figure 3C** shows a bar plot for the average VL-absolute muscle CSA (cm^2) for the three groups with different spasticity scores. The first group (MAS score 0) had an average absolute CSA of 3.9 cm^2, the second group (MAS score 1–2) had an average CSA of 9.7 ± 1.7 cm^2, and the third group (MAS score 3–4) had an average CSA of 13.9 ± 2.6 cm^2; with no statistical difference between the second and the third groups.

Linear regression between stiffness and peak torque
For the SCI group ($n = 10$), outlier analysis was performed as described in the methods section. One participant was identified as an outlier and the data was omitted from both the isometric and isokinetic torque analyses. For the remaining nine participants, paired t-test showed significant difference between the isometric PTs at 30 and 100 Hz ($P < 0.05$). Repeated measure ANOVA showed significant differences among the isokinetic torques at PT_{60}, PT_{90}, and PT_{180} ($P = 0.027$). Pairwise comparisons using Bonferroni's adjustment indicated that there was a trend ($P = 0.053$) of lower isokinetic torque at PT_{180} compared to PT_{60}, without any differences between PT_{60} and PT_{90} ($P = 0.33$) or PT_{90}, and PT_{180} ($P = 0.16$).

Stiffness values were positively related to both isometric ($n = 9$) and isokinetic ($n = 9$) PTs (**Figure 4**). Moreover, the relationships appeared to be almost consistent despite changing frequencies and angular velocities for both isometric and isokinetic torque respectively. **Figure 4** shows linear regression between the average stiffness values of VL muscle ($n = 9$) with a) isokinetic PT_{60} at 60°/s ($r^2 = 0.48$, $P = 0.039$) , isokinetic PT_{90} at 90°/s ($r^2 = 0.46$, $P = 0.044$), isokinetic PT180 at 180°/s ($r^2 = 0.47$, $P = 0.039$), b) isometric PT_0 at 30 Hz ($r^2 = 0.57$, $P = 0.02$) and isometric PT_0 at 100 Hz ($r^2 = 0.55$, $P = 0.05$).
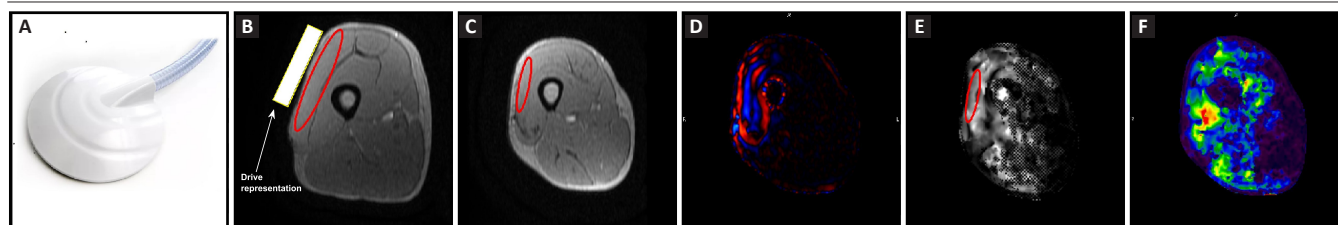
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Figure 1 | Steps to compute magnetic resonance elastography (MRE) stiffness values in SCI and AB groups.
(A) External drive used to send mechanical shear-waves. (B) Anatomical magnetic resonance imaging (MRI) for thigh muscle of an able-bodied (AB) participant, with elliptical region of interest (ROI) to capture vastus lateralis (VL) muscle group and a representation of the drive position. (C) Anatomical MRI for thigh muscle of a SCI participant, with elliptical ROI. (D) Propagation of the mechanical shear waves into the thigh tissue where red and blue represent the waves peaks and troughs. (E) Replicated elliptical ROI on a mapped stiffness view; the average pixel intensity of the ROI is representative of the stiffness value. (F) Final color heat elastogram for the whole thigh region with red spots represent regions with higher stiffness values. Red oval in B, C, and E: elliptical ROI.
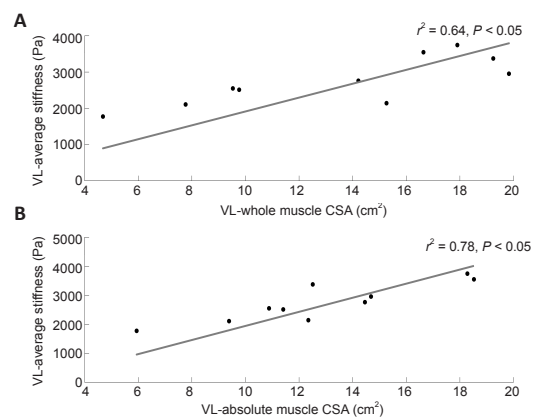
Figure 2 | Linear regression analyses between stiffness values and VL-whole muscle CSA and -absolute muscle CSA.
Linear regression analyses between stiffness values and VL-whole muscle CSA ($r^2 = 0.64$, $P = 0.0057$; A) and VL-absolute muscle CSA ($r^2 = 0.78$, $P = 0.0007$; B) for the spinal cord injury participants ($n = 10$). CSA: Cross-sectional area; VL: vastus lateralis.

Table 3 | Clinical and physical characteristics for both chronic SCI and able-bodied (AB) control groups

Characteristics	SCI ($n = 10$)	AB ($n = 8$)	P-values
Age (yr)	39.5±14	38.25±17	0.8
Weight (kg)	66.7±14.4	78.2±12.3	0.09
Height (cm)	180±4.9	176±10.2	0.6
BMI (kg/m^2)	23±5	25.21±3.0	0.09
Sex (M/F, n)	9/1	7/1	1
Race (AA/W, n)	6/4	3/5	0.6
Neurological level of injury	C5–T8	N/A	
Time since injury (yr)	11.5±11	N/A	
AIS classification	A, B and C	N/A	

Values are presented as the mean ± SD. AA: African American; AIS: American Spinal Injury Association Impairment Scale; BMI: body mass index; f: female; M: male; N/A: not applicable; SCI: spinal cord injury; W: White.

Figure 3 | Spasticity measurements related to stiffness and muscle CSA.
Bar plot of the three different spasticity score levels (MAS = 0, 1–2 and 3–4) for VL stiffness (Pa) and individual data points ($n = 10$; $r = 0.30$, $P = 0.43$; A), VL-whole muscle CSA (cm^2; B), and VL-absolute muscle CSA (cm^2; C), for the chronic spinal cord injury participants. $n = 1$, 5, and 4 for MAS 0, 1–2, and 3–4, respectively. CSA: Cross-sectional area; MAS: modified Ashworth scale; VL: vastus lateralis.

Figure 4 | Linear regression analysis between the average stiffness values of VL muscle with various isokinetic and isometric PT.
Isokinetic PT (PT$_{60}$) at 60°/s ($r^2 = 0.48$, $P = 0.039$), PT90 at 90°/s ($r^2 = 0.46$, $P = 0.044$), PT180 at 180°/s ($r^2 = 0.47$, $P = 0.039$), and B) isometric PT (PT$_0$) at 30 Hz ($r^2 = 0.57$, $P = 0.02$) and 100 Hz ($r^2 = 0.55$, $P = 0.05$). PT: Isokinetic peak; VL: vastus lateralis.

Discussion

The primary findings of the current study suggested that SCI group had non- statistically significant 12.5% lower VL stiffness compared to the matched AB controls. Furthermore, absolute muscle CSA (i.e. after excluding IMF) explained a higher variance in muscle stiffness compared to the whole thigh muscle CSA. This finding may have supported our hypothesis that muscle composition may influence muscle stiffness via altering the density of the paralyzed muscles. A noticeable step increase in stiffness was detected with increasing spasticity scores as measured by MAS; however, there was no significant relationship between stiffness and MAS. Because of the small samp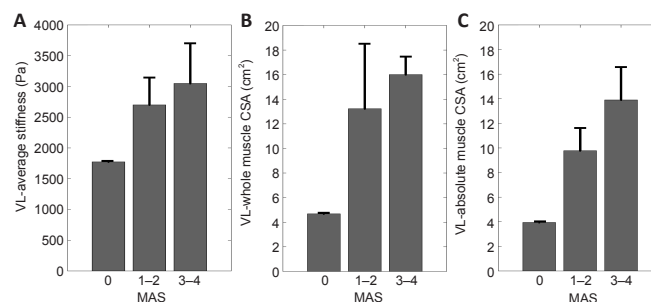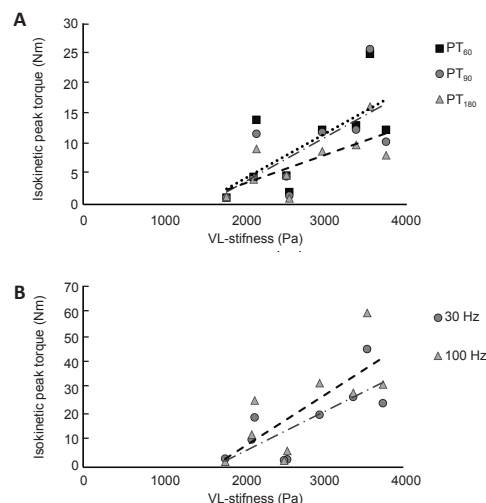le size, statistical difference was not attained in MRE stiffness between intermediate and highly spastic SCI groups. Finally, positive relationships were noted between muscle stiffness and PTs of the knee extensor muscle group both during isometric and isokinetic actions. These relationships did not seem to be altered by changing torque parameters via altering the frequency (30 Hz *vs.* 100 Hz) or examining different angular velocities.

Rationale of the work

For the past two decades, efforts have been aimed at developing non-invasive techniques for the assessment of mechanical properties of human tissues (Basford et al., 2002;

Table 4 | Stiffness values and elliptical region of interest area for both chronic SCI and AB control groups

Group/ID	Stiffness (Pa)	CSA (mm^2)
SCI-001	3548.33±146.3	518.72±50.38
SCI-002	2761.36±152.2	346.19±46.93
SCI-003	2549.82±8.4	413.92±14.77
SCI-004	3375.67±59.1	417.67±37.45
SCI-005	1773.38±37.70	285.47±10.57
SCI-006	2141.58±38.2	540.84±41.22
SCI-007	2107.4±58.8	273.91±13.74
SCI-008	2955.01±32.2	694.18±51.45
SCI-009	2511.94±58.7	334.56±9.29
SCI-010	3745.56±97.0	888.97±65.96
AB-01	3456.91±101.3	912.57±21.19
AB-02	2702.45±16.0	699.29±45.65
AB-03	1901.7±119.0	486.85±21.08
AB-04	2433.5±129.25	448.19±46.64
AB-05	3061.11±5.1	885.62±48.78
AB-06	2851.79±116.1	607.53±50.6
AB-07	4838.15±95.6	648.29±51.51
AB-08	3438.55±51.9	703.13±78.3

Values are presented as the mean ± SD. AB: Able-bodied control; CSA: cross-sectional area; SCI: spinal cord injury.

Litwiller et al., 2012). Imaging elastography is a non-invasive technique that can measure tissue mechanical properties (Litwiller et al., 2012). Studies have used ultrasound elastography to assess skeletal muscle stiffness in different physiological and pathological conditions such as muscle fatigue (Witte et al., 2006; Siracusa et al., 2019), muscle spasticity in multiple sclerosis (Illomei et al., 2017) and the assessment of tendon integrity and muscle quality (Drakonaki et al., 2012; Rosskopf et al., 2016). Moreover, studies have used MRE to assess altered muscle stiffness in different clinical conditions such as aging (Debernard et al., 2011) and other neuromuscular disorders (Basford et al., 2002), however there is limited evidence that examined the direct effects of SCI and the associated factors, such as spasticity (Gorgey and Dudley, 2007) or muscle quality (Holman and Gorgey, 2019), on muscle stiffness as measured by MRE.

Understanding muscle stiffness remains a major research focus after SCI, because of its direct link to musculoskeletal health (Basford et al., 2002; Jiang et al., 2017). This assumption is based on previous work that highlighted the role of decreasing muscle stiffness on altering muscle architecture and decreasing the mechanical force on osteocytes; which may subsequently lead to bone porosity and development of osteopenia and then osteoporosis (Qin et al., 2013; Ferrucci et al., 2014). For example, we have previously shown that persons with SCI have two folds greater yellow bone marrow adiposity associated with cortical thinning compared to healthy AB controls (Gorgey et al., 2013). Furthermore, knee extensor muscle CSA was the only variable that was related to leg bone mineral density as measured by dual energy X-ray absorptiometry and negatively related to bone marrow adiposity (Gorgey et al., 2013). Therefore, it is possible to assume that altered mechanical properties may abruptly influence bone quality after SCI.

Stiffness in SCI *vs.* AB and the role of spasticity
MRE had expanded on the assessment of muscle stiffness to account for tissue density; as shear wave propagation speed increases in denser tissues (Dao et al., 2014; Illomei et al., 2017). This may likely explain the higher stiffness in AB compared to SCI who are characterized with greater muscle atrophy and infiltration of IMF. It is well known that an increase in muscle mass is associated with increased fiber density (Schoenfeld, 2010); in which shear waves propagate faster in denser tissue and resulting in higher stiffness values. Another important aspect is stemmed from the established relationship between spasticity and altered mechanical properties after SCI. Changes in mechanical properties are likely to be influenced by muscle stiffness (Litwiller et al., 2012; Jiang et al., 2017). The current findings clearly suggested that muscle stiffness is also a function of altered muscle density as result of increasing IMF in the paralyzed muscles (Gorgey and Dudley, 2007).

It is still unclear how muscle stiffness may change following neuromuscular disorders, such as spasticity, or may influence neuromuscular rehabilitation in persons with SCI. A previous study showed higher stiffness of the spastic gastrocnemius muscles; which is also negatively correlated with the percentage of type I and II fibers in rat models with SCI (Jiang et al., 2017). Another study indicated that shear waves velocity was statistically higher in spastic muscles compared with non-spastic muscles in persons with acute SCI (Cho and Nam, 2015). Previous studies have used ultrasound to measure stiffness (Rosskopf et al., 2016; Siracusa et al., 2019); which is limited to a smaller field of view for the anatomical regions of interest. Moreover, ultrasound shear waves were manually introduced to the tissue, which may lack consistency as compared to MRE that relies on a constant drive frequency. Our results agreed with previous studies (Cho and Nam, 2015; Jiang et al., 2017), despite the lack of statistical significance findings, there was a clear step rise in the stiffness values with increasing MAS in persons with SCI. It is also interesting to note that the lowest MRE stiffness and MAS score (0) values were observed in an SCI woman with an extensive muscle atrophy and implanted rod as a result of mid-femur fracture prior to participation in the study.

Stiffness and peak torque
There is limited evidence concerning the relationship between muscle stiffness and peak torque in persons with SCI Our results demonstrated relationships between muscle stiffness and both isometric and isokinetic PTs. We were primarily interested in examining the hypothesis that altering PT via either modifying the stimulation parameters or angular velocities will impact muscle stiffness. In cats, soleus muscle stiffness decreased with increasing temperature from 26°C to 38°C without altering PT. The findings suggested that despite the well-established torque-stiffness relationship, muscle stiffness can be dissociated from PT (Bernabei et al., 2020).

It is interesting to note that alerting the stimulation frequency did not impact the relationships between PT and muscle stiffness. This may suggest that muscle stiffness is not a function of the number of attached cross-bridges to the active sites at the level of sarcomere, but rather is a function of muscle density. Frequency of the pulses increases PT via enhancing calcium kinetics and increasing the number of the attached cross-bridges without increasing muscle recruitment. Furthermore, cross-bridges stiffens is related to the number of attached cross-bridges which play important role in the energetics of muscle contraction (Ettema and Huijing, 1994). This finding has important clinical implication that setting the frequency at 30 Hz is equally as effective as 100 Hz during training participants with SCI. Previous work suggested that higher frequency of 40 Hz is recommended over 20 Hz to increase strength, dexterity and endurance in the thenar muscles of persons with chronic hemiplegia (Doucet and Griffin, 2013). We have continuously used 30 Hz to train the paralyzed muscle to evoke muscle hypertrophy and to offset for the onset of muscle fatigue commonly observed with higher frequencies. Another important implication is that the PT-stiffness relationship is not impacted in the paralyzed muscles after SCI (Ettema and Huijing, 1994).

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Furthermore, the relationships between muscle stiffness and PT remained unaltered after introducing different angular velocities in persons with SCI. The inverse logarithmic force-velocity relationship is a function of cross-bridges at the level of sarcomere (Ettema and Huijing, 1994). The concentric isokinetic actions during different angular velocities mimic the training program commonly apply to evoke muscle hypertrophy in persons with SCI. We have recently shown that 16 weeks of electrically evoked resistance training in conjunction with androgen replacement therapy enhanced isokinetic torques in similar fashion at angular velocities of 60, 90 and 180°/s (Holman and Gorgey, 2019). Contraction velocity is an important determinant of the outcomes of any resistance training program. The preliminary findings did not support the hypothesis that altering angular velocities from slow to fast may influence the outcomes of knee extensor concentric actions as demonstrated by similar relationships between peak isokinetic torque and stiffness. Further research is warranted to examine the same relationship during slow *versus* fast eccentric actions.

Limitations and future considerations

Several limitations are highlighted in the current study; this is a pilot study that was limited to 18 participants due to budget constraints. The current preliminary data can be used to adequately power future studies with large sample size in this population. Every effort was made to match SCI and AB-controls based on age, gender and body mass index; however; it is worth noting that failure to match based on race may have influenced fat distribution between both groups and contributed to the non-statistical findings in the current study. MRI analysis to measure muscle composition and PTs were only limited to persons with SCI and not to AB controls. The analyses were part of a parent study and limited resources precluded the expansion of such methodological approaches to the enrolled AB controls.

Additionally, the current work successfully used MRE to non-invasively measure muscle stiffness in persons with SCI. Future work may also evolve on study design and incorporate a randomized clinical trial, as that may improve the level of evidence compared to a case control design. One physician was assigned to perform the spasticity MAS tests, future studies should involve multiple physicians to perform the test and perhaps calculate coefficient of variation to avoid any bias.

Seven participants with SCI were on anti-spastic medications for over a 1-year period before enrollment in the current study. It is possible that different anti-spastic medications may have influenced muscle stiffness. However, we believe that the current cross-sectional design is unlikely to address such concern. A future longitudinal design is highly warranted to determine how anti-spastic medications are likely to influence muscle stiffness in persons with SCI.

The passive drive is designed of a plastic circular flat material to assess liver stiffness. We believe a more non-flat custom designed drive would have accounted for the anatomical conical shape of the mid-thigh region in both groups. This would have led to a better delivery of the shear waves to the ROI and expand the shear wave propagation to other muscle groups of the knee extensors. Despite every effort to maintain a consistent placement of the drive on the belly of the VL muscle, it was challenging because of the significant muscle atrophy in the SCI population. This may explain the high variability in the stiffness values in this group. Shear wave frequency was set at 120 Hz compared to other studies that used multiple frequencies (Kennedy et al., 2017). Based on a preliminary work in a healthy AB control, we have determined that 120 Hz elicited the highest wave penetration as compared

to 60 and 90 Hz. Temperature is suggested to influence stiffness (Kennedy et al., 2017). Previous study showed that muscle temperature in persons with SCI is lower compared to AB-controls (Song et al., 2015), this variable may need to be considered in future studies to ensure that would not influence the stiffness measurements between both groups.

The primary available ROI geometry for measuring stiffness is an ellipse. Future software versions should consider more flexible ROI tools to account for different muscle groups as well as irregular anatomical boundaries. Other thigh MRI regions were segmented, and the whole muscle, absolute muscle and IMF CSAs were measured for participants with SCI but not for AB control group. The current study focused primarily on measuring passive stiffness of VL muscles in a supine lying position. Future studies should consider measuring muscle stiffness during different knee joint to alter the elasticity of muscle-tendon complex or during isometric or dynamic actions.

Conclusions

MRE is a non-invasive technique capable of measuring muscle stiffness after SCI. The current work expanded our understanding of the effects of SCI on stiffness and its association with spasticity and peak isometric and isokinetic torques. Stiffness is likely to be influenced by IMF infiltration and altered muscle density of the paralyzed muscles. The current preliminary data indicated the relationship between muscle stiffness and PT is not altered with modifying the frequency of pulses or angular velocities. The findings could be of clinical implications on selection of rehabilitation protocols that are likely to enhance muscle mechanical properties after SCI. Finally, despite absence of significant relationship between muscle stiffness and spasticity, the current findings may be of clinical relevance to the SCI population considering the effect of spasticity on quality of life in persons with SCI.

Additional files:
Additional file 1: *Informed consent form.*
Additional file 2: *IRB Approval.*
Additional file 3: *STROBE checklist.*

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STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract **Reply: Page :1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found **Reply: Page : 1-2**
Introduction		
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported **Page : 2 Lines: 43-104**
Objectives	3	State specific objectives, including any prespecified hypotheses **Page :4 Lines: 105-111.**
Methods		
Study design	4	Present key elements of study design early in the paper **Page :5 Lines:114**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection **Page : 5 Lines:115-137**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants **Page : 5 Lines:115-137**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable **Page : 7 Lines:148-244**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group **Page : 7 Lines:148-244**
Bias	9	Describe any efforts to address potential sources of bias
Study size	10	Explain how the study size was arrived at
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding **Page : 11 Lines:247-259**
		(*b*) Describe any methods used to examine subgroups and interactions
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was

addressed

Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy

(*e*) Describe any sensitivity analyses

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed **Page : 12 Lines:262-264**
		(b) Give reasons for non-participation at each stage
		(c) Consider use of a flow diagram
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders **Page : 12 Lines:262-264**
		(b) Indicate number of participants with missing data for each variable of interest
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included **Page : 12 Lines:262-318**
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses

Discussion

Key results	18	Summarise key results with reference to study objectives **Page : 13 Lines:320-331**
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias **Page : 19 Lines:425-467**
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence **Page : 15 Lines:333-423**
Generalisability	21	Discuss the generalisability (external validity) of the study results **Page : 21 Lines:470-480**

Other information

Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.