EVALUATION OF MUSCLE FUNCTION AND PATHOLOGIES USING ULTRASOUND SHEAR WAVE ELASTOGRAPHY

A Dissertation in
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by

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Abstract

Several pathologies have been found to change muscle force production in the musculoskeletal system. Changes in shear modulus are linearly proportional to the force produced by the muscle. Therefore, accurate assessment of shear modulus may help early detection, diagnosis, and prognosis of several pathologies. Ultrasound shear wave elastography (SWE) is a promising technique for measurement of shear modulus in soft tissues. The overall goal of this thesis is to develop the ultrasound SWE-based protocol for evaluation of muscle function (force production) and pathologies. Chapter 1 is a general overview of the background information about the muscle function evaluation and limitations of current techniques. It will then discuss the application of ultrasound shear wave (SWE) elastography as an emerging technique for measuring muscle function. In this thesis, a particular focus will be given to muscle function evaluation using ultrasound shear wave elastography for two essential diseases: compartment syndrome (CS) and low-back pain. Chapter 1 will conclude by specifying the general aims of the thesis. Chapter 2 will present the development of a customized of supersonic shear wave imaging code implemented in the Verasonics research scanner. Additionally, the safety measurements conducted to ensure the acoustic output parameters satisfy the FDA regulatory limits and the reliability of the developed code will be also be presented. Chapter 3 will develop and evaluate a novel protocol to quantify muscle force production capability of the multifidi muscles using ultrasound SWE. Chapter 4 will develop and evaluate a novel protocol to quantify dysfunction of the multifidus muscle after radiofrequency neurotomy and posterior lumbar fusion surgery using ultrasound SWE. Chapter 5 will quantify changes in lower-leg stiffness induced by variation of intracompartmental pressure using ultrasound SWE in healthy individuals. Chapter 6 will propose a new SWE-based protocol to quantify changes in lower-leg compartment shear modulus after running in healthy individuals.
and two CS patients for diagnosis of CS. Chapter 7 will introduce a method that combines numerical simulations and shear modulus measurements to evaluate the actual shear modulus of fascia in lower-leg compartments for the prognosis of CS. Chapter 8 will conclude the thesis with a summary of the contributions and explore the directions for future work.
# TABLE OF CONTENTS

**LIST OF FIGURES** ......................................................................................... viii  
**LIST OF TABLES** ....................................................................................... xii  
Acknowledgements ......................................................................................... xiii  

**Chapter 1** Introduction ......................................................................................... 1  
1.1 Muscle function ............................................................................................ 2  
1.2 Multifidus dysfunction after spinal fusion surgery and radiofrequency ablation  4  
1.3 Compartment Syndrome .............................................................................. 5  
1.4 Ultrasound for musculoskeletal tissues ....................................................... 8  
1.5 Ultrasound elastography ............................................................................. 9  
   1.5.1 Ultrasound SWE applications .............................................................. 11  
1.6 Objectives of this Ph.D. dissertation research ........................................... 14

**Chapter 2** Developing SSI sequencing code for Verasonics ultrasound scanner  15  
2.1 Introduction ................................................................................................. 17  
2.2 Verasonics Hardware Architecture ............................................................ 17  
2.3 Verasonics software sequencing and parameter specification ................... 18  
   2.3.1 Angular compounding ........................................................................ 22  
   2.3.2 Axial velocity estimation .................................................................... 23  
   2.3.3 Directional filtering .......................................................................... 23  
   2.3.4 Shear wave speed estimation .............................................................. 24  
2.4 Safety measurements .................................................................................. 24  
   2.4.1 Equipment and Set up ....................................................................... 26  
2.5 Reliability and validity of the developed technique .................................... 28  
2.6 Conclusion .................................................................................................. 30

**Chapter 3** Developing a new protocol for quantifying active contraction of lumbar multifidus muscle .......................................................... 31  
3.1 Introduction .................................................................................................. 32  
3.2 Methods ....................................................................................................... 35  
   3.2.1 Overview ........................................................................................... 35  
   3.2.2 Shear Wave Elastography System ..................................................... 35  
   3.2.3 Proposed Protocol to Evaluate Multifidus Muscle Function .......... 36  
   3.2.4 Quantifying changes in shear modulus in different postures .......... 38  
   3.2.5 Reliability of the Proposed Protocol .................................................. 38  
   3.2.6 Localized Changes of Contraction in the Multifidus Muscle .......... 38  
3.3 Results ......................................................................................................... 40  
   3.3.1 Results of reliability study ................................................................. 40  
   3.3.2 Results of multifidus activation study ................................................. 40  
   3.3.3 Results of Localized Changes of Contraction in the Multifidus Muscle 41
Chapter 4 Quantifying Dysfunction of the Lumbar Multifidus Muscle after Radiofrequency
Neurotomy and Fusion Surgery .................................................. 49

4.1 Introduction .............................................................................. 50
4.2. Methods ................................................................................. 52
   4.2.1 Overview ........................................................................ 52
   4.2.2 Measurement procedure .................................................... 52
   4.2.3 Statistical analysis ............................................................. 54
4.3 Results ...................................................................................... 55
4.4 Discussion ............................................................................... 58
4.5 Conclusion ............................................................................... 61

Chapter 5 Shear modulus of lower-leg muscles and intracompartmental pressure are correlated................................................. 63

5.1 Introduction .............................................................................. 64
5.2 Methods ................................................................................... 66
   5.2.1 Overview ........................................................................ 66
   5.2.2 Shear Wave Elastography System ...................................... 67
   5.2.3 Proposed Protocol to quantify changes in shear modulus at different blood pressure ......................................................... 67
5.3 Results ...................................................................................... 69
5.4 Discussion ............................................................................... 74
5.5 Conclusion ............................................................................... 78

Chapter 6 Feasibility study of Shear Wave Elastography for Compartment Syndrome .. 79

6.1 Change in shear modulus of healthy lower legs after treadmill running .......... 80
6.2 Methods ................................................................................... 83
   6.2.1 Protocol to quantify temporal changes in shear modulus after running ...... 84
   6.2.2 Protocol to quantify changes in shear modulus of all compartments ........ 85
6.3 Results ...................................................................................... 86
6.4 Discussion ............................................................................... 93
6.5 Conclusion ............................................................................... 96

Chapter 7 Measurement of the Shear Modulus in Muscle Fascia of Lower Leg As a Prognostic
Indicator of Compartment Syndrome ..................................................... 97

7.1 Introduction .............................................................................. 98
7.2 Overview .................................................................................. 100
   7.2.1 Numerical Simulation of SWE ........................................... 100
   7.2.2 Simulation of SWE in agarose gels and validation ...................... 103
7.3 In-vivo measurement .................................................................. 103
7.4 Results ...................................................................................... 105
   7.4.1 Simulation and validation results ........................................... 105
   7.4.2 In-vivo results .................................................................. 108
7.5 Discussion ............................................................................... 111
7.6 Conclusion ............................................................................... 114
Chapter 8  Summary and Future Work .................................................................115

Bibliography .......................................................................................................119
Appendix A: SSI sequencing in Verasonics Scanner using L7-4 transducer ..........129
Appendix B: SWE-based Protocol for the lumbar multifidus elastography ..........140
Appendix C: SWE-based Protocol for the running on treadmill study .................145
LIST OF FIGURES

Figure 1-1. a) Multifidus muscle anatomy (b) retraction process after PLF surgery[14] (c) RFN on the superior location of the L3 medial branch nerve at the L4 transverse process [22]...5

Figure 1-2. Lower leg compartment anatomy.................................................................6

Figure 1-3. Needle manometry for diagnosis of CS through measuring ICP. ....................7

Figure 1-4. Schematic push pulse configurations for (a) ARFI shear wave imaging with a single focused ultrasound push (b) SSI method with consecutive ultrasound push pulses at different time points [53]...........................................................................................................10

Figure 1-5. Demonstration of the elastography measurement of the tibialis anterior muscle..10

Figure 1-6. Ultrasound SWE measurement in passive stretched and rested positions of calf muscle [65]..........................................................................................................................................................12

Figure 1-7. Ultrasound SWE Measurement of the liver performed with the Philips system through intercostal access [69]. ...............................................................................................................................................13

Figure 2-1. Schematic view of Verasonics research scanner [71]. ......................................18

Figure 2-2. Shear wave speed estimation procedure [72]................................................22

Figure 2-3. Acoustic output measurement setup..................................................................27

Figure 3-1. Imaging with the transducer located lateral to the spinous processes and angled medially to view the left L4-5 facet joint at (a) prone (b) sitting up (c) sitting up with the right arm lifted in a horizontal position........................................................................................................37

Figure 3-2. Ultrasound B-mode image of the left L4-5 multifidus.....................................37

Figure 3-3. Location of ROI for the elastography measurement of the L4-5 multifidus muscle at the entire multifidus thickness .................................................................39

Figure 3-4. The shear modulus of the L4-5 multifidus (median, interquartile range) in 3 body postures: prone (left side), sitting up (left side), and sitting up with lifted right arm (LA) position for both the left and right sides.................................................................41

Figure 3-5. Comparing the shear modulus (median, interquartile range) of the average of the superficial and deeper layer of the L4-5 multifidus with the shear modulus of the bigger ROI in 3 body postures: prone (left side), sitting up (left side), and sitting up with lifted right arm (LA) position for both the left and right sides.................................................................42
Figure 3-6. Demonstration of the elastography measurement of the L4-5 multifidus muscle. The shear modulus levels of the tissues are represented in the shear modulus map: a superficial and deeper layer of the multifidus (top image), the entire multifidus thickness (bottom image). 43

Figure 4-1. Experimental set up for the shear modulus measurement of multifidus muscle with the transducer located lateral to the spinous processes and angled medially at prone (a), sitting up (b), and sitting up with the arms lifted a horizontal position (c). 54

Figure 4-2. Representative B-mode ultrasound image of the multifidus in the prone position. 54

Figure 4-3. Representative shear modulus maps of the L4-5 multifidus muscle in the sitting up with the lifted arms position: PLF patient (a), RFN patient (b), and age- and gender-matched healthy participants (c) The lower shear modulus in the RFN and PLF patient compared to the healthy individual indicates multifidus dysfunction. 56

Figure 4-4. The shear modulus of the affected multifidus (median, interquartile range) increased from the prone to sitting up, and from sitting up to sitting up with lifted arms position in the RFN group and matched healthy controls. 57

Figure 4-5. The shear modulus of the affected multifidus (median, interquartile range) increased from the prone to sitting up, and from sitting up to sitting up with lifted arms position in the PLF group and matched healthy controls. 57

Figure 4-6. The patient reported outcomes in the PLF and RFN groups: (a) PCS score, (b) VAS score, and (c) ODQ score. 58

Figure 5-1. The experimental setup for elastography while (a) TA muscle is pressurized with the pressure cuff around thigh (b) one leg is elevated (c) ICP measurement inside TA muscle is performed. 68

Figure 5-2. ICP measured in the TA muscle increased as a function of the cuff pressure (p < 0.01) (median, interquartile range). 71

Figure 5-3. Change in shear modulus (kPa) of TA muscle (median, interquartile range) at different levels of blood pressure (values at each pressure level represent changes with respect to shear modulus at zero pressure level). 71

Figure 5-4. Change in shear modulus (kPa) of PL muscle (median, interquartile range) at different levels of blood pressure (values at each pressure level represent changes with respect to shear modulus at zero pressure level). 72

Figure 5-5. The change in shear modulus of TA and PL muscles as a function of cuff pressure (values at each cuff pressure level represent changes with respect to shear modulus at zero cuff pressure) (median, interquartile range). 72

Figure 5-6. The shear modulus maps of the TA muscle (top) and PL muscle (bottom) at each cuff pressure level: (a) 0 (b) 40 mmHg (c) 80 mm Hg and (d) 120 mmHg. 73

Figure 5-7. The shear modulus of TA increased as a function of ICP (median, interquartile range) (p < 0.01). The horizontal bars represent the interquartile range in ICP, and the vertical bars represent the interquartile range of shear modulus. 73
Figure 5-8. The shear modulus of PL increased as a function of ICP (median, interquartile range) (p < 0.01). The horizontal bars represent the interquartile range in ICP, and the vertical bars represent the interquartile range of shear modulus.

Figure 6-1. The experimental setup for elastography of the TA muscle (left) and the PL muscle (right). The ankle is placed in a support block to avoid applying contact pressure to the calf muscles. The transducer was placed at a point 30% of the distance from the head of the fibula to the tip of the lateral malleolus.

Figure 6-2. The shear modulus maps of the (top) TA and (bottom) PL muscles at pre-running (left) immediately (center), and 5 minutes after cessation of exercise (right).

Figure 6-3. The change in shear modulus for the (top) TA and (bottom) PL muscles (mean + standard deviation) over time in healthy individuals shows an initial increase of shear modulus after treadmill running followed by gradual decrease back to pre-exercise values.

Figure 6-4. The curve fitting of the Weibull cumulative distribution function to the average change in shear modulus for the (top) TA and (bottom) PL muscles over time after cessation of treadmill running. The median, calculated from the Weibull parameters, was 3.6 and 5.1 minutes for the TA and PL muscles, representing the time needed for a decrease of 50% of the change in shear modulus after exercise.

Figure 6-5. Results of a Pedowitz-like protocol using the shear modulus (kPa) measured before, immediately and 5 minutes after cessation of exercise show that shear modulus can be used a surrogate measurement of intra-compartment pressure (median and interquartile range).

Figure 6-6. The change in shear modulus for the (top) TA and (bottom) PL muscles in the first CECS patient over time shows an initial increase of shear modulus after treadmill running followed by gradual decrease back to pre-exercise values.

Figure 6-7. The change in shear modulus for the (top) TA and (bottom) PL muscles in the second CECS patient over time shows an initial increase of shear modulus after treadmill running followed by gradual decrease back to pre-exercise values.

Figure 7-1. Schematic image of a thin layer with the thickness H bounded between two substrates used for the simulation of shear wave propagation.

Figure 7-2. The experimental setup for SWE measurement in TA muscle, fat layer, and fascia of the TA. A gel pad was used since the fascia is close to the skin.

Figure 7-3. The Representative shear modulus maps of the substrate and 1 mm layer for one of the agarose constructs measured by SWE.

Figure 7-4. The pressure field of the push pulses at the elevation plane (z = 10 mm) at different focal depths. Five push pulses are successively focused from 10 to 40 mm (Figure 4 a-e). The 1 mm thin layer is located at a depth of 20 mm (marked with two horizontal black lines). The pressure amplitude was normalized by its maximum amplitude.

Figure 7-5. Shear wave propagation at different time points: 8.08 ms (left), 13.53 ms (center), and 19.67 ms (right) in a layered tissue. The 1 mm thin layer is located at a depth of 20 mm (marked with two horizontal black lines). The actual shear modulus of the layer and substrates was 16
kPa and 3.28 kPa, respectively. The displacement amplitude was normalized by its maximum displacement.

Figure 7-6. The relationship between the apparent and actual shear modulus for the 1 mm layered constructs of agarose gels obtained by simulations and SWE experiments. A power fit of the simulation results was performed for better visualization of the numerical data.

Figure 7-7. The shear modulus maps of the fat layer, TA muscle, and fascia of the TA muscle.

Figure 7-8. The simulation results showing the relationship between the apparent and actual shear modulus of the fascia of the TA muscle. A power fit of the simulation results was performed for better visualization of the numerical data. The average and SD values are marked with the solid black lines and the shaded grey area, respectively.
LIST OF TABLES

Table 2-1. Parameters of the imaging methods used in the calculation of the acoustic intensity and MI. 27

Table 2-2. Acoustic outputs for each elastography technique for C5-2 transducer. FDA limits of these parameters are $I_{SPTA,3} \leq 720 \text{ mW/cm}^2$, and $I_{SPPA,3} \leq 190 \text{ W/cm}^2$ or MI $\leq 1.9$. MI $\leq 1.9$ was taken as a limit. 28

Table 2-3. Acoustic outputs for each elastography technique for L7-4 transducer. FDA limits of these parameters are $I_{SPTA,3} \leq 720 \text{ mW/cm}^2$, and $I_{SPPA,3} \leq 190 \text{ W/cm}^2$ or MI $\leq 1.9$. MI $\leq 1.9$ was taken as a limit. 28

Table 2-4. Measured shear wave speed of elasticity phantom for background materials at different depths and inclusion using the L7-4 transducer. 29

Table 2-5. Measured shear wave speed of elasticity phantom for background materials at different depths and inclusion using C5-2 transducer. 29

Table 3-1. Obtained ICCs and 95% CIs for evaluation of superficial and deeper layers of all postures of the L4/5 multifidus muscle. 40

Table 5-1. Post hoc Bonferroni test results for ICP (mmHg) between different pressure cuff conditions. 69

Table 5-2. Post hoc Bonferroni test results for shear modulus (kPa) between different pressure cuff conditions. 70

Table 7-1. Obtained shear modulus in SWE measurements in the fat, TA muscle, and fascia of TA (mean ± SD). 109
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Chapter 1
Introduction
Several pathologies have been found to change the muscle function in the musculoskeletal system. To improve our understanding of muscle pathologies, clinical tools to quantify muscle function are needed. Current techniques for measuring muscle function such as surface electromyography (EMG) and dynamometry cannot evaluate change in muscle force production locally. This limitation hinders the evaluation of pathologies, such as multifidus dysfunction after fusion surgery. Ultrasound elastography is a promising technique that can overcome this limitation by quantifying individual muscle function independent of the neighboring muscles. Shear wave elastography (SWE) is one of the ultrasound elastography techniques that uses shear waves to measure tissue stiffness quantitatively. Ultrasound SWE has shown promising results for various clinical applications. It has been used to directly measure individual muscle stiffness during active and passive muscle contraction. It has been used for identifying several pathologies and musculoskeletal disorders. We aim to develop SWE-based protocols for evaluation of muscle function in compartment syndrome (CS) patients and multifidus function in patients with lower back pain.

1.1 Muscle function

Muscle function refers to changes in muscle force production. A muscular function is an important parameter of rehabilitation programs and a strong predictor of survival (e.g., not encountering heart attack, cancer or death) in middle age [4]. Altered muscle function has been associated with several pathological conditions. For example, compartment syndrome (CS) is an exercise-induced condition, in which high pressure develops in one or more lower-leg compartments, resulting in muscle dysfunction. Spinal fusion surgery causes a localized injury in the multifidus muscle, resulting in atrophy and dysfunction [5]. Dysfunctional muscle tissue often demonstrates abnormal mechanical properties, which highlights the clinical importance of obtaining accurate information about individual muscle mechanical properties for physiological conditions [6, 7].
Nonetheless, muscle mechanical properties such as stiffness have traditionally been evaluated through indirect methods such as palpation and manual muscle testing. While such assessments have provided valuable information and played a fundamental role in the physical examination of patients, their accuracy is limited by the experience of the tester, making these methods subjective. Besides, such assessments are unable to differentiate the mechanical properties of individual muscles from the muscle group in musculoskeletal tissues.

Dynamometers provide accurate assessments of dynamic and also static muscle strengths [4]. However, they suffer from some limitations. The biodex medical system cannot evaluate muscle function locally. It measures the resultant moment produced by the muscle groups rather than individual muscles. This limitation hinders the ability to evaluate local muscle function in low back pain patients. For example, multifidus muscle (a series of muscles attached to the spinal column providing spinal stability) dysfunction is magnified after surgical procedures such as fusion surgery. Assessment of localized multifidi function, independent of the erector spinae and adjacent hip musculature, can help clinicians identify the contribution of that muscle to clinical outcomes. Therefore, there is a need for clinical tools that can evaluate individual muscle function in clinical practice to overcome the limitations of dynamometers.

Surface electromyography (EMG) is also a valuable tool that can provide information on the relative contribution of the superficial muscles during movement through clinical gait analysis [8]. In particular, the assessment of the timing of muscle activation from the surface EMG along with kinematic analysis can help select the appropriate therapeutic or surgical treatments in neurological patients [9]. However, there are several limitations with EMG for muscle function evaluation. First, surface EMG is affected by many methodological factors, such as electrode location, which may change across experimental sessions [10, 11]. Another limitation of EMG is muscle cross talk, which occurs when the EMG signal from one muscle interferes with that of another, limiting the reliability of the signal of the muscle being tested [10]. Surface EMG is only limited to measuring the superficial muscles, and even sometimes, it is even hard to narrow down the signal to a single muscle. On the other hand, deep muscles require intramuscular wires that are
intrusive in order to achieve an EMG signal. Additionally, under extreme fatigue conditions, muscles are unable to produce force, while EMG signals are still produced, meaning that EMG is a poor indicator of force. Therefore, there is a need for clinical methods that can evaluate muscle function in clinical practice to overcome the limitations of current techniques.

1.2 Multifidus dysfunction after spinal fusion surgery and radiofrequency ablation

Multifidi are a series of muscles that attach to the spinal column and span 2 to 4 vertebrae and provide active stability of the spine (Figure 1-1. (a)). These muscles may be divided into two parts: superficial and deeper. Lumbar multifidi are thought to play an essential role in active stabilization and movement of the spine [12, 13]. Due to the complex anatomy of the multifidi, however, understanding the biomechanics of the multifidi is essential in clinical practice. Posterior lumbar fusion (PLF), which is the most common fusion approach for deformity, traumatic instability, and other spinal disorders (Figure 1-1. (b)), injures the paraspinal muscles, including the multifidi muscles [14]. Muscle atrophy and increased (intramuscular fat) IMF have often been reported in the multifidi of patients with low back pain (LBP) and more specifically, after PLF surgery [15-19]. Evaluating multifidus muscle function and injuries is challenging. Thus, there is a need for clinical methods that can quantify the function of individual paraspinal muscles post-operatively. Additionally, pain caused by lumbar facet joints is commonly treated with a treatment procedure, called medial branch radiofrequency neurotomy (RFN). RFN is a non-surgical, minimally-invasive pain treatment, in which an electrical pulse produced by radiofrequency waves directs heat to a precise area of the nerve tissue in the lower back (Figure 1-1. (c)). It has been shown that RFN leads to localized atrophy in multifidus muscle [20]. The multifidus atrophy after RFN may have an impact on multifidus function and segmental stability, accelerating disc degeneration [21]. Evaluating multifidus muscle function and injuries is challenging. Reduced strength and endurance of the paraspinal muscles are globally assessed using isometric or isokinetic tests (i.e., dynamometry), which lack the ability to quantify multifidi contribution independent of the erector spinae (and adjacent hip musculature). Thus, there is a need for
clinical methods that can quantify localized multifidus biomechanics changes occurring due to disease or injury post-operatively. Further details regarding multifidus function evaluation will be provided in Chapter 3 and 4.

Figure 1-1. a) Multifidus muscle anatomy (b) retraction process after PLF surgery[14] (c) RFN on the superior location of the L3 medial branch nerve at the L4 transverse process [22].

1.3 Compartment Syndrome

Compartment Syndrome (CS) occurs when excessive pressure develops inside an tissue enclosure (compartment) in the body, such as lower-leg compartments (Figure 1-2). Chronic exertional compartment syndrome (CECS) is an exercise-induced condition, which occurs in 14-27% of those with chronic anterior lower leg pain [23], with the anterior and lateral compartments being most often affected bilaterally in 95% of cases [24-27]. CECS imposes decreased tissue perfusion and increased intracompartmental pressure (ICP) due to the inadequacy of muscle fascia compliance [28, 29]. Inadequate blood flow to the tissues results in pain, muscle weakness, numbness, and difficulty in moving the foot [23]. The most effective treatment for CECS focuses on reducing the ICP using the fasciotomy technique by cutting the fascia of each affected compartment [27]. Current diagnostic methods of CECS rely on clinical evaluation assisted by ICP measurement through needle manometry [27]. However, they have been associated with high false-positive rates [30], emphasizes the limitations of current diagnostic methods.
Needle manometry is used to assist the diagnosis of CS, but its accuracy has been questioned (Figure 1-3). Needle manometry directly measures ICP by inserting a needle into the affected area and recording the pressure in the tissue (Figure 1-2). Various diagnostic thresholds for ICP have been suggested for the diagnosis of CECS of the lower leg [31]. The modified Pedowitz criteria are the most commonly used method to diagnose CECS in the lower leg; a pre-exercise ICP of 15 mmHg or greater, a 1 min post-exercise ICP of 30 mmHg or higher, and a 5 min post-exercise ICP of 20 mmHg or greater [26, 32]. However, there is still substantial disagreement over which value should be used as a threshold pressure for the diagnosis of CS.

On the other hand, Barrera-Ochoa et al. [33] conducted a retrospective cohort study on patients with suspected forearm CECS, introducing the "recovery time" parameter (the time between maximum ICP and return to resting pressure) as a more accurate technique than direct measurement of ICP. Overall, needle manometry is an invasive and painful method of diagnosis and suffers from significant variability depending on the depth of needle insertion, amount of fluid introduced, and soft tissue occlusion of the needle. A recent study evaluated the error in the technique and measured ICP values between physicians in a level I trauma center [34]. It was found that only 31% of the measurements were correctly performed, and only 60% of the measurements made with the correct technique were accurate. Accuracy dropped to 42%
for measurements taken with small errors in technique and 22% when a catastrophic error was committed. The current criteria have overlapping confidence intervals between healthy participants and patients with CECS [35]. Nearly 30% of patients with CECS fail to meet any of the current diagnostic ICP cutoff criteria [35]. Therefore, there are several weaknesses with the current CS diagnostic method.

![Figure 21-3. Needle manometry for diagnosis of CS through measuring ICP.](image)

Assessment of lower leg CECS has also been studied in both research and clinical practice through other methods such as near-infrared spectroscopy (NIRS) and magnetic resonance imaging (MRI). There are, however, several inherent limitations to these techniques. Although several recent studies have shown that near-infrared spectroscopy (NIRS) is capable of demonstrating a significant inverse correlation between ICP and oxyhemoglobin level, it has trouble measuring oxygen saturation in deep compartments. Additionally, the instrumentation required for NIRS is relatively expensive [36-38]. MRI has also shown increased T2 signal intensity with increased anterior compartment pressure [39-41]. However, it is not widely used due to its cost. Therefore, there is a need for non-invasive and more accurate diagnostic methods for CS. Further details regarding lower-leg compartments force production evaluation will be provided in Chapter 5 and 6.

Altered mechanical properties of layered musculoskeletal tissues, such as fascia are a consequence or the cause of several pathologies. For example, increased stiffness in muscle fascia of the lower leg has been
associated with compartment syndrome [42]. Therefore, measuring the mechanical properties of muscle fascia may help the diagnosis or evaluation of compartment syndrome. Shear wave speed inside the tissue is correlated to the tissue shear modulus. Shear waves are the waves that the oscillations of the particles of the medium is perpendicular to the direction of wave propagation. Shear wave elastography (SWE) is a non-invasive method to quantify the mechanical properties of soft tissues [43-46]. Shear modulus obtained using SWE are based on bulk wave theory assuming that the wavelength of the shear wave is much smaller than the dimension of the medium. However, the thickness of some thin-layered tissues is comparable to or smaller than the wavelength of the shear waves. Therefore, the thickness and the properties of the surrounding tissues have an important influence on the wave speed of layered tissues [47]. In many applications, the energy and motion in the layer are transferred to surrounding tissues or media, which have an important effect on the wave speed. For instance, a recent study reported that the shear wave propagation speed in tendons immersed in a saline bath was 22% lower than that of the tendon tested in the air [48]. Therefore, the presence of adjacent tissues causes the wave propagating in the layer to have an ‘apparent’ wave speed that is different from ‘actual’ wave speed associated with its mechanical properties. Therefore, in order to accurately measure the shear modulus of layered tissues, it is necessary to consider the thickness and the properties of surrounding media or tissues. Chapter 7 introduces a method to evaluate the mechanical properties of the fascia of the lower leg to potentially use it as a prognostic indicator of CS.

1.4 Ultrasound for musculoskeletal tissues.

The application of ultrasound (US) imaging in many medical specialties is now widely regarded as an indispensable tool. US imaging is the subject of intense research activity to provide new information and potential clinical value about tissues. US imaging is a real-time, safe, generally less expensive technique compared to other imaging modalities and capable of imaging moving organs.
1.5 Ultrasound elastography

Although most contemporary US imaging techniques are limited to the anatomy display, tissue motion and the soft tissue elasticity aims at providing information about the mechanical properties of tissues (their stiffness) by using ultrasound elastography. Ultrasound elastography shows promise for direct measurement of the mechanical properties of muscle. In the context of ultrasound elastography measurements, muscle mechanical properties are typically reported as "muscle shear modulus."

Ultrasound elastography relies on determining the mechanical properties of tissue, including measurements of deformation in response to applied stress or force. The stress can be produced by external mechanical compression such as vibration [49, 50]; by internal physiological motions [51] or by a high-intensity long-duration (hundreds of ultrasound cycles) focused ultrasound “push” beam [43, 44, 52]. The tissue deformation detected by pulse-echo ultrasound causes a small time shift in ultrasound echoes, which is utilized to measure the tissue deformation from push pulses.

SWE is one of the ultrasound elastography techniques that uses shear waves induced by push pulses to measure tissue stiffness quantitatively. Various SWE techniques are available which rely on different methods to produced shear waves. Nightingale et al. [53] proposed an acoustic radiation force impulse (ARFI) technique using long and high energy pulses capable of exciting tissue. A single ultrasound transducer first generates a push beam within the tissue to apply stress, while the same transducer measures the tissue displacement along the push beam, and shear wave speed will be calculated. Another method was also proposed by Bercoff et al. [43], called supersonic shear imaging (SSI), in which several successive focused pushes can be generated along a line (Figure 1-4). This pushing sequence is called “supersonic” since the shear wave source (the focal points), moves faster than the shear wave propagation speed, generating a "mach-cone," caused by the constructive interference of the individual ARFI pushes [43]. SSI uses an ultrafast plane-wave imaging technique for shear-wave detection with a high frame rate, allowing the capturing of 2D shear-wave propagation real-time (Figure 1-5). One advantage of SSI over ARFI method is that it can cover a larger area in just one sequence [43]. Therefore, in this thesis, the custom
The implementation of SSI technique has been used. The details regarding the employed SSI technique will be provided in Chapter 2.

Figure 1-4. Schematic push pulse configurations for (a) ARFI shear wave imaging with a single focused ultrasound push (b) SSI method with consecutive ultrasound push pulses at different time points [54].

Figure 1-5. Demonstration of the elastography measurement of the tibialis anterior muscle.
It should also be noted that all SWE techniques generally assume that the underlying tissue is isotropic, elastic, and locally homogenous. However, muscle tissue is highly anisotropic, and the mechanical properties across the fibers differ from those along the muscle fibers. Hence, the transducer must be oriented longitudinally to the muscle fibers so as to achieve accurate and reliable SWE measurements.

1.5.1 Ultrasound SWE applications

Ultrasound SWE has shown promising results in the musculoskeletal field for various clinical applications. It has been used to directly measure individual muscle stiffness during active muscle contraction, showing increasing stiffness with increasing force [53, 55, 56]. Nordez et al. [57] showed that the SWE-derived elastic modulus of the biceps brachii muscle increased linearly with the isometric contraction intensity in the range from 0 to 40% of maximal voluntary contraction (MVC). Ateş et al. [58] recently showed that the shear modulus is linearly related to muscle force over the full range of 0–100% of MVC. Therefore, these results suggest that the shear modulus of individual muscles can be used to estimate the force production of an individual muscle reliably. Furthermore, ultrasound SWE has been used for measuring passive stiffness in various musculoskeletal muscles (Figure 1-6) [55, 59, 60]. The reliability of passive stiffness measurements for SSI appeared to be better than the ARFI method [61]. Muscle measurements with passive stretch showed a pattern of exponentially increasing stiffness using SWE [62, 63]. Ultrasound elastography studies of dynamic muscle stretch (measurements during passive stretching of muscle) have shown that the shear modulus may provide an indirect estimation of passive muscle force [64, 65].
Ultrasound elastography also shows promise for identifying abnormal muscle stiffness in neuromuscular and musculoskeletal disorders [67-69]. SWE can also be used as a potential biomarker for measuring liver fibrosis in a patient with the chronic liver disease since normal fibrosis is found to have different stiffness compared to significant fibrosis [70]. Ultrasound SWE may be used to measure the response to spasticity treatment or to measure specific areas of increased stiffness inside muscle [69]. This information improves our understanding of muscle injuries and provides insight for targeted strengthening to diagnose disease in patients with muscle disorders.
Overall, SWE offers several potential application for practitioners. First, this is a localized method, which is capable of quantifying individual muscle function independent of the neighboring muscles, while other conventional methods, such as EMG has some limitations for evaluating muscles function. The individual assessment of individual muscle stiffness may be helpful to quantify the efficacy of new therapies, including stretching and massage. Another potential application of SWE is that it may help clinicians diagnose or monitor the recovery process of muscles noninvasively. While some stiffness may be necessary for performance, either too little or too much stiffness may cause injury. For example, higher stiffness was found to be beneficial to the athletic performance of football players [71]. Therefore, it may be beneficial for practitioners working with athletes, particularly the ones required to perform dynamic activities such as running to consider the contribution of stiffness to athletic performance. Therefore, SWE techniques provide noninvasive, quantitative, and reliable measurements suitable for clinical assessment and rehabilitation purposes.

Motivated by the necessities of addressing the mentioned challenges in muscle function assessment, this thesis aims at developing protocols for muscle function evaluation based on SSI technique. Ultrasound SWE provides the opportunity to improve our understanding of the interaction between muscle structure and function by allowing measurement of the mechanical properties of individual muscles. It is important
to systematically compare the results of SWE performed on patients with healthy individuals in the same experimental setting to understand better the relationship between SWE derived data. Therefore, the objectives of this thesis can be divided into three categories.

In this Ph.D. dissertation, the Verasonics research scanner has been used. The main advantage of Verasonics system over other conventional ultrasound scanners is that it is programmable and different types of image processing techniques can be implemented in the software part of the system. Shear waves are not able to propagate inside multifidus muscles at higher frequencies (more than 5 MHz). By using the curvilinear transducers with lower center frequencies, we may be able to visualize shear wave propagation inside multifidus. However, there is no shear wave elastography script available in the Verasonics system for the curvilinear transducers. Therefore, there is a need for implementing shear wave elastography technique in the Verasonics research scanner for the curvilinear transducers.

1.6 Objectives of this Ph.D. dissertation research

In this proposed Ph.D. dissertation research project, the following fundamental research objectives will be addressed.

1- Development of SSI technique on Verasonics research scanner for curvilinear transducers

2- Developing SWE-based protocol for diagnosis of compartment syndrome

3- Developing SWE-based protocol for evaluation of multifidus muscle function in patients with lower back pain
Chapter 2
Developing SSI sequencing code for Verasonics ultrasound scanner
The Verasonics research scanner is a research ultrasound system, which is different from clinical ultrasounds. The main advantage of Verasonics system over other conventional scanners is that it is programmable and different types of image processing techniques can be implemented in the software part of the system. Because there is no SSI elastography script available in the Verasonics system, we implemented this technique in the Verasonics research scanner for the linear L7-4 transducer. Although the developed SSI code for the L7-4 transducer was able to measure the function of lower leg compartments successfully, it was not successful for measurement of the multifidus muscle function, since shear waves were not able to propagate inside deeper tissues. Therefore, we also implemented the SSI technique for the curvilinear C5-2 transducer with lower center frequency so the shear wave propagation can be observed in multifidus muscle. The main contribution of this chapter is that the programming of SSI technique for the curvilinear transducer was performed in the Verasonics system. Programming the Verasonics system has the advantage of modifying and optimizing the script for the particular applications (e.g., multifidus muscle and compartment syndrome evaluation). Optimizing the elastography parameters for obtaining more robust shear wave speed was part of this thesis. For example, the locations of push pulses, the duration between push pulses, and the duration between push pulses and imaging pulses were modified to obtain reliable and robust shear wave speed. We first introduce the Verasonics research scanner and its hardware structure, followed by the detailed procedure of SSI programming for C5-2 transducer. Additionally, the data processing steps that were used for shear wave speed calculation, including axial velocity measurement, directional filtering, and shear wave speed estimation will be discussed. Furthermore, in order to ensure that the acoustic output parameters are within the FDA guidelines, the safety measurements will be conducted. Finally, the reliability of the developed elastography technique will be evaluated using the elasticity phantom.
2.1 Introduction

The Verasonics research system (Verasonics, Inc., Kirkland, WA, USA) has been widely adopted by different research labs for ultrasound research. The Verasonics system is compatible with different kinds of the transducer such as L7-4, P4-2, and C5-2 arrays and offers full flexibility in programming and sequence design of the system. The major advantage of Verasonics research scanner over a clinical ultrasound system is that it provides direct access to the raw radio frequency (RF) data from each element of the array. Additionally, different types of image processing techniques can be implemented in the software part of the system to improve the ultrasound images quality. The Verasonics system consists of two parts: the Verasonics data acquisition system, as dedicated hardware, and a software program running on a host computer. The hardware system is designed to transmit and acquire ultrasound pulses. The signal will then be compressed and transferred to the host computer. All beamforming and further image processing are done in the software.

The objective of this chapter was to develop a SSI code for the curvilinear transducer in Verasonics ultrasound scanner. The optimization of elastography parameters such as locations of push pulses, the duration between push pulses, the duration between push pulses and imaging pulses were also performed. The safety measurements were performed for translating the developed code into clinical research, because Verasonics is not marketed for clinical use. Finally, the reliability and validity of the developed SSI code was evaluated using the elasticity phantom.

2.2 Verasonics Hardware Architecture

The components of the Verasonics hardware is shown in Figure 2-1 [72]. The scan head interface is connected to the transducer and the acquisition modules, which are responsible for transmitting and receiving ultrasound on each channel. Received RF data is stored in the local memory of these acquisition
modules. The data acquisition system is attached to the host computer through a PCI cable, which is also connected to the acquisition modules through the backplane. The power transmit controller is responsible for supplying appropriate voltage levels to the system. The voltages used for ultrasound transmission can be adjusted by the software. To transmit a pulse, the waveform generators in the acquisition modules receive waveform parameters from the written MATLAB code. The waves are amplified and transmitted through the scan head interface. In receiving modes, the RF data is processed by the acquisition modules and going through time gain control, A/D conversion, low pass filtering, Band-pass filtering, and apodization.

Figure 2-1. Schematic view of Verasonics research scanner [72].

2.3 Verasonics software sequencing and parameter specification

Software sequencing of the Verasonics system was performed in a MATLAB programming environment. In order to generate an imaging sequence, a programming script for C5-2 and L7-4 transducers was written by a collection of objects defined using MATLAB structures. The written MATLAB file needs to be loaded into the system by a loader program (named VSX) to execute the sequence during runtime. When the script is run, the Verasonics system enters real-time B-mode imaging environment. When the user clicks the "SWI ON" button on the GUI environment, the sequence then enters shear wave elastography mode and executes the SSI sequence. The setup C5-2 script developed in this
thesis can serve as a template for researchers interested in using shear wave techniques in deeper tissues such as spine muscles or abdominal applications.

A sequence of pushing, imaging, and reconstruction was implemented in a set-up file through a structure called "Events," which contains a list of commands. When the program executes, these events are processed sequentially. Each event includes one or more structures for transmission, receiving, reconstruction, and processing of IQ data. It should be noticed that the sequences in our custom-implementation of SSI technique were different from the original SSI code introduced by Bercoff et al. [43]. In order to produce a stronger signal and better SNR, we first transmitted seven push pulses at different depths, and then we started collecting image acquisitions for shear wave estimation instead of performing image acquisition after each push pulse.

Each transmission structure (TX) contains the delays and apodization for each transducer elements, along with reference to a specific transmission waveform structure (TW). The push and acquisition frequencies were specified in separate TW structures. The acquisition frequency and push transmit frequency were both set to the center frequency of the transducer (3.10 MHz and 5.80 MHz for the C5-2 and L7-4 transducers respectively) to allow maximum transmission efficiency to deliver acoustic radiation force to the tissue. The general approach is to use a push frequency that is within the lower −6-dB bandwidth of the transducer to broaden the push beam while maintaining high transmit efficiency. The push pulse duration is associated with the number of cycles of the push waveform in the TW(2) structure. Therefore, increasing the push duration will increase tissue displacement, resulting in a higher shear wave signal-to-noise ratio (SNR) and a higher likelihood of successful shear wave speed estimation. The push duration was set to 500 cycles, which corresponds to a duration of 161 μs at 3.10 MHz and 96 μs at 5.80 MHz for the C5-2 and L7-4 transducers respectively. A single TX (1) structure was used to describe the identical transmit characteristics for the acquisition events. However, each repeated receive event was described by a unique Receive structure, which contained the unique memory location of the received channel data. Each acquisition receive event had its corresponding Receive structure entry, with a unique combination of the
variables Receive.framenum, Receive.acqNum and Receive.bufnum, specifying memory location of the received channel data. The transmit aperture and focal configurations were specified in the TX structures. Specifically, the transmit aperture location and the focal depth were defined by TX. Origin and TX.focus, respectively. The acquisition configuration was defined in TX (1), where TX (1).focus was set to 0, which corresponds to plane wave (no transmit delays) for the acquisition pulses. All elements were used in plane wave acquisition pulses, set by TX (1).Apod. The push beam configuration was specified in TX (2). The central 64 elements of the transducer were active for the push beam as specified by TX (2).Apod.

The pulse repetition interval (PRI) determines the sampling rate chosen to track shear wave propagation. For the C5-2 transducer, the PRI between acquisition frames in the B-mode image was set to 10 ms, corresponding to a pulse repetition frequency (PRF) of 100 Hz. The PRI between acquisition frames in the elastography mode was set to 100 μs, corresponding to a pulse repetition frequency (PRF) of 10000 Hz. In addition, the time between the push and the first acquisition frame was set to 250 μs, and the time between the push pulses was set to 333 μs. For the L7-4 transducer, the PRI between acquisition frames in the B-mode image was set to 10 ms, corresponding to a pulse repetition frequency (PRF) of 100 Hz. The PRI between acquisition frames in the elastography mode was set to 100 μs, corresponding to a pulse repetition frequency (PRF) of 10000 Hz. The time between the push and the first acquisition frame was set to 500 μs, and the time between the push pulses was set to 200 μs. The sampling frequency for both transducers was set to four times the center frequency of the transducer.

The reconstruction structures (“Recon”) describe the memory locations of the RF data for converting to IQ data and image reconstruction. The Recon structure defines the general characteristics of the reconstruction, including the source and destination buffers, and also which ReconInfo structures to apply to the reconstruction process. The ReconInfo structures specify individual reconstruction actions that apply to the individual regions of the IQ data. The data is reconstructed once the event referencing a recon structure is reached. The processing structures (“Process”) was used to run custom functions for processing IQ data and estimating shear wave speed. Finally, events referenced sequence control structures (“SeqControl”) to perform a variety of commands, including jumping to another event in the list,
introducing delays, synchronizing the hardware and software sequencers, changing power supply and transmitting data from the Verasonics hardware to the host computer. The event sequence in the set-up script consists of the following steps:

1. The power transmit controller was adjusted to use the power supply adapted for long pushing sequences for elastography.

2. A period of plane-wave imaging records the initial state of the medium. Plane wave images were transmitted at a pulse repetition frequency of 10 kHz. For each plane wave, the entire transducer region was insonified.

3. Waves were transmitted according to the pushing sequences in order to generate shear waves.

4. The shear wave propagation was recorded.

5. The recorded RF data were transferred to the host computer and were transformed into IQ data by the beamformer provided by Verasonics.

6. The external function that estimated shear wave velocity was called with the IQ data as input.

7. Processing jumped to step 2 until the user presses the "Freeze" button.

The schematic figure of the overall procedure for estimating shear wave speed is shown in Figure 2-2. The Matlab code for defining the SWE parameters and sequences in SSI is provided in Appendix A.
2.3.1 Angular compounding

Angular compounding was conducted to compensate for the loss in resolution caused by ultrafast imaging. The plane waves were transmitted at three different angles: $-15^\circ$, $0^\circ$, and $15^\circ$. The first plane wave was tilted at $-15^\circ$, the second was vertical, and the third was tilted at $15^\circ$. The fourth plane waves the same as the first plane wave was tilted at $-15^\circ$, and so on. The angled plane waves were generated by delaying the transducer elements linearly. In post-processing, the results were averaged in order to improve image quality by reducing the acoustic artifact. IQ data was obtained from three different angles and was subsequently averaged using a third-order moving-average filter across frames [74]. Thus, each averaged
frame is the mean of three original frames from different angles, and each original frame contributes to three averaged frames.

### 2.3.2 Axial velocity estimation

There have been several time-delay estimators suggested for tissue motion tracking. They can be broadly characterized into correlation-based approaches that operate on RF data and phase-shift algorithms that operate on IQ data [75]. In this thesis, the 2D Loupas autocorrelator [76], which takes better advantage of the dimensionality of the data, was implemented to estimate the local axial particle velocity. Equation 2-1 shows the final expression for the axial velocity $v$.

$$v = \frac{c}{2 T_s} \tan^{-1} \left\{ \frac{\sum_{m=0}^{M-1} \sum_{n=0}^{N-2} (Q(m,n)I(m,n+1) - I(m,n)Q(m,n+1))}{\sum_{m=0}^{M-1} \sum_{n=0}^{N-2} (I(m,n)I(m,n+1) + Q(m,n)Q(m,n+1))} \right\}$$

where $I$ and $Q$ denote the IQ matrices, respectively, containing IQ data from $M$ different depths and $N$ different frames, with the constant lateral position. In this study, $M = 5$ and $N = 5$ were chosen. $C$ denotes the speed of sound in the tissue (1540 m/s), $t_s$ represents the sampling period of the RF signal (50 ns), $T_s$ represents the pulse repetition frequency and $F_{dem}$ the number of wavelengths of the center frequency per RF sample (1/4).

### 2.3.3 Directional filtering

Directional filtering was applied to reduce the artifacts from the reflected waves at boundaries. A 2-D directional filter in Fourier $(k, \omega)$ space was implemented to separate rightward and leftward traveling waves. The 2D discrete Fourier transform of a lateral-temporal slice can be written as a function of angular frequency $\omega$ and wavenumber $k$ with the phase velocity $c = \omega / k$ [77]. Therefore, the energy of the right-
propagating shear waves corresponds to the first and third quadrants of the spectrum, where $c > 0$, while that of the left-propagating shear waves corresponds to the second and fourth quadrants, where $c < 0$. The directional filtering was performed by keeping only the leftward traveling waves for each slice, followed by inverse transformation to the spatiotemporal domain [77].

2.3.4 Shear wave speed estimation

Estimating the shear wave speed at a target pixel was conducted by picking two reference neighbor pixels and comparing the time-dependent axial velocity between the two reference pixels to estimate the time taken for the shear wave to travel between them. The reference pixels were picked at the same depth as the target pixel and four pixels away from it in each lateral direction. The cross-correlation of the resulting functions was calculated. The average shear wave speed between the reference pixels was calculated by dividing the distance the wave traveled by the time obtained by cross-correlation and considered as the local shear wave speed at the target pixel.

The Verasonics system is not marketed for clinical use. Therefore, for translating the developed code into clinical research, the safety measurements need to be performed to ensure that the acoustic output parameters are within the FDA guidelines.

2.4 Safety measurements

The FDA established limits on acoustic output parameters to avoid bioacoustic effects that are damaging to tissues [78]. These parameters include derated spatial peak time average intensity ($I_{SPTA,3}$), derated spatial peak pulse average intensity ($I_{SPPA,3}$), and mechanical index (MI). For musculoskeletal applications, the safety limits of these parameters are $I_{SPTA,3} \leq 720$ mW/cm$^2$, and $I_{SPPA,3} \leq 190$ W/cm$^2$ or MI $\leq 1.9$. For elastography applications, the acoustic output parameters are typically close to the FDA limits. Therefore,
it is important to understand the relationships between acoustic parameters and characteristics of the push pulses. The measurement procedures for obtaining acoustic output parameters were performed according to the NEMA testing and labeling standard [79]. The relationships between the frequency and acoustic output parameters may serve as guidelines for the implementation of SSI methods for musculoskeletal applications.

The measurements were derated by 0.3 dB cm\(^{-1}\) to consider the difference between in-tissue and in-water measurement effect. The maximum derated pulse intensity integral (PII\(_3\)) and the position at which it takes place was determined by scanning the region of interest. The derated spatial-peak pulse-average intensity (I\(_{SPPA,3}\)) was calculated at the location of the maximum value of PII\(_3\) as Eq. 2-2 suggests:

\[
I_{SPPA,3} = \frac{\text{PII}_3}{\text{PD}} \quad (2-2)
\]

where PD is pulse duration expressed in a sec, and the derated pulse intensity integral is calculated as Eq. 2-3:

\[
\text{PII}_3 = \exp(-0.23 \times 0.3 \times f_c \times z) \times \text{PII} \quad (2-3)
\]

where \(z\) is the distance from the transducer assembly to the measurement point along the beam axis, and \(f_c\) is the push pulse frequency expressed in MHz. The pulse intensity integral (PII), which is equal to the energy flow per pulse, is the time integral of instantaneous intensity, for any specific pulse, integrated over the time interval in which the envelope of acoustic pressure for the specific pulse is nonzero and is calculated using the Eq. 2-4:

\[
\text{PII} = \int_{t_0}^{t_2} V_h^2(t) dt \quad (2-4)
\]

\[
\text{PII} = \frac{\int_{t_0}^{t_2} V_h^2(t) dt}{10^4 \rho c M_L^2(f_c)}
\]

where \(\rho\) is density (kg/m\(^3\)), \(c\) is the speed of sound (m/s), \(M_L(f_c)\) is the hydrophone loaded sensitivity expressed in V/Pa, and \(v_h(t)\) is the output voltage of the hydrophone. The derated spatial-peak temporal-average intensity (I\(_{SPTA,3}\)) was calculated at the location of the maximum value of PII\(_3\) as Eq. 2-5:

\[
I_{SPTA,3} = \text{PII}_3 \times \text{PRF} \quad (2-5)
\]
where PRF is the pulse repetition frequency in Hz. Finally, MI gives an estimation of the risk of the non-thermal effects (cavitation and streaming) and is defined as Eq. 2-6:

\[
MI = \frac{|p_3|}{\sqrt{f_c}}
\]  

(2-6)

where \(p_3\) is the derated peak rarefaction pressure of the ultrasound wave (MPa) and is calculated as Eq. 2-7:

\[
p_3 = \exp(-0.115 \times 0.3 \times f_c \times z) \times p
\]  

(2-7)

2.4.1 Equipment and Set up

L7-4 and C5-2 probes were used for transmitting the push pulses. The probe was located perpendicular to the water surface in a water tank (Figure 2-3). The driving voltage and the number of push cycles were 40 V and 1000 cycles, respectively. This maximum driving voltage was selected because higher voltages did not result in increased values of acoustic intensity or mechanical index. This saturation effect may have been caused by a limitation in the amount of power delivered by the Vantage system. These frequencies were chosen to be in the -6 dB range of the ultrasound probe used.

Acoustic pressure was measured using an HGL-0200 hydrophone along with AH-2010-025 amplifier (ONDA Corp., Sunnyvale, CA). The hydrophone was attached to the 3D positioning system with a spatial resolution of 0.1 mm. An MDO3012 oscilloscope (Tektronix Inc., Beaverton, OR, USA) with a sampling rate of 2.5 GHz was used to record data from the hydrophone. A Matlab (Mathworks, Natick, MA) script was coded to read the waveform directly from the oscilloscope and calculate the acoustic output parameters. Measurements were performed in a tank lined with 10 mm thick polyurethane acoustic absorber. De-ionized and degassed water at room temperature was used.
The measured ultrasound acoustic intensity and MI are shown in Tables 1. MI and $I_{SPTA,3}$ values at various frequencies are below FDA regulatory limits.

Table 2-1. Parameters of the imaging methods used in the calculation of the acoustic intensity and MI.

<table>
<thead>
<tr>
<th>Technique</th>
<th>PRF</th>
<th>Pulse duration (ms)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSI for transducer C5-2</td>
<td>2</td>
<td>2.16</td>
<td>3.1025</td>
</tr>
<tr>
<td>SSI for transducer L7-4</td>
<td>2</td>
<td>1.29</td>
<td>5.2080</td>
</tr>
</tbody>
</table>
Table 2-2. Acoustic outputs for each elastography technique for C5-2 transducer. FDA limits of these parameters are $I_{SPTA,3} \leq 720$ mW/cm$^2$, and $I_{SPPA,3} \leq 190$ W/cm$^2$ or MI $\leq 1.9$. MI $\leq 1.9$ was taken as a limit

<table>
<thead>
<tr>
<th>Technique</th>
<th>$I_{SPTA,3}$</th>
<th>$I_{SPPA,3}$</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSI</td>
<td>648.18</td>
<td>150.04</td>
<td>2.63</td>
</tr>
</tbody>
</table>

Table 2-3. Acoustic outputs for each elastography technique for L7-4 transducer. FDA limits of these parameters are $I_{SPTA,3} \leq 720$ mW/cm$^2$, and $I_{SPPA,3} \leq 190$ W/cm$^2$ or MI $\leq 1.9$. MI $\leq 1.9$ was taken as a limit

<table>
<thead>
<tr>
<th>Technique</th>
<th>$I_{SPTA,3}$</th>
<th>$I_{SPPA,3}$</th>
<th>MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSI</td>
<td>520.76</td>
<td>200.29</td>
<td>1.72</td>
</tr>
</tbody>
</table>

The acoustic parameters satisfied the FDA regulatory limits for the driving voltage and timing parameters used in this study. Results show that the obtained acoustic output parameters are well below the limits established by the FDA. The FDA requires either the $I_{SPPA,3}$ or the MI to be under the regulated limits. In the case of L7-4 transducer, the $I_{SPPA,3}$ exceeded the limit of 190 W/cm$^2$, but the MI for all experiments was under 1.9. Therefore, both transducers can be implemented for clinical research on human subjects using the specified SSI parameters.

2.5 Reliability and validity of the developed technique

The developed SSI codes for L7-4 and C5-2 transducers were validated on a commercially available CIRS elasticity phantom (nominal shear wave speed = 2.94 m/s, Model 040GSE, CIRS, Norfolk, Virginia, USA) with inclusion masses at different locations. An aperture of 64 central transducer elements emitted focused push pulses (500 push cycles at 5.208 MHz for L7-4 transducer and 500 push cycles at 3.125 MHz for C5-2 transducer) successively at seven increasing, equally spaced focal depths, creating quasi-plane
shear waves in the sample. The ROI size was 7.39 × 7.39 cm. The shear wave speed measurements were conducted at different depths and were compared with values from nominal values released by the company. For measuring the shear wave speed at each depth, the center of ROI was located at that depth. For each position, the measurement was repeated five times, and the mean and standard deviation of shear wave speed values were calculated (Table 2-4 and 2-5).

Table 2-4. Measured shear wave speed of elasticity phantom for background materials at different depths and inclusion using the L7-4 transducer.

<table>
<thead>
<tr>
<th>Position (mm)</th>
<th>Shear wave speed (m/s) mean± SD</th>
<th>Nominal value (m/s)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth=7.98</td>
<td>2.98±0.07</td>
<td>2.93</td>
<td>1.7</td>
</tr>
<tr>
<td>depth=13.3</td>
<td>2.86±0.01</td>
<td>2.93</td>
<td>2.4</td>
</tr>
<tr>
<td>depth=18.6</td>
<td>2.94±0.02</td>
<td>2.93</td>
<td>0.3</td>
</tr>
<tr>
<td>depth=23.9</td>
<td>3.01±0.02</td>
<td>2.93</td>
<td>2.7</td>
</tr>
<tr>
<td>inclusion 1</td>
<td>3.55±0.01</td>
<td>3.65</td>
<td>2.7</td>
</tr>
<tr>
<td>Inclusion 2</td>
<td>1.89±0.04</td>
<td>1.83</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Table 2-5. Measured shear wave speed of elasticity phantom for background materials at different depths and inclusion using C5-2 transducer.

<table>
<thead>
<tr>
<th>Position (mm)</th>
<th>Shear wave speed (m/s) mean± SD</th>
<th>Nominal value (m/s)</th>
<th>Difference (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth=10</td>
<td>3.13±0.04</td>
<td>2.93</td>
<td>6.8</td>
</tr>
<tr>
<td>depth=20</td>
<td>3.22±0.01</td>
<td>2.93</td>
<td>9.9</td>
</tr>
<tr>
<td>depth=30</td>
<td>3.21±0.02</td>
<td>2.93</td>
<td>9.6</td>
</tr>
<tr>
<td>depth=35</td>
<td>3.15±0.03</td>
<td>2.93</td>
<td>7.5</td>
</tr>
<tr>
<td>inclusion</td>
<td>3.60±0.03</td>
<td>3.65</td>
<td>1.37</td>
</tr>
</tbody>
</table>
Table 2-4 and 2-5 show that the developed SSI sequences for both C5-2 and L7-4 transducers provided robust shear wave speed estimates of the background and inclusion with an excellent agreement to the nominal value (the errors in all measurement were below 10%). The measurements produced consistent measurements with low variances for both C5-2 and L7-4 transducers.

2.6 Conclusion

This chapter developed the SSI code for the curvilinear C5-2 transducer in Verasonics ultrasound scanner. The optimization of elastography parameters such as locations of push pulses, the duration between push pulses, the duration between push pulses and imaging pulses were also performed. The safety measurements were conducted and showed all acoustic output parameters satisfied the FDA regulatory limits for the driving voltage and timing parameters used in this study. The reliability of the developed codes was measured using commercially available elasticity phantom, and the obtained mean values at different depths were within 10% of the nominal values released by the company.
Chapter 3

Developing a new protocol for quantifying active contraction of lumbar multifidus muscle

The content of this Chapter has been published in the Journal of Biomechanical Engineering [2].
The multifidus is an important paraspinal muscle providing active stability of the spine. Multifidus function is compromised in patients with chronic low back pain and other spine pathologies. The function of the back muscles is often evaluated using isometric or isokinetic tests, which cannot quantify multifidus contribution independent of the erector spinae and adjacent hip musculature. In this study, a new protocol will be presented and evaluated to quantify localized force production capability in multifidus muscle using the SSI method in healthy individuals. The protocol consists of measuring the multifidus shear modulus during three different positions: lying prone, sitting up, and sitting up with the right arm lifted. Measurements are taken of the superficial and deeper layers of the multifidus muscle. Additionally, repeatability and possible sources of error of the shear modulus measurements will be analyzed. Experiments and results of elastography measurement on healthy individuals will be presented, followed by a discussion of the results.

3.1 Introduction

Quantifying localized contraction of the multifidus, including contraction characteristics of the two parts of the multifidus, may enhance our understanding of relationships among multifidus anatomy, function, and resultant spine stability. The anatomical features of the multifidus (a high cross-sectional area and a low fiber length-to-muscle length ratio) suggests that it is uniquely designed as a stabilizer to produce large forces [80]. The superficial and deeper fibers of the multifidus are differentially active during single and repetitive movements [81], providing evidence of functional and anatomical differences between the layers. It has been suggested that the superficial region of the multifidus mainly controls spinal orientation, i.e., the extension of lumbar spine and control of lumbar lordosis, while the deeper layer controls shear and torsion motions via intervertebral compression [81]. Due to the structural differences between the superficial and deeper layers of the multifidus, different force production may be expected at each layer during various tasks that challenge spinal stability. Despite the biomechanical differences between multifidus layers, few studies have compared their recruitment and activation. Consequently, an assessment
method capable of estimating muscle force production of the two multifidus layers may be useful in evaluating regional spinal stability.

Assessment of lower back muscle function in both research and clinical practice may occur through physical examination, strength testing, EMG and endurance of the paraspinal muscles may be assessed using isometric or isokinetic strength tests (i.e., dynamometry), which lack the ability to quantify multifidus contribution independent of the erector spinae (and adjacent hip musculature). Rehabilitative Ultrasound Imaging (RUSI) has been used to evaluate multifidus function in patients with chronic back pain [82-84]. RUSI evaluates multifidus function by quantifying changes in muscle thickness during volitional contractions facilitated with specific exercises or during automatic contractions facilitated with limb lifts. However, the anatomy may be altered during fusion surgery, making identification of muscle boundaries difficult post-surgery and precluding RUSI for evaluation of multifidus function post-PLF. The majority of RUSI studies exclude individuals with a history of spinal surgery [85, 86]. Although needle EMG addresses some of the problems related to surface EMG, (i.e. maintaining robust electrode contact with skin, evaluating multifidus muscle independent of erector spinae input), it is invasive and its amplitude is affected by many factors, such as the location of electrodes relative to muscle fiber direction [87, 88]. MRI has primarily been used to evaluate changes in muscle morphology and structure, but the expense and accessibility of such imaging preclude its use in clinical practice. Thus, there is a need for a low-cost, non-invasive clinical assessment technique that can quantify the force production capability of individual muscles, such as the multifidus, both in the presence of spinal pathology and post-operatively.

As previously discussed, Ultrasound Shear Wave Elastography (SWE) is a reliable, noninvasive imaging technique that can provide information about muscle function [44, 45, 89]. Changes in shear modulus are linearly proportional to the force produced by the muscle [57, 90, 91]. Therefore, SWE can be used to evaluate the force production capability of individual muscles in motor tasks with muscle redundancy, which cannot be achieved with current clinical tests [92]. SWE has been extensively used to characterize muscles function in the limbs [46, 57, 93], but there has been little focus on the evaluation of multifidus
muscles using SWE. Recently, Creze et al. [94] quantified resting shear modulus in the paraspinal muscles (i.e., multifidus, longissimus, and iliocostalis) in vivo at level L3-4. The longissimus muscle had a higher shear modulus than the multifidus muscle, i.e., 6.9 ± 2.7 kPa versus 5.4 ± 1.6 kPa. Moreau et al. [95] investigated the reliability of ultrasound SWE in the assessment of shear modulus of the lumbar multifidi muscle at level L2–3 and L4-5 at rest and during passive stretching (sitting on an ergonomic forward leaning massage chair without actively contracting trunk muscles). Shear modulus in the passive stretching posture at level L2-L3 (L4-L5) was found to be higher than in the resting position, i.e., 13.8 ± 2.9 kPa (22.7 ± 3.8 kPa) versus 8.5 ±1.9 kPa (6.8 ± 1.2 kPa), providing evidence of increased shear modulus due to muscle activation. These studies, however, were limited to measurements at rest and with passive stretching, which fails to capture actively contracted muscle function that is of interest to clinicians developing a rehabilitation exercise program. SWE can measure the active function of the multifidus since the region of interest (ROI) can be adjusted to allow quantification of changes of stiffness in both the superficial and deeper layers of this muscle. An ROI covering the entire muscle thickness may also offer information about the overall function of the muscle. Additionally, evaluating the reliability and repeatability of SWE for active measurements in back muscle is essential for future clinical application of this technique.

The objective of this study is to develop and evaluate a new protocol to quantify muscle force production capability of the multifidus muscles using ultrasound SWE. The proposed protocol quantifies active contraction of the muscle by measuring changes in shear modulus of the multifidus in three body postures: lying prone, sitting up, and sitting up with the right arm lifted, for both the superficial and deeper layers of the muscle. An important advantage of the proposed protocol is that it uses functional tasks requiring less muscle activity when compared to classically used strength and endurance tests, and thus, may be used when evaluating injured patients (i.e., those with chronic LBP), including those recovering from spine surgery. The pilot study consists of three aims: 1) evaluating shear modulus in three different postures and comparing shear modulus of the superficial and deeper layer of multifidus for the left and right sides, 2) calculating ICCs to determine the reliability of the method, and 3) comparing values of shear modulus for
different ROI sizes so that regional spinal stability could be evaluated. We hypothesized that shear modulus would increase from lying prone, to sitting up, to sitting up with the arm lifted, regardless of the muscle layer assessed. We hypothesized that the deeper layer of the multifidus would be less active than the superficial layer of the muscle due to its proposed stabilizing role and the low-level contractions required of the selected tasks.

3.2. Methods

3.2.1 Overview

The Institutional Review Board (IRB) of the Pennsylvania State University approved the study, and all participants gave informed consent before any evaluation. Participants were excluded if they had received services for LBP within the past six months; ever had low back surgery; had difficulty performing the requested tasks; had experienced a recent traumatic event such as a motor vehicle collision; had a history of any neurological disease, or had a terminal illness.

3.2.2 Shear Wave Elastography System

A Verasonic ultrasound system (Verasonic Inc., Redmond, WA, USA) with a C5-2 transducer (128 elements, beamwidth = 2-5 MHz, center frequency = 3.1025 MHz) was used. A custom implementation of the supersonic SWE method proposed by Bercoff et al. [43] was used to measure muscle shear modulus. Acoustic output intensity was measured to ensure the method satisfied the Food and Drug Administration (FDA) limits for intensity for use in human subjects. Sixty-four central elements of the transducer emitted focused ultrasound pushing beam (500 push cycles at 3.1025 MHz frequency; push duration = 161 μs) at seven focal points at different depths. The propagation speed of shear wave was calculated within the ROI.
using a frame rate of 10000 frames/s. Finally, the corresponding shear modulus map was constructed based on the obtained shear wave speed. The SWE method was validated on a commercially available homogeneous elasticity phantom (nominal shear wave speed = 2.94 m/s, Model 040GSE, CIRS, Norfolk, Virginia, USA) with the dimensions of 17.8 cm × 12.7 cm × 20.3 cm [96]. This technique has also been successfully used in several previous studies [1, 97, 98].

3.2.3 Proposed Protocol to Evaluate Multifidus Muscle Function

Participants laid prone with the back muscles in a fully relaxed position (Fig. 1(a)). The transducer was placed in long-axis just lateral to the spinous processes and angled medially (10-15 degrees), minimizing contact pressure through the use of an adjustable fixture. The L4-5 facet joint and adjacent multifidus muscle was identified. For measurement of left L4-5 multifidus shear modulus during multifidus activation in sitting, each participant was first asked to sit up (Fig. 1(b)). They were asked to sit upright in a normal resting posture, while their feet were not supported on the floor. The transducer was placed back on the skin. Then the participant was instructed to sit up with their right arm horizontal to measure the multifidus shear modulus on the left and right sides (Fig. 1(c)).

During SWE measurements, the investigator monitored the real-time brightness-mode (B-mode) ultrasound images to ensure that there was no noticeable body movement. Once the multifidus muscle was identified in the B-mode image (Fig. 2), the elastography mode was then activated to measure the shear modulus of the muscle. The measurements in all studies were completed by locating the ROI 2 mm above the facet joint (for shear modulus measurements of the deeper and full thickness of the multifidus muscle) in order to remove artifacts from the bone edge. The muscle's fascial line was chosen as the upper limit of the ROI for the superficial multifidus measurement. Fig. 2 shows the ROI location of the superficial, deeper, and the entire thickness of the L4-5 multifidus. The shear wave speed was measured five times in each round of measurements to calculate a robust average. Further details regarding the protocol for multifidus function evaluation is provided in Appendix B.
Figure 3-1. Imaging with the transducer located lateral to the spinous processes and angled medially to view the left L4-5 facet joint at (a) prone (b) sitting up (c) sitting up with the right arm lifted in a horizontal position.

Figure 3-2. Ultrasound B-mode image of the left L4-5 multifidus
3.2.4 Quantifying changes in shear modulus in different postures

Eighteen healthy participants who met the study’s inclusion criteria were recruited (mean age ± SD, 35.50 ± 18.06 years; mean BMI ± SD, 21.73 ± 2.34 kg/m\(^2\)). The changes in shear modulus of the multifidus muscle were quantified in three body postures (i.e., prone, sitting up, and sitting up with the right arm lifted). The difference between shear modulus of the right and left multifidus in the right arm lifted state was evaluated using the linear mixed effect model with the lme4 package in R program [99].

3.2.5 Reliability of the Proposed Protocol

Participants were required to undergo the described protocol three times (twice on day 1 to evaluate within-day reliability, and again four days later to evaluate between-days intra-examiner reliability) with the same examiner. The within and between-days intra-examiner reliability of the proposed method was analyzed by calculating ICCs (model 3,3) with 95% confidence intervals (CIs) for the 18 participants for shear modulus assessment of both superficial and deeper layers of the muscle; a similar method was used by Dieterich [100]. Absolute reliability was expressed by the standard deviation (SD) and the standard error of measurement (SEM).

3.2.6 Localized Changes of Contraction in the Multifidus Muscle

In this portion of the study, we compared the average value of shear modulus obtained from the superficial and deeper layers to the value obtained with a bigger ROI covering the entire thickness of the muscle (Fig. 3). Five healthy subjects (four male, one female) ranging in age from 21 to 34 years (Mean age ± SD, 26.8 ± 4.76; Mean BMI ± SD, 22.97 ± 1.08) were recruited. The shear modulus of the superficial
and deeper layers of the multifidus was measured by adjusting the ROI size to 20 mm * 12 mm and placing the ROI within each layer. An ROI with the same width (20 mm) but variable height to cover the entire thickness of the multifidus muscle (from the muscle’s fascial line to 2 mm above the facet joint) was considered. However, only the ROI size of 20 mm * 12 mm was chosen for the superficial and deeper multifidus layers in other portions of the study. In order to compare the shear modulus of the bigger ROI and the average of the two smaller ROI windows, a Two One-Sided Test (TOST) procedure was used. To ensure an alpha level of 0.05 for the equivalence test, a 90% CI generated from a paired t-test was used. It was predicted that the two measures (shear modulus of the bigger ROI and the average of the two smaller ROI windows) would vary by at least delta, where delta is defined as a clinically meaningful difference. This statistical approach is more appropriate than the traditional definition in which delta is zero [101].

Figure 3-3. Location of ROI for the elastography measurement of the L4-5 multifidus muscle at the entire multifidus thickness
3.3 Results

3.3.1 Results of reliability study

The within-day ICC point estimates for the superficial and deeper layer were found to be 0.76 and 0.80, respectively, suggesting good-to-excellent reliability (Table 3-1). Between-days reliability for the deeper layer of the multifidus was lower than for evaluation of the superficial layer of the multifidus.

<table>
<thead>
<tr>
<th></th>
<th>Within-day ICC&lt;sub&gt;3,3&lt;/sub&gt; (95% CIs)</th>
<th>SD, SEM (kPa)</th>
<th>Between-days ICC&lt;sub&gt;3,3&lt;/sub&gt; (95% CIs)</th>
<th>SD, SEM (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial</td>
<td>0.76 (0.58-0.85)</td>
<td>18.23, 8.93</td>
<td>0.77 (0.59-0.87)</td>
<td>21.47, 10.31</td>
</tr>
<tr>
<td>Deeper</td>
<td>0.80 (0.63-0.88)</td>
<td>18.15, 8.12</td>
<td>0.63 (0.38-0.78)</td>
<td>14.44, 8.78</td>
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</table>

Table 3-1. Obtained ICCs and 95% CIs for evaluation of superficial and deeper layers of all postures of the L4/5 multifidus muscle.

3.3.2 Results of multifidus activation study

The measurement results of the multifidus shear modulus in the three body postures are summarized in Fig. 4. Data are presented as median (interquartile range) due to Shapiro–Wilk test results (p=0.02). The average shear modulus of the superficial and deeper multifidus increased from prone, i.e., 16.15 (6.69) kPa, to sitting up, i.e., 27.28 (15.72) kPa, to sitting up with the right arm lifted, i.e., 45.02 (25.27) kPa, indicating increased activity and force production. The shear modulus for the deep multifidus layer for the left side in prone, sitting up and lifted arm postures was 14.18 (7.65) kPa, 24.24 (16.05) kPa, and 29.93 (17.55) kPa, respectively; for the superficial multifidus, values were 16.52 (6.16) kPa, 29.93 (17.55) kPa, and 50.38 (20.44) kPa, respectively. The shear modulus in the deeper layer of the multifidus was significantly lower than that of superficial layer (p<0.05) in all three body postures, suggesting inhomogeneous relaxation and contraction of the layers of the multifidus muscle. The median shear modulus for the left side was found to
be 12.73 kPa higher than that of the right side when lifting the right arm (Figure 3-4). The linear mixed effect model analysis indicated that this side-to-side difference in shear modulus is significant (p< 0.05).

![Image](image.png)

Figure 3-4. The shear modulus of the L4-5 multifidus (median, interquartile range) in 3 body postures: prone (left side), sitting up (left side), and sitting up with lifted right arm (LA) position for both the left and right sides.

### 3.3.3 Results of Localized Changes of Contraction in the Multifidus Muscle

A summary of the differences between the shear modulus of the entire thickness and average of the superficial and deeper layer of the L4-5 multifidus muscle are depicted in Figure 3-5. The shear modulus (median, (interquartile range)) of the average of superficial and deeper multifidus at the prone, sitting up and sitting up with right arm lifted positions on the left and right side were found to be 24.65 (10.16), 27.27 (18.72), 51.29 (15) and 28.66 (3.67) kPa respectively. These values for the bigger ROI covering the entire multifidus thickness were 32.42 (10.64), 28.76 (34.13), 53.27 (28.55), and 28.01 (22.30) kPa. Based on the statistical analysis using the TOST procedure, it was found that if the clinically meaningful difference between shear modulus of bigger ROI and the average of the two smaller ROI windows was larger than 8.839 kPa, the two measures could be considered equivalent. Figure 3-6 shows an example of the
elastography measurement results at the superficial, deeper, and entire layers of the L4-5 multifidus. The shear modulus map within the rectangular ROI shows the shear modulus levels of the tissues.

Figure 3-5. Comparing the shear modulus (median, interquartile range) of the average of the superficial and deeper layer of the L4-5 multifidus with the shear modulus of the bigger ROI in 3 body postures: prone (left side), sitting up (left side), and sitting up with lifted right arm (LA) position for both the left and right sides.
3.4 Discussion

A new protocol was developed to quantify changes of shear modulus during contraction of the multifidus muscle. Changes in muscle shear modulus are important because they are linearly related to change in muscle force production. The proposed protocol consisted of measuring shear modulus using SWE at three different postures: lying prone, sitting up, and sitting up with the single arm lifted. It was observed that the
multifidus shear modulus was greatest in the lifted arm position, followed by the sitting up and lying prone positions. The change in median shear modulus from lying prone to sitting up with the right arm lifted was more pronounced compared to changes in the other postures. During lifting a single arm, the higher shear modulus of the contralateral multifidus shows increased force production to keep the torso balanced. The deeper layer of the multifidus showed lower shear modulus in all cases, suggesting a reduced muscle contraction in the deeper layer. This difference in contraction suggests that the superficial layer of the multifidus plays a larger role in the extension of the back, while the lower layer provides a stabilizer function. There was not a significant difference between measurement of the whole multifidus shear modulus using the bigger ROI and averaging the shear modulus of the upper and lower multifidus layers using the smaller ROI. It was also observed that the superficial and deeper multifidus layers have excellent within-day reliability. Therefore, this study confirms procedural reliability of our protocol for assessments of the multifidus shear modulus at different levels of muscle contraction in healthy individuals, demonstrating that the new technique could be a useful tool in the assessment of force production and function of the lumbar multifidi.

Few studies have explored the shear modulus of the multifidus muscle in a group of healthy adults. Dieterich et al. [100] found a shear modulus of 14.9 kPa for the cervical spine multifidus during resting posture in prone, which is very similar to our results for the prone position. Additionally, they showed an increasing value of shear modulus as a function of loading of the neck. Alis et al. [102] used a convex low-frequency transducer to evaluate the lumbar multifidus muscle in a relaxed prone position in patients with disc herniation. They reported mean stiffness values of the multifidus muscle on the affected side (observer 1, 14.08 ± 3.57 kPa; observer 2, 13.70 ± 4.05 kPa) that was significantly lower compared to the contralateral side (observer1, 18.81 ± 3.95 kPa; observer 2, 18.28 ± 4.12 kPa). These values are also very close to our measurements of shear modulus in a prone position. Chan et al. [103] reported a shear modulus of 12 kPa at level L4-5 using strain elastography. Moreau et al. [95] found a shear modulus of 6.8 ± 1.2 kPa at level
L4-5 in the prone position. Creze et al. [94] reported 5.4±1.6 kPa for the L3-4 multifidus shear modulus in the prone position. These values are lower than our reported values of 16.15 (6.69) kPa.

Such differences might be explained by differences with elastography methods (strain vs. shear wave elastography), and technical parameters, such as transducer type, frequency, the location of the ROI and the angle between the transducer and muscle fibers. For instance, Moreau et al. [95] employed a linear transducer with a frequency of 8 MHz, which is higher than what we used in this study (3.1 MHz). Several studies have demonstrated that parameters such as ultrasound frequencies, transducer types, and imaging depths contribute to biases in the shear wave speed measurements in transient methods like the one used in this study [104-106]. These biases can be explained, in part, by the effect of those parameters on the bandwidth of the transient shear waves used to measure mechanical properties of the tissue. Several authors reported that it is not easy to identify the proper orientation of the fibers due to the thinness of the fascicles and their arrangement [94, 107, 108]. Multifidi are poly-articular and multipennate with multiple fiber orientations, given the random layering of millimetric fascicles and fatty spaces. Such geometrical complexity implies that transducer angle is influential on shear modulus values. Our protocol used a transducer orientation similar to that used in previous studies for USI of the multifidus [12, 16, 109, 110]. In the B-mode image, the multifidus is traditionally considered to span superiorly from the facet joint to the last dark pixel before the muscle's fascia. However, in our experiments, we noticed that the superficial and deeper multifidus layer represent different morphological appearances and mechanical properties (lower shear modulus in the deeper layer). Therefore, the location of the ROI may be another factor causing differences between reported studies.

The shear modulus changed with increasing contraction of the multifidus. Several studies have suggested a linear relationship between muscle force production and shear modulus [57, 90, 91]. Our measurements show an increase of shear modulus from prone to sitting up, and from sitting up to arm lifted, which were expected since those positions require different levels of muscle force production to maintain the stability of the torso. For the sitting with the right arm lifted position, the left multifidus showed higher shear
modulus compared to the right. This difference was expected since lifting the right arm cause a force moment towards the right side of the subject that needs to be balanced out by a moment caused by increased force production of muscle at the left side of the spine. These observations suggest that our protocol can capture patterns of muscle contraction caused by different functional tasks. In this study, the deeper layer of L4-5 multifidus showed lower shear modulus in all cases, suggesting an inhomogeneous contraction of the multifidus. This difference in contraction suggests that the superficial layer of the multifidi plays a more significant role in the extension of the back. From the clinical point of view, an overall evaluation of the function of the multifidus may be more relevant. Therefore, for comparison purposes, the bigger ROI size, including the whole multifidus muscle, was also employed. However, there was not a significant difference between measurement of the whole multifidus shear modulus using the bigger ROI and averaging the shear modulus of the upper and lower multifidus layers using the smaller ROI. Although L4-5 multifidus shear modulus was quantified in the previously mentioned studies, the present paper is the first to publish shear modulus data for the lumbar multifidus muscle using SWE at different levels of muscle activation in healthy individuals.

A comprehensive analysis of the procedural reliability for the proposed protocol was performed in this study. The proposed protocol yielded excellent reliability in the assessment of lumbar multifidus shear modulus. ICCs were obtained to provide information about within-day and between-days variation. Lower obtained ICCs suggests that the majority of the variation is introduced due to the wave attenuation and smaller shear wave amplitude for deeper muscles. This is consistent with previous studies showing increased variability between ex-vivo and in-vivo measurements of the shear modulus of the supraspinatus muscle [94, 95]. In order to have consistent measurements, we placed the transducer in long-axis just lateral to the spinous processes and angled medially (10-15 degrees) and minimized the contact pressure through the use of an adjustable fixture. Therefore, applying a consistent method of transducer placement is essential for data accuracy in future studies.
Our protocol offers several advantages over other conventional methods for evaluating force production in multifidus muscle. First, this is a localized method that is capable of quantifying multifidus muscle function independent of the erector spinae (and adjacent hip musculature), while other conventional methods, such as isometric or isokinetic tests (i.e., dynamometry), cannot evaluate muscle function locally. This can help clinicians diagnose or monitor pathological conditions of paraspinal muscles. Besides, this is the first time that differences in activation within layers of the multifidus are reported, which highlights the importance of developing techniques to quantify localized force production capability of spinal muscles.

RUSI has been used to evaluate multifidus function in patients with chronic back pain \([111-113]\). RUSI evaluates multifidus function by quantifying changes in muscle thickness during volitional contractions facilitated with specific exercises or during automatic contractions facilitated with limb lifts. However, the linearity between change in thickness and muscle activation is limited to a range between 18 and 50% of the maximum voluntary contraction \([12, 16, 111]\). This is opposite to shear modulus, for which the linearity over the entire range of voluntary contraction has been shown. Additionally, the anatomy of the spine may be altered during surgical procedures like fusion, making identification of muscle boundaries difficult post-surgery and precluding RUSI \([42-44]\) for evaluation of multifidus function. The majority of RUSI studies exclude individuals with a history of spinal surgery \([17, 18]\). Thus, our proposed protocol may provide a clinical tool that can quantify the function of individual paraspinal muscles post-operatively. Further, utilization of limb movements to elicit automatic multifidus contractions, rather than volitional exercises to elicit multifidus activity, is also advantageous. In healthy spines, proximal, stabilizing muscles such as the multifidus, should activate automatically before more distal muscles (e.g., shoulder, hip) that move joints. Consequently, clinicians may be more interested in evaluating automatic muscle performance (rather than volitional performance), which may provide better insight into muscle function during everyday tasks. Another advantage is that the functional tasks introduced in this protocol are less demanding compared to the strength and endurance tests, which may be of crucial importance for evaluating injured patients, patients recovering from spine surgery, and older adult patients who may not be able to perform physically demanding tasks.
There are several limitations to this study. The first limitation includes a relatively small sample size. Additionally, gender effects were not investigated, which may have influenced the variability of the data. Lastly, in this study, mostly younger subjects with normal BMI were recruited.

### 3.5 Conclusion

To summarize, this chapter presented a new protocol to quantify localized force production capability in multifidus muscle using ultrasound SWE in healthy individuals. The postures of the subjects were influential on the multifidus shear modulus. The highest multifidus shear modulus was obtained during the sitting up position with the right arm lifted. The change in median shear modulus from lying prone to sitting up with the right arm lifted was more pronounced compared to changes in the other postures. Superficial multifidus had higher shear modulus compared with the deeper layer in all three postures. Furthermore, during a single arm lifted, the shear modulus of the contralateral multifidus was higher. The superficial and deeper multifidus layers were found to have excellent within-day reliability. Comparison between our measurements and those from other studies suggest that ultrasound equipment and parameters may cause bias in the measured values of shear modulus. However, our measurements suggest a strong relationship between changes in shear modulus of multifidus and force production. Additionally, this study quantified localized lumbar multifidus muscle dysfunction after RFN and PLF procedures using ultrasound SWE. Overall, the proposed protocol using SWE has the potential of assessing individual spine muscles in response to post-surgical rehabilitation protocols.
Chapter 4
Quantifying Dysfunction of the Lumbar Multifidus Muscle after Radiofrequency Neurotomy and Fusion Surgery

The content of this Chapter has been submitted to the Journal of Orthopaedic Research Spine.
Multifidus muscles play an important role in active stabilization and movement of the spine. Surgical procedures such as posterior lumbar fusion (PLF) and radiofrequency neurotomy (RFN) cause injury to these muscles affecting their function. These iatrogenic injuries have been evaluated using electromyography, cross-sectional area, and intramuscular fat content. However, changes in the force production of the multifidus after RFA and PLF have not been studied. In this chapter, the localized lumbar multifidus muscle dysfunction after RFN and PLF procedures will be evaluated using ultrasound SWE during three different positions: lying prone, sitting up, and sitting up with the arms lifted. Finally, experiments and results of elastography measurement on PLF, RFN, and healthy matched controls will be presented and discussed.

4.1 Introduction

After the reliable collecting data in healthy participants, next step was to repeat the protocol in a group of patients with lower back pain, particularly the patients with the history of posterior lumbar fusion (PLF) or medial branch radiofrequency neurotomy (RFN). Chronic low back pain is one of the most common sources of disability in the middle-aged and elderly population with significant associated medical expenditures [114]. RFN and PLF and are common procedures for the treatment of different spine conditions. The lumbar facet joints are the primary source of pain in approximately 10–40% of chronic low back pain patients [115-117]. Common interventional treatment for pain caused by lumbar facet joints is medial RFN. Unfortunately, RFN also causes denervation of the multifidus muscle along with localized atrophy in multifidus muscle [20]. Since multifidus muscle is considered as one of the primary active stabilizers of the lumbar spine [80], multifidus dysfunction after RFN may have an impact on segmental stability, possibly affecting other structures of the spine like the intervertebral disc [21]. Similarly, muscle retraction during PLF causes a localized multifidus iatrogenic injury that results in atrophy [15] and reduced trunk strength [17]. Atrophy in paraspinal muscles has been associated with worse clinical outcomes after
PLF [18]. Therefore, evaluating multifidus dysfunction is important to understand and improve the long-term outcomes of these treatments.

RFN and PLF procedures have been shown to affect the multifidus muscle in different ways. In RFN, electrical pulse produced by radiofrequency waves damages the nerve tissue in order to disrupt the pain signals being transferred to the brain. However, it was shown that the damage to the lumbar spinal nerves leads to multifidus muscle atrophy [20]. Additionally, RFN may be effective for pain relief for up to 12 months, since coagulated nerve regeneration may occur after 12 months [22]. Therefore, multiple subsequent RFN may be needed to relieve the pain, causing the subsequent multifidus muscle atrophy [20].

Multifidus injury may also be caused by damage to the dorsal ramus of the segmental spinal nerve [3]. PLF is also a common surgical treatment for degenerative spinal conditions, such as traumatic instability. The surgery injures the paraspinal muscles, including the multifidus muscles [14]. The localized muscle injury causes muscle atrophy and increased intramuscular fat in the multifidus of patients with PLF surgery [15-19]. These results suggest that RFN and PLF procedures may influence multifidus muscle force production.

The objective of the second part of this chapter was quantify localized lumbar multifidus muscle force production after RFN and PLF procedures using ultrasound SWE. We hypothesized that the shear modulus in multifidus muscle after PLF and RFN procedures would be lower compared to healthy participants. Results from this preliminary study will provide the background information needed for the design of appropriate rehabilitative strategies and recovery procedures for improving multifidus muscle function in RFN and PLF patients.
4.2. Methods

4.2.1 Overview

The Institutional Review Board (IRB) of the Pennsylvania State University approved the study (STUDY00010509), and all participants gave informed consent before any data was collected. This study quantified multifidus shear modulus in three groups: 1) patients who have received RFN within the past two years, 2) patients who have received PLF within the past five years, and 3) age- and gender-matched healthy participants. RFN and PLF Participants were excluded if they had previous spinal surgeries other than RFN or PLF; had a history of scoliosis; were pregnant, or had a history of any neurological disease. Healthy participants were excluded if they had a history of low back surgery; had received services for LBP within the past six months; had difficulty performing the requested tasks; had experienced a recent traumatic event such as a motor vehicle collision; had a history of any neurological disease, or had a terminal illness.

A customized supersonic SWE method [43] was used to measure multifidus muscle shear modulus using a C5-2 transducer [2]. This method was validated on a commercially available homogeneous elasticity phantom (nominal shear wave speed = 2.94 m/s, Model 040GSE, CIRS, Norfolk, Virginia, USA) with the dimensions of $17.8 \text{ cm} \times 12.7 \text{ cm} \times 20.3 \text{ cm}$ [96]. This technique has also been successfully used in several previous studies [1, 97, 98].

4.2.2 Measurement procedure

Thirteen patients (six men and seven women) who have received RFN within two years before ultrasound evaluation were recruited (mean age ± SD, 61.15 ± 11.09 years). Thirteen healthy age- and gender-matched controls (six men and seven women) who met the study’s inclusion criteria were recruited to match the RFN group (mean age ± SD, 60.31 ± 9.00 years). Ten patients (six men and four women) who have received PLF within the past five years were recruited (mean age ± SD, 60.90 ± 11.08 years). Ten
healthy age- and gender-matched controls (six men and four women) who met the study’s inclusion criteria were recruited to match the PLF group (mean age ± SD, 60.80 ± 9.59 years). Participants were asked to fill out the pain catastrophizing scale (PCS) [118], modified Oswestry low back pain disability questionnaire (ODQ) [119] and visual analog scale (VAS) pain intensity rating questionnaire [120] to assess their pain intensity and perceived disability. The changes in shear modulus of the multifidus muscle were quantified in three body postures (i.e., prone, sitting up, and sitting up with the arms lifted).

Participants laid prone with the back muscles in a fully relaxed position (Figure 4-1 (a)). For consistency in measurements, the transducer was placed in long-axis just lateral to the spinous processes and angled medially (10-15 degrees), similar to that used in previous studies for the multifidus [12, 16, 109, 110]. The facet joint at the procedure level and adjacent multifidus muscle were identified, and the contact pressure of the transducer was minimized through the use of an adjustable fixture. For measurement of multifidus shear modulus during multifidus activation in sitting, each participant was first asked to sit up (Figure 4-1(b)). They were asked to sit upright in a normal resting posture, while their feet were not supported on the floor. The transducer was placed back on the skin. Then the participant was instructed to sit up with their arms horizontal to measure the multifidus shear modulus (Figure 4-1 (c)).

During SWE measurements, the investigator monitored the real-time brightness-mode (B-mode) ultrasound images to ensure that there was no noticeable body movement. Once the multifidus muscle was identified in the B-mode image (Figure 4-2), the elastography mode was then activated to measure the shear modulus of the muscle. An ROI with the 20 mm width, but variable height to cover the entire thickness of the multifidus muscle (from the muscle’s fascial line to 2 mm above the facet joint) was considered [2]. The first dark pixel before the muscle’s fascial line was chosen as the upper limit of the ROI. Figure 4-2 shows the representative B-mode image of the multifidus in an RFN, PLF, and matched healthy participant. The shear wave speed was measured five times in each round of measurements to calculate a robust median.
Figure 4-1. Experimental set up for the shear modulus measurement of multifidus muscle with the transducer located lateral to the spinous processes and angled medially at prone (a), sitting up (b), and sitting up with the arms lifted a horizontal position (c).

Figure 4-2. Representative B-mode ultrasound image of the multifidus in the prone position.

4.2.3 Statistical analysis

Shapiro–Wilks test was used to analyze the normality of data distribution. A linear mixed effects model was used for the statistical analysis, with shear modulus as a dependent variable and the body posture (lying
down, sitting up, and sitting up with the arms lifted) and group type (RFN patients and matched healthy controls) as fixed variables to find possible significant differences between multifidus shear modulus in RFN patients and matched healthy controls. A linear mixed effects model was used, with the body posture (lying down, sitting up, and sitting up with the arms lifted) and group type (PLF patients and matched healthy controls) as fixed variables to find possible significant differences between multifidus shear modulus in PLF patients and matched healthy controls, while the shear modulus was used as the dependent variable. Additionally, a linear mixed effects model followed by post-hoc Bonferroni correction was used to find possible significant differences between multifidus shear modulus in RFN and PLF patients, with shear modulus as a dependent variable and the body posture (lying down, sitting up, and sitting up with the arms lifted) and group type (RFN patients and PLF patients) as fixed variables. The Spearman's rank correlation coefficient was applied to analyze the correlation between the multifidus shear modulus and PCS score in the RFN and PLF group, between the multifidus shear modulus and ODQ score in the RFN and PLF group. For all analyses, the level of statistical significance was set at \( p < 0.05 \). All statistical analyses were performed using SPSS statistics software (v24, IBM, Chicago, IL, USA).

4.3 Results

The representative shear modulus maps of the multifidus muscle in healthy controls, RFN, and PLF patients in the sitting up with lifted arms position are shown in Figure 4-3. From the normality test, it was determined that the shear modulus data were not normally distributed, therefore data are presented as median (interquartile range). The shear modulus for the affected multifidus in the RFN patients for the prone, sitting up and lifted arms postures was 14.44 (6.62) kPa, 16.57 (9.59) kPa, and 20.07 (10.96) kPa, respectively; for the healthy controls, values were 18.55 (5.59) kPa, 27.14 (5.08) kPa, and 38.45 (16.71) kPa, respectively (Figure 4-4). The shear modulus for the affected multifidus in PLF patients for the prone, sitting up and lifted arms postures was 9.81 (3.75) kPa, 17.26 (8.68) kPa, and 21.85 (13.83) kPa,
respectively; for the healthy controls, values were 16.08 (11.85) kPa, 28.11 (3.87) kPa, and 37.26 (11.87) kPa, respectively (Figure 4-5). The shear modulus of the multifidus muscle in the RFN and PLF patients was significantly lower than that of in healthy controls (p < 0.01) in all three body postures, suggesting lower force production in RFN and PLF patients. There was a significant increase in shear modulus of the multifidus muscle in the PLF patients from prone to sitting up and from sitting up to the lifted arms posture (p < 0.01), while there was no significant difference in shear modulus of the multifidus muscle between different postures in the RFN patients (p = 0.13).

The total PCS score for the RFN and PLF group was 15.0 (13.0) and 13.5 (16.75), respectively (Figure 4-6(a)); the VAS score for the RFN and PLF group was 4.0 (2.85) and 5.55 (5.66), respectively (Figure 4-6 (b)); and the ODQ score for the RFN and PLF group was 42.0% (19%) and 28.0% (22.5%), respectively (Figure 4-6 (c)). Although there was no statistical difference in PCS, VAS score and ODQ score between RFN and PLF, the median ODQ score of the RFN group was in the severe disability range (41% - 60%), while the PLF group was in the moderate disability range (21% - 40%). There was not enough power to determine the possible correlations between multifidus function and patient-reported outcomes.

Figure 4-3. Representative shear modulus maps of the L4-5 multifidus muscle in the sitting up with the lifted arms position: PLF patient (a), RFN patient (b), and age- and gender-matched healthy participants (c) The lower shear modulus in the RFN and PLF patient compared to the healthy individual indicates multifidus dysfunction.
Figure 4-4. The shear modulus of the affected multifidus (median, interquartile range) increased from the prone to sitting up, and from sitting up to sitting up with lifted arms position in the RFN group and matched healthy controls.

Figure 4-5. The shear modulus of the affected multifidus (median, interquartile range) increased from the prone to sitting up, and from sitting up to sitting up with lifted arms position in the PLF group and matched healthy controls.
Figure 4-6. The patient reported outcomes in the PLF and RFN groups: (a) PCS score, (b) VAS score, and (c) ODQ score.

4.4 Discussion

This study evaluated localized lumbar multifidus muscle force production after RFN and PLF procedures using ultrasound SWE. Changes in shear modulus are essential because they are linearly related to force production. The multifidus shear modulus in the healthy participants was higher than those in the
PLF and RFN group. The change in median shear modulus from lying prone to sitting up and sitting up with the arms lifted was more pronounced in the healthy participants compared to changes in the other groups. During lifting arms in healthy controls, the higher shear modulus of the multifidus shows increased force production to keep the torso balanced. The multifidus muscle showed lower shear modulus in RFN and PLF group in all postures, suggesting a reduced muscle contraction after RFN and PLF procedures. The preliminary results in this study demonstrate that the new protocol is a useful tool in the assessment of force production and lumbar multifidi dysfunction after RFN and PLF procedures.

Few studies have explored the shear modulus of the multifidus muscle in a group of healthy individuals. Sadeghi et al. [2] evaluated the reliability of SWE for quantifying L4-5 multifidus shear modulus in 18 healthy individuals (mean age ± SD, 35.50 ± 18.06 years) at prone, sitting up and sitting up with the right arm postures, reporting shear modulus values of 16.15 (6.69), 27.28 (15.72) and 45.02 (25.27), respectively. These numbers are very similar to the multifidus shear modulus in matched healthy controls in this study (mean age ± SD, 60.31 ± 9.00 years). The similarity of the shear modulus between these two groups with different age range suggests that age may not have a significant effect in the multifidus muscle shear modulus. The similarity of muscle shear modulus between different age groups has been reported in other studies as well. For example, Akagi et al. [121] investigated the age-related differences between the shear moduli in the lower extremity of younger and elderly individuals, reporting no age-related differences between the soleus shear modulus in two groups. Dieterich et al. [100] reported a shear modulus of 14.9 kPa for the cervical spine multifidus during resting posture in prone, which is also very similar to our results in healthy individuals for the prone position. Additionally, they reported an increasing value of shear modulus as a function of loading of the neck. Our measurements also show an increase of shear modulus from prone to sitting up, and from sitting up to arms lifted, which were expected since those positions require different levels of muscle force production to maintain the stability of the torso. Alis et al. [102] evaluated the lumbar multifidus muscle in a relaxed prone position in patients with disc herniation. They reported a mean shear modulus values of the multifidus muscle on the affected side that was significantly
lower than the contralateral side. These values are higher than our values for patients with PLF and RFN. The differences may be because of the severity of nerve compression after disc herniation. The presence of lumbar disc herniation compresses the adjacent nerve root, causing a change in multifidus function depending on the compression severity. Alis et al. [102] reported a negative correlation between multifidus shear modulus and the severity of the nerve compression. Therefore, multifidus shear modulus may change differently after different spinal pathologies.

The changes in multifidus shear modulus in the PLF patients was different from that observed in RFN patients reflecting differences muscle functionality for two cases. A moderate increase in shear modulus from lying prone to sitting up and from sitting up to the sitting up with the lifted arms posture was observed in the PLF patients, while the shear modulus remained relatively constant at all three positions in the RFN patients. These results suggest that the force production of multifidus muscle slightly increases during multifidus activation in PLF patients, while it does not change in RFN patients. The difference may be explained by the different mechanisms involved in the PLF and RFN procedures affecting multifidus function. In the PLF, a direct injury to the multifidus due to the incision during surgery and retraction with long hours of excessive pressure [122] may contribute to the reduced functionality. On the other hand, the nerve damage caused by RFN seems to completely inhibit muscle contraction. These results suggest that SWE-based protocol used in this study can discriminate between different patterns and severity of multifidus dysfunction after injury or disease.

Quantifying multifidus force production may clarify possible relationships between multifidus dysfunction and spinal pathologies. For instance, it has been shown that there is an increased load on adjacent segments of an injured level after PLF procedure, causing adjacent segment disease (ASD) [123]. The etiology of ASD is not fully understood [124]. SWE can be used to evaluate the decreased multifidus force production affect the loading of adjacent intervertebral discs or facet joints. Wu et al. [125] conducted
a prospective case study in an individual with chronic low back pain to determine whether the lumbar multifidus muscle is polysegmentally innervated; showing that the medial branch nerve of the lumbar root innervates the multifidus muscle at multiple levels. Therefore, our protocol can be used to determine the extent of muscle dysfunction after RFN. The ability of SWE of evaluating function from individual muscles can be exploited to understand interactions between back muscles. For instance, Kong et al. [126] suggested that multifidus muscle dysfunction affects the normal functioning of other paraspinal muscles, causing spinal disorder. SWE can potentially evaluate compensation mechanisms after multifidus dysfunction. Our proposed protocol may also help clinicians evaluate the health status and monitor the recovery process of the muscle after interventions are applied to mitigate the severity of its dysfunction.

There are several limitations to this study. The first limitation includes a relatively small sample size. The underpowered correlation analysis might have contributed to the lack of significant correlation between the shear modulus results and pain symptom scores. However, the sample size did not compromise the linear mixed effects model results. Patients in this cross-sectional study underwent RFN or PLF at different time points over the last year, which may have influenced the variability of the data. Future studies focus on longitudinal studies evaluating multifidus function before and after the RFN/PLF procedure.

4.5 Conclusion

To summarize, this chapter quantified localized lumbar multifidus muscle dysfunction after RFN and PLF procedures using ultrasound SWE. The patterns of multifidus contraction were different between PLF and RFN patients. A moderate increase in multifidus shear modulus from lying prone to sitting up and from sitting up to sitting up with lifted arms posture was observed in PLF patients,
while the shear modulus remained relatively constant in RFN patients. The preliminary results in this study suggest that RFN and PLF cause reductions in the force production of multifidus muscles. Overall, the proposed protocol using SWE has the potential of assessing individual spine muscles in response to post-surgical rehabilitation protocols.
Chapter 5
Shear modulus of lower-leg muscles and intracompartmental pressure are correlated

The content of this Chapter has been published in the Journal of Biomechanics [1].
CECS is an exercise-induced condition, in which high pressure develops in one or several lower-leg compartments, resulting in pain, numbness, and difficulty moving the foot. Diagnosis of CECS is done through clinical evaluation and values of ICP measured via needle manometry before, 1-minute and 5-minutes after cessation of exercise (Pedowitz criteria). However, needle manometry is a painful procedure, and its accuracy depends on many parameters. The purpose of this study is to explore if SWE can potentially be used for the diagnosis of CECS. To this aim, first, we introduce an SWE-based protocol to evaluate the effect of variation of ICP on the lower leg compartment stiffness. First, an introduction about ICP and developed models for ICP measurement will be provided, followed by the Methods section, which gives the details of the designed protocol and SWE parameters. Experiments and results of elastography measurement on healthy individuals are presented, followed by a discussion of the results. The rationale for this study is that changes in shear modulus due to the variation of ICP support our hypothesis about the shear modulus changes after running exercise in CECS patients since CECS imposes increased ICP due to low muscle fascia compliance.

5.1 Introduction

Blood pressure is the amount of force created inside the arteries and veins. This force varies between a maximum and minimum value during the cardiac cycle. Elevated ICP occurs due to the variation of internal force in the tissue, which may change muscle mechanical properties, particularly the ones near arteries. Therefore, muscle mechanical properties can potentially be used as biomarkers for ICP status. The objective of the first part of this chapter is to quantify changes in lower-leg stiffness induced by variation of ICP using ultrasound SWE in healthy individuals. Changes in lower-leg compartment stiffness due to variation of ICP strengthen our hypothesis about the shear modulus changes after running exercise in CS patients.
Models of abnormally increased ICP, including changes in elevation of the limb and the use of tourniquets, have been used in several previous studies. Ashton [127] evaluated the effect of employing inflatable plastic splints on the blood flow of the arms and legs at various pressures in a healthy group. He reported inflation of a splint to 40 mmHg caused a considerable reduction in blood flow in the limb, while inflation to 30 mmHg caused a similar but less pronounced reduction in blood flow. Moreover, he argued that the combination of leg elevation and splint-inflation further reduce blood flow [128]. Matsen et al. [129] suggested measuring nerve-conduction velocity and muscle action potential amplitude instead of interstitial compartment pressure to reflect the physiological status of intracompartmental tissues. Wiger et al. [130] proposed a model employing vein stasis of one leg in a plaster cast and external compression of the contralateral leg to investigate the effects of increased ICP on nerve and muscle function in the leg and foot in a group of healthy controls. They reported that although the function of the extensor digitorum and hallucis brevis muscles after increasing ICP remained normal on both legs, impaired function of the Tibialis Anterior (TA) muscle was induced by elevation of a vein obstructed leg in a plaster cast. Zhang et al. [131] evaluated the effect of limb elevation and increased ICP on TA muscle blood flow using photoplethysmography and a custom-designed probe before, during, and after vein obstruction. They increased ICP to 60-65 mmHg by employing a tourniquet on both legs and elevating one leg, reporting that limb elevation combined with venous stasis reduced perfusion pressure and induced muscular weakness and dysfunction. These studies suggest that muscle function may change after an increase in ICP.

SWE can potentially help to quantify changes in the leg muscles’ stiffness in healthy individuals due to the variation in ICP, but it has not received significant attention in ultrasound SWE and, in general, in most diagnostic imaging modalities yet. Rominger et al. [132] evaluated the use of MRI for diagnosis and therapy management of compartment syndromes in a case-control study, reporting that MRI is unable to differentiate between the edema of affected muscles with high ICP and that of soft-tissue injury after trauma. Recently, Chaudhry et al. [133] investigated the effect of ICP contrast on ultrasound axial strain and axial shear strain imaging using finite element analysis. They showed that an underlying contrast in ICP creates a new contrast mechanism in elastographic images, which might be important for an improved
interpretation of elastographic images of tumors. Their study, however, was limited to the simulation environment and was not validated experimentally. Therefore, there is a need for clinical tools to quantify local changes in muscles’ function as a function of ICP.

The objective of this study was to quantify changes in lower-leg stiffness induced by variation of ICP using ultrasound SWE in healthy individuals. The proposed protocol quantified the TA and Peroneus Longus (PL) muscle stiffness at different levels of ICP. The ICP of TA muscle was increased using a blood pressure cuff around the thigh and an invasive blood pressure monitor. SWE was also performed at each pressure point, and when one of the legs was elevated. We hypothesized that TA and PL shear modulus would increase from the elevated leg to the lying supine posture. We also hypothesized that the increasing ICP using blood pressure cuff would increase muscle stiffness, which could be detected using ultrasound SWE. These results may represent the first step toward the acknowledgment of the important role that ICP may have in SWE results and in general, assist in the interpretation of ultrasound SWE elastograms obtained in tumor-related applications.

5.2 Methods

5.2.1 Overview

The IRB of the Pennsylvania State University approved the study (STUDY00007849) all subjects gave informed consent before any evaluation. The proposed protocol evaluates changes of the shear modulus of lower leg compartments at different levels of ICP using pressure cuff inflation and leg elevation. Participants were excluded if they ever had cardiovascular conditions; easily bruised; had diagnosed with an injury in their leg muscles; had a history of any neurological disease, or had a terminal illness.
5.2.2 Shear Wave Elastography System

A Verasonic ultrasound system with a linear L7-4 transducer (128 elements, beamwidth = 4-7 MHz, center frequency = 5.2080 MHz) was used in this study. A developed SSI code for L7-4 transducer in Chapter 2 was used to measure muscle shear modulus. Sixty-four central elements of the transducer emitted focused ultrasound pushing beam (500 push cycles at 5.2080 MHz frequency; push duration = 96 µs) at seven focal points at different depths. The propagation speed of shear wave was calculated within the ROI using a frame rate of 10,000/s. The ROI size of 7.39 mm * 7.39 mm was chosen for measurement of shear modulus in compartments. Finally, the corresponding shear modulus map could be constructed based on the obtained shear wave speed.

5.2.3 Proposed Protocol to quantify changes in shear modulus at different blood pressure

Nineteen healthy Participants (Mean age ± SD, 23.84±6.64; Mean BMI ± SD, 23.00±2.89) were recruited in this portion of the study. Participants were asked to rest for 15 minutes, and ultrasound images of the TA muscle were taken before applying a blood pressure cuff at resting supine position. Subjects laid supine with the hip and knee in full extension, and the ankle was resting on a fixture made of foam to constraint the ankle movement. An initial measurement was performed by placing the transducer in a longitudinal view along the length of muscle fibers, minimizing contact pressure through the use of an adjustable fixture. Scan images were taken at a point 30% of the distance from the head of the fibula to the tip of the lateral malleolus. The location of the ultrasound probe was marked on the skin for accurate placement of the probe after running. The ICP of TA muscle was increased using a blood pressure cuff around the thigh to 40, 80, and 120 mmHg, respectively (Fig. 5-1 (a)). After keeping the thigh pressurized for 2 minutes at each pressure point, and ensuring changes in lower-leg muscle pressure, ultrasound images were then taken. Besides, the effect of leg elevation on TA muscle stiffness was investigated (Fig. 5-1 (b)). One of the legs was elevated using a leg holder, and the knee and hip joints were flexed to 90°. The leg was
kept elevated for five minutes to stabilize the pressure inside the muscle, and then the elastography was carried out. In order to increase accuracy, the muscle location was marked in all positions. The same protocol was also applied on PL muscle at different pressure levels to evaluate the change in shear modulus of the lateral compartment. Further details regarding the SWE-based protocol for evaluating lower leg function is provided in Appendix C.

Figure 5-1. The experimental setup for elastography while (a) TA muscle is pressurized with the pressure cuff around thigh (b) one leg is elevated (c) ICP measurement inside TA muscle is performed.

Additionally, in order to measure the ICP corresponding to each cuff pressure level applied around the thigh, the invasive blood pressure system was employed (Fig. 5-1(c)). The invasive blood pressure system was comprised of a pressure transducer kit, which captures the amount of produced pressure within the TA muscle induced by blood pressure cuff. The amount of pressure in muscle was monitored in the patient monitor. An experienced clinician conducted insertion of the catheter on the leg to measure and control the blood measure inside TA muscle under local anesthesia on the skin. The catheter, which itself is plastic, was inserted into the skin using a 22-gauge needle inside of the catheter. This depth was approximately 3 cm inside the compartment of the muscle, indicating the short distance penetration of catheter into the muscle when inserted. The thigh muscle was pressurized using pressure cuff, and it was kept for 2 minutes at 0, 40, 80, and 120 mmHg, respectively to ensure changes in lower-leg muscle pressure. The ICP of TA muscle was then recorded at each pressure point, respectively.
5.3 Results

Data are presented as median (interquartile range) for TA and PL muscles due to Shapiro–Wilk test results (p 0.03 and <0.01 respectively). ICP increased as a function of cuff pressure for the TA muscle (Figure 5-2). The results of the linear mixed effects model indicated that cuff pressure has a significant effect on the ICP of TA muscle (p < 0.01). Additionally, from the Bonferroni post-hoc analysis, a significant difference was obtained between the ICP at 0 and 120 mmHg cuff pressure (Table 5-1). ICP returned to the normal value (zero cuff pressure) within five minutes after the release of cuff pressure in all participants.

Table 5-1. Post hoc Bonferroni test results for ICP (mmHg) between different pressure cuff conditions.

<table>
<thead>
<tr>
<th>Pressure cuff (mmHg)</th>
<th>Mean difference</th>
<th>Standard deviation</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
</tr>
<tr>
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<tr>
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</tbody>
</table>

The results of changes in shear modulus for TA and PL muscles at four levels of ICP with respect to shear modulus at zero pressure level are shown in Figure 5-3 and 5-4. The shear modulus values (median (interquartile range)) for the TA muscle at elevated leg, 0 mmHg, 40 mmHg, 80 mmHg and 120 mmHg cuff pressure were 13.77 (3.94) kPa, 15.83 (2.46) kPa, 17.80 (4.52) kPa, 19.79 (4.65) kPa and 21.88 (4.33) kPa respectively; for the PL muscle, values were 8.21 (1.73) kPa, 9.64 (1.97) kPa, 10.32 (1.41) kPa, 12.30 (5.83) kPa and 12.88 (5.99) kPa respectively, suggesting increased shear modulus with increased ICP level. The change in shear modulus (relative to the shear modulus at zero pressure) for TA and PL muscles as a function of ICP are shown in Figure 5-5. Specifically, the change in shear modulus (median and interquartile range) at 40 mmHg, 80 mmHg and 120 mmHg cuff pressure was 1.22 (2.09) kPa, 3.02 (4.94) kPa, and 5.10 (5.02) kPa, respectively, for the TA muscle; and 1.12 (1.64) kPa, 3.02 (3.74) kPa and 3.22 (4.96) kPa for
the PL muscle. Figure 5-6 shows representative shear modulus maps of the TA and PL muscles at each cuff pressure level. From the linear mixed effects model, it was observed that muscle and cuff pressure had a significant effect on muscle shear modulus (P < 0.01 and P < 0.01, respectively), while gender did not. Additionally, from the Bonferroni post-hoc analysis, the shear modulus was significantly different between the 0 cuff pressure and the cuff pressure at both 80 and 120 mmHg. Significant differences for shear modulus were also found between cuff pressure at 40 mmHg and cuff pressure at both 80 and 120 mmHg (Table 5-2). Additionally, the shear modulus for both the TA and PL muscles were found to increase as a function of ICP (Figure 5-7 and 5-8). Specifically, there were significant positive correlations found between the median shear modulus and ICP for the TA muscle (rho = 0.99, p < 0.01), and the PL muscle (rho = 0.99, p < 0.01).

Table 5-2. Post hoc Bonferroni test results for shear modulus (kPa) between different pressure cuff conditions.

<table>
<thead>
<tr>
<th>Pressure cuff (mmHg)</th>
<th>Mean difference</th>
<th>Standard deviation</th>
<th>95% CI</th>
<th>Significance</th>
</tr>
</thead>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Lower bound</td>
<td>Upper bound</td>
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<td>40-120</td>
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<td>3.78</td>
<td>-6.20</td>
<td>-1.57</td>
</tr>
</tbody>
</table>
Figure 5-2. ICP measured in the TA muscle increased as a function of the cuff pressure (p < 0.01) (median, interquartile range).

Figure 5-3. Change in shear modulus (kPa) of TA muscle (median, interquartile range) at different levels of blood pressure (values at each pressure level represent changes with respect to shear modulus at zero pressure level).
Figure 5-4. Change in shear modulus (kPa) of PL muscle (median, interquartile range) at different levels of blood pressure (values at each pressure level represent changes with respect to shear modulus at zero pressure level).

Figure 5-5. The change in shear modulus of TA and PL muscles as a function of cuff pressure (values at each cuff pressure level represent changes with respect to shear modulus at zero cuff pressure) (median, interquartile range).
Figure 5-6. The shear modulus maps of the TA muscle (top) and PL muscle (bottom) at each cuff pressure level: (a) 0 (b) 40 mmHg (c) 80 mm Hg and (d) 120 mmHg.

Figure 5-7. The shear modulus of TA increased as a function of ICP (median, interquartile range) (p < 0.01). The horizontal bars represent the interquartile range in ICP, and the vertical bars represent the interquartile range of shear modulus.
Figure 5-8. The shear modulus of PL increased as a function of ICP (median, interquartile range) (p < 0.01). The horizontal bars represent the interquartile range in ICP, and the vertical bars represent the interquartile range of shear modulus.

5.4 Discussion

This study quantified changes in lower-leg stiffness induced by variation of ICP using ultrasound SWE in healthy individuals. The proposed protocol consisted of measuring shear modulus using SWE for the TA and PL muscles at different levels of ICP, including elevation of one leg and using pressure cuff inflation around the thigh at 40mmHg, 80 mmHg and 120 mmHg. It was found that elevated leg ICP has a significant effect on the TA and PL muscles’ stiffness. It was observed that the TA and PL shear modulus was highest while a cuff pressure of 120 mmHg was applied around the thigh, while it was lowest when the leg was elevated. Additionally, the change in median shear modulus from zero to the 120 mmHg pressure point was more pronounced compared to changes in the other pressure points for the TA and PL muscles. Overall, SWE could effectively measure the mechanical properties of lower leg muscles at various ICP levels.
Therefore, SWE could be a potential candidate for evaluating physiological changes of muscles at different levels of ICP.

Few studies have explored the effect of ICP on muscle function in vivo and ex-vivo. Weaver et al. [134] studied the effect of ICP on the shear modulus of the porcine brain using magnetic resonance elastography (MRE). They acquired an MRE sequence on the pre-mortem brain of farm-strain Yorkshire swine, reporting that the pre-mortem shear modulus was, on average, 1.43 times greater than the post-mortem values. They attributed the significant reduction of shear modulus primarily to the decrease in ICP. Wiger et al. [130] developed the model employing vein stasis of one leg in a plaster cast and external compression of the contralateral leg to study the relationship among increased ICP, muscle blood flow, and muscle function. The ICP in the test leg (vein stasis model) was 5.4 mmHg at resting position, while it was 5.2 mmHg in the contralateral leg. After limb elevation and applying a plaster cast and tourniquet on the legs, the ICP increased to 38 mmHg and 39.3 mmHg in the test leg and contralateral leg, respectively. They reported that function of the extensor digitorum and hallucis brevis muscles remained normal after increasing ICP on both legs, but an impaired function of the TA muscle was induced by elevation of a vein obstructed leg in a plaster cast. Zhang et al. [131] investigated the effects of limb elevation and increased ICP on blood flow in the TA muscle and leg neuromuscular function in healthy controls. Muscle blood flow (MBF) was measured by photoplethysmography using a custom-designed probe. They increased ICP bilaterally by vein obstruction (60-65 mmHg) lasting 30 min, induced by a thigh tourniquet of casted legs. ICP increased to 40 mmHg in the vein-obstructed casted legs. Perfusion pressure decreased from a mean of 65 (SD 9.9) mmHg to 43 (SD 8.4) mmHg in the non-elevated leg and from 42 (SD 5.8) mmHg to 17 (SD 6.4) mmHg in the elevated leg, while MBF decreased by 50% in the elevated leg and by 42% in the non-elevated leg. Their results are concordant with our findings, confirming the hypothesis that applying pressure cuff inflation around the thigh decreases the blood flow out of the limb, causing the TA and PL muscles to become stiffer due to the accumulated blood in the lower limb. Overall, a comprehensive comparison of our protocol with similar protocol models for measurement of muscle shear modulus was not possible. To
our knowledge, this is the first study to provide information about the effect of ICP on shear modulus of lower leg muscles using SWE.

The change in ICP may be caused by several mechanisms. The immediate increase in ICP was postulated to a linear increase of contraction force in skeletal muscles [135, 136]. Since most muscles are tightly packed in compartments and fascia cannot expand, the increase in pressure within the compartment limits blood flow to the muscles. These reversible deformations of muscle architecture may cause additional transverse forces (perpendicular to the line of action) in between contracting muscles or between muscles and surrounding tissues, leading to variations in the ICP [135]. Reinhardt et al. [135] examined the relationship between ICP and muscle force generated during isometric contractions (i.e., at a constant muscle-tendon unit length) in synergistic muscles of New Zealand rabbits and reported that ICP changes with muscle length. Other explanations for the change in ICP of lower leg muscles have been associated with a change in the stiffness of soft tissue around blood vessels, changing direct pressure on blood vessels [137]. When the pressure inside the compartment is elevated, blood flow through the capillaries slows down. If the blood flow to the compartment is limited enough, the tissue will begin to swell and develop edema in the limb due to the accumulation of fluids. Moreover, blood circulation insufficiency causes hypoxic conditions in tissues, the production, and release of algesic substances, and tissue fibrosis, leading to pain and muscle spasms [137]. Detection of this progression and promoting tissue oxygenation are clinically crucial for improvements of the tissue and muscle recovery [138-140]. Therefore, using a sensitive and specific biomarker for diagnosing the variation of ICP in musculoskeletal tissues would be of crucial importance.

Our proposed protocol using SWE offers several potential applications for practitioners. First, this is a non-invasive method, which is capable of quantifying ICP without needle injection for tumor-related applications. Most malignant tumors have been shown to have elevated interstitial fluid pressure (IFP) with respect to healthy tissue [133]. This has been the case for lung carcinoma [141], breast carcinoma [142], carcinoma of the cervix [143, 144] and colon-rectal carcinoma [145], indicating the fundamental role of ICP in the response of cancers to treatments. However, the effects of ICP on the ultrasound SWE parameters
have not received significant attention. Therefore, the results of this study may help clinicians better interpret ultrasound elastogram obtained in tumor-related applications and monitor pathologies associated with increased ICP. In addition, SWE may also be beneficial for studying the effect of elevated ICP on nerve function and different types of muscular activities. This protocol also enables a better characterization of the mechanical behavior of muscles as a function of ICP using bi-phasic models. Another potential application of SWE is that it may help clinicians diagnose or monitor the recovery process of Compartment Syndrome (CS) disease, while other conventional methods, such as needle manometry, have some limitations for diagnosing CS. For example, needle manometry directly measures ICP by inserting a needle into the affected compartment, which is an invasive and painful method of diagnosis and suffers from significant variability depending on the depth of needle insertion, amount of fluid introduced, and soft tissue occlusion of the needle [26]. The blood pressure cuff inflation around the thigh may simulate CS with reversible neuromuscular dysfunction. Therefore, the results of this study may be beneficial for practitioners to consider the contribution of stiffness on the diagnosis of CS disease using ultrasound SWE, which could provide better insight into compartment function at different levels of blood pressure. Further studies are warranted to validate the findings of this study in an attempt to explore the feasibility of ultrasound SWE as a diagnostic tool for chronic CS.

There are some limitations to this study. First, the majority of the subjects recruited were under age 30. Progressive muscular weakness linked to a shrinking of muscle mass and change in muscle cross-section is reported in the elderly [146]. Denervation of motor units also occurs with aging, which can transition type II muscle fibers into type I fibers [146]. Hence, these physiological changes and denervation of motor units with aging may be factors affecting muscle function and ICP. Another limitation is that the effect of pennation angle on the reliability of the results was not evaluated. Our future studies will investigate these effects on the shear modulus of the lower leg muscles at different ICP levels.
5.5 Conclusion

In conclusion, this paper has described a protocol to quantify changes in lower-leg compartment stiffness induced by variation of ICP using ultrasound SWE in healthy individuals. The ICP level was influential on the TA and PL shear modulus. The lowest TA and PL shear modulus were obtained during the leg elevated position. The change in median shear modulus from zero to the 120 mmHg pressure point was more pronounced compared to changes in the other pressure points for TA and PL muscles. Evaluating lower leg shear modulus at various pressure levels could also be extended to other musculoskeletal muscles to evaluate muscle's function elicited by different models of elevated ICP. Overall, the preliminary results presented in this study suggest that SWE has the potential to quantify shear modulus changes of muscles under various blood pressure levels and help provide the background information needed to more accurately interpret the clinical results.
Chapter 6
Feasibility study of Shear Wave Elastography for Compartment Syndrome

The content of this Chapter has been published in the Journal of Engineering and Science in Medical Diagnostics and Therapy [3].
In this chapter, we will quantify temporal changes in shear modulus of muscle in lower-leg compartments of healthy individuals before and after running exercise, and evaluate a Pedowitz-like criterion for the diagnosis of CECS using muscle shear modulus as a biomarker. An introduction about CECS and current assessment tools of CECS will be provided, followed by the Methods section, which gives the details of the designed protocol and SWE parameters. Experiments and results of elastography measurement on healthy individuals are presented, followed by a discussion of the results. Finally, the results of the proposed SWE-based protocol on three patients with a history of CECS will be presented to evaluate the change in shear modulus of their lower leg after cessation of running.

6.1 Change in shear modulus of healthy lower legs after treadmill running

It was found that SWE could help to quantify changes in the leg muscles' shear modulus due to the variation in ICP. The obtained results imply that SWE may be a reliable tool to quantify shear modulus changes at different levels of ICP induced by running exercise. The results from this study represent the first step towards the development of a non-invasive protocol for the diagnosis of CS.

As previously mentioned, CS occurs when excessive pressure develops inside an enclosed tissue (compartment) in the body, such as lower-leg muscle compartments. CECS occurs in 14-27% of those with chronic anterior lower-leg pain [23], in the anterior and lateral compartments bilaterally in 95% of cases [24-27]. CECS imposes decreased tissue perfusion and increased intracompartmental pressure (ICP) due to exercise effects and low muscle fascia compliance [28, 29]. Current diagnostic methods of CECS rely on clinical evaluation assisted by ICP measurement through needle manometry [27]. However, needle manometry is an invasive procedure with high false-positive rates [30]. We showed a positive linear correlation between shear modulus and ICP (Spearman's correlation coefficient = 0.99) measured via SWE [1]. Therefore, shear modulus might be used as a non-invasive measurement of ICP.
Needle manometry directly measures ICP by inserting a needle into the affected area and recording the pressure in the tissue. The most commonly used method to diagnose CECS in the lower-leg is modified Pedowitz criteria. A pre-exercise ICP of 15 mmHg or above, a 1-minute post-exercise ICP of 30 mmHg or above, and a 5-minute post-exercise ICP of 20 mmHg or above is used for diagnosis of CECS [26, 32]. However, there is still noticeable disagreement over which value should be used as a threshold pressure for the diagnosis of CS. Needle manometry has shown to have considerable variability depending upon the depth of needle, soft tissue occlusion of the needle and amount of fluid injected. Therefore, there is a need for more reliable methods for diagnosis of CECS.

Assessment of lower-leg CECS has also been studied in both research and clinical practice through other methods, including near-infrared spectroscopy, magnetic resonance imaging (MRI), and ultrasound imaging. There are, however, several inherent limitations to these techniques. Although several recent studies have shown that near-infrared spectroscopy is capable of demonstrating a significant inverse correlation between ICP and oxyhemoglobin level, it has limitations in measuring the saturation of oxygen in deep compartments. Additionally, the instrumentation required for near-infrared spectroscopy is relatively expensive [36-38]. MRI also provides increased T2 signal intensity with increased anterior compartment pressure [39-41]. However, it is not widely used due to its cost. The ultrasound imaging technique has been employed for evaluating the changes in muscle thickness, pennation angle, and muscle fascicle length during static and dynamic contractions. Wiemann et al. [147] employed a non-invasive ultrasound device to correlate ICP with a fascial displacement waveform in acute CS patients. However, the range of ICPs in the model was narrow (10-40 mmHg), which may be lower than the ICPs in some CS patients. Garabekyan et al. [148] used a novel digital ultrasound device to measure fascial displacement in a porcine model of acute CS over a broader range of ICPs, reporting increased fascial displacement in a compartment with elevated ICP relative to a contralateral compartment with standard ICP. However, since the porcine skin was considerably thicker than human, higher power settings were applied, causing it
difficult to distinguish the fascia and surrounding tissues. Birtles et al. [149] evaluated the effect of eccentric and isometric exercises on the change in the TA muscle thickness in patients with CECS and healthy individuals using ultrasound, reporting no significant differences in TA muscle thickness between two groups. Several methods have been used to measure blood perfusion in muscles. Although Doppler ultrasound has been used to quantify flow in relatively large blood vessels supplying blood to the muscles, it is not typically used to evaluate perfusion inside the muscle [150].

Ultrasound SWE is a reliable imaging technique that can evaluate several muscle properties by quantifying changes in the shear modulus [43-45]. Brandenburg et al. [151] investigated the reliability of SWE by measuring passive bilateral lateral gastrocnemius muscle shear modulus in children, reporting a good to excellent reliability (mean [95% confidence interval]) range of reliability, 0.67 [0.44-0.83] to 0.80 [0.63-0.90]) when probe was placed on the skin with minimal pressure. Lacourpaille et al. [152] used SWE to evaluate the reliability of shear modulus in lower leg muscles, reporting excellent reliability (Intraclass Correlation Coefficient (ICC) = 0.871 ± 0.045 for the day to day reliability and ICC = 0.815 ± 0.065 for the inter-day reliability). Sadeghi et al. [1] quantified the relationship between ICP and shear modulus within the lower-leg muscles by inflating pressure cuff around the thigh at 40mmHg, 80 mmHg, and 120 mmHg in healthy individuals, reporting that the shear modulus for both the TA and PL muscles increase with increasing ICP. There were significant positive correlations found between the ICP and median shear modulus for the TA and PL muscles (Spearman's correlation coefficient = 0.99 and 0.99, respectively). These results demonstrate that changes in muscle shear modulus are correlated to intramuscular pressure. Therefore, these studies suggest that SWE can potentially overcome the limitations of reliability associated with needle manometry and improve the diagnosis of CECS.
The objective of this study was to quantify changes in shear modulus of muscles in lower-leg compartments of healthy individuals before and after a treadmill running. Specifically, we investigated the minute-by-minute changes in the shear modulus of TA and PL muscle after exercise. Additionally, the shear modulus was measured in all lower-leg compartments bilaterally in a procedure that resembles the ICP measurements associated with the Pedowitz criteria. The results from this study may show the feasibility of the design of a Pedowitz-like criterion for non-invasive diagnosis of CECS. We hypothesized that the increased compartment pressure, produced by treadmill running, would result in a temporary increase of shear modulus of the muscles consistent with changes of ICP observed in healthy individuals. The results from this study represent the first step towards the development of a non-invasive protocol for the diagnosis of CS.

6.2 Methods

The Institutional Review Board of the Pennsylvania State University approved the study (STUDY00005848). All participants signed the informed consent and filled out the Physical Activity Readiness Questionnaire form before any evaluation. Participants were excluded if they had a history of cardiovascular conditions; any injury in their leg muscles; or a history of any neurological disease; were pregnant or not adult or had a terminal illness. The proposed protocol evaluates changes in shear modulus of the lower-leg compartments after a running exercise on a treadmill. This study was divided into two parts: 1) quantifying temporal changes in TA and PL muscle shear modulus after the running exercise using SWE; and 2) quantifying changes in shear modulus of all lower-leg compartments 1- and 5-minutes post-cessation of exercise in a procedure that resembles that of ICP measurements to evaluate the Pedowitz criteria.
6.2.1 Protocol to quantify temporal changes in shear modulus after running

Sixteen healthy volunteers (Mean age ± SD, 21.5±2.8; Mean BMI ± SD, 22.58±2.99) were recruited. Additionally, in a case study, two patients with a history of compartment syndrome (Age = 19 and 21, and BMI= 21.66 and 23.04) were also recruited. Subjects were asked to rest for 15 minutes before testing. Elastography of TA muscle was performed before and after the running exercise. Participants laid supine with the knee and hip in full extension with the ankle resting on a fixture made of foam to constrain ankle movement (Figure 6-1). Subjects were instructed to remain relaxed during the shear modulus measurement. A baseline level for TA and PL shear modulus was obtained by an initial elastography measurement parallel to the muscle fibers, using an adjustable fixture to minimize contact pressure. Scan images of the TA muscle were taken at a point 30% of the distance from the tip of the lateral malleolus to the head of the fibula. The ultrasound probe location was marked on the skin for accurate placement of the probe after running. When the pennation of the fibers was identifiable on the screen, the elastography measurements were conducted to measure the muscle shear modulus. The depth of measurement from the transducer surface to the bottom of the ROI was 3 cm. Participants were then asked to run for 10 minutes on the treadmill with the speed of 6.5~7 miles per hour. Ultrasound elastography measurements were performed at the same location in the muscle immediately after running (within ten seconds of cessation of exercise) and repeated every minute for ten minutes. After ten minutes of resting, the same protocol was repeated for the PL muscle to quantify the shear modulus changes of the lateral compartment. A one-way repeated measures ANOVA was employed using SPSS statistics software (v24, IBM, Chicago, IL, USA) to evaluate the effect of time on the shear modulus values of TA and PL muscles. Significance was considered at $p < 0.05$ for all analyses. Additionally, the shear modulus changes relative to the shear modulus values at pre-running for TA and PL muscle were represented using cumulative distribution function (CDF) curve fitting of a Weibull distribution expressed by Eq. (1)

$$1 - F(x) = ce^{-\left(\frac{x}{\alpha}\right)^\beta}$$

where $c$, $\alpha$, and $\beta$ are the amplitude, scale, and shape parameters, respectively.
6.2.2 Protocol to quantify changes in shear modulus of all compartments

In order to quantify the shear modulus of four compartments of both legs after running, a similar protocol was employed. Nineteen healthy volunteers (Mean age ± SD, 23.33±4.53; Mean BMI ± SD, 22.83±3.07) were recruited for this purpose. Subjects were asked to rest for 15 minutes before testing and elastography of four compartments, including the TA, PL, Soleus and Tibialis posterior muscles, were performed bilaterally before running. Subjects were then asked to run for 10 minutes on the treadmill with the speed of ~7 miles per hour. The measurement of shear modulus was conducted on four compartments bilaterally 1- and 5-minutes after running, respectively. The order of shear modulus measurement at each time point was chosen as follows: TA, PL, Soleus, and Tibialis posterior muscles, respectively. In this protocol, the probe was hand-held instead of using an adjustable fixture. For the statistical analyses, a one-way repeated measures ANOVA followed by post hoc Bonferroni correction was applied on each muscle to investigate the effect of time on the shear modulus values of all compartments. Significance was considered at $p < 0.05$ for all analyses.
6.3 Results

The representative shear modulus maps of the TA and PL muscles at pre-running, immediately and 5 minutes after cessation of exercise is shown in Figure 6-2. The average of the shear modulus of the TA and PL muscles in healthy individuals increased from 14.12 ± 2.11 kPa and 8.56 ± 1.71 kPa before running, to the peak value of 17.75 ± 5.58 kPa and 11.81 ± 3.95 kPa 1-minute after running, respectively (Figure 6-3). Shear modulus returned to pre-running values about 5-minutes after the cessation of exercise. The results of repeated measured ANOVA indicated that time had a significant effect on PL (p < 0.01) and TA (p < 0.01) muscles. The amplitude, scale and shape parameters of the modified CDF of the Weibull distribution for the average of shear modulus changes of TA muscle were 4.50, 5.47 and 0.89; for the PL muscle were 3.98, 7.24 and 1.08 (Figure 6-4). The median, calculated from the Weibull parameters, was 3.6 and 5.1 minutes for the TA and PL muscles. The median represents the time after treadmill running for a decrease of 50% of the initial change in shear modulus. Therefore, it can be considered as the ‘transition time’ between high and low values of shear modulus. The average shear modulus values and standard deviations in this study will serve as a shear modulus range for healthy individuals.
Figure 6-2. The shear modulus maps of the (top) TA and (bottom) PL muscles at pre-running (left) immediately (center), and 5 minutes after cessation of exercise (right).
Figure 6-3. The change in shear modulus for the (top) TA and (bottom) PL muscles (mean + standard deviation) over time in healthy individuals shows an initial increase of shear modulus after treadmill running followed by gradual decrease back to pre-exercise values.
Figure 6-4. The curve fitting of the Weibull cumulative distribution function to the average change in shear modulus for the (top) TA and (bottom) PL muscles over time after cessation of treadmill running. The median, calculated from the Weibull parameters, was 3.6 and 5.1 minutes for the TA and PL muscles, representing the time needed for a decrease of 50% of the change in shear modulus after exercise.

The changes in shear modulus of four compartments of both legs using the Pedowitz-like protocol are shown in Figure 6-5. There was a noticeable increase from the pre-running values to immediately after cessation values for all compartments. The shear modulus of all compartments in both legs returned to approximate pre-running values 5-minutes after the cessation of exercise. The results of repeated measured ANOVA indicated that time had a significant effect on TA (p < 0.01), PL (p = 0.01), Soleus (p < 0.01) and
Tibialis posterior (p = 0.01) muscles. Additionally, the Bonferroni post-hoc analysis performed on individual muscles showed a significant difference in shear modulus between the pre-running and immediately after cessation of running time points for the TA (p = 0.02), PL (p = 0.03), Soleus (p < 0.01) and Tibialis posterior (p = 0.02), with no meaningful differences between pre-running and five minutes post-cessation of exercise time points.

Figure 6-5. Results of a Pedowitz-like protocol using the shear modulus (kPa) measured before, immediately and 5 minutes after cessation of exercise show that shear modulus can be used a surrogate measurement of intra-compartment pressure (median and interquartile range).

In the case studies, the average of the shear modulus of the TA and PL muscles for the first CECS patient increased from 10.69 kPa and 8.14 kPa before running, to the peak value of 14.44 kPa and 11.83 kPa after running, respectively (Figure 6-6). The average of the shear modulus of the TA and PL muscles for the second CECS patient increased from 11.41 kPa and 6.82 kPa before running, to the peak value of 22.56 kPa and 19.89 kPa 1-minute after running, respectively (Figure 6-7). Shear modulus returned to pre-running values about 11 to 15 minutes after the cessation of exercise, showing the difference between shear modulus changes after cessation of running in CECS patients and healthy individuals.
Figure 6-6. The change in shear modulus for the (top) TA and (bottom) PL muscles in the first CECS patient over time shows an initial increase of shear modulus after treadmill running followed by gradual decrease back to pre-exercise values.
Figure 6-7. The change in shear modulus for the (top) TA and (bottom) PL muscles in the second CECS patient over time shows an initial increase of shear modulus after treadmill running followed by gradual decrease back to pre-exercise values.
6.4 Discussion

Changes in lower-leg compartments shear modulus after a treadmill running were measured using SWE in healthy individuals. A significant increase of shear modulus immediately after cessation of running was observed for all compartments compared to pre-running values. Shear modulus of all compartments in both legs gradually returned to pre-running after cessation of exercise. It took between 3 and 5 minutes for the shear modulus to decrease 50% of the initial increase caused by exercise. Overall values returned to pre-running values in about 10 minutes. These preliminary results indicate that SWE is sensitive to transient changes in muscle ICP. Our protocol used an SWE technique to quantify the change in shear modulus of the lower-leg compartments, which differs from previous ultrasound techniques. To our knowledge, this is the first time a new protocol based on SWE has been used to quantify temporal changes in the TA muscle shear modulus after a running exercise. SWE can potentially provide equivalent measures to those obtained using needle manometry so that it may be useful for the diagnosis of CS.

Several mechanisms may be responsible for the shear modulus changes of the compartment muscles after running. Sadeghi et al. [1] reported a linear correlation between shear modulus and ICP in the range of 10-40 mmHg, which is relevant for pathological conditions such as CECS. Additionally, the symptoms associated with CECS are thought to arise from progressive neurovascular dysfunction and transient muscular ischemia, caused by excessive ICP during running [153, 154]. This implies that the increase in TA and PL shear modulus immediately after running could be due to the lower-leg compartments are pressurized during exercise by increased blood perfusion, leading to stiffening of the muscles in the compartment. Besides, the decreasing shear modulus trend after exercise might be due to the gradual stabilization of blood pressure and perfusion after the cessation of running. The temporal changes in shear modulus after exercise follow the expected trends in healthy individuals. It can be observed that by the 5-minute mark, the shear modulus significantly decreases as specified in the Pedowitz criteria for healthy
individuals. Consequently, the change in ICP is likely the driving mechanisms for the observed changes in shear modulus.

There was considerable variability in shear modulus values at the 1-minute post-exercise in our protocol. This may have been caused by the rapid change in ICP and blood pressure, and its associated change in shear modulus, after the cessation of exercise. This is evidenced by the decrease in shear modulus based on the order of measurement of the four compartments. It takes a few seconds to measure all the compartments, causing a slight delay in the measurement of the other compartments. The measurement in the TA muscle (anterior compartment) was always the first compartment measured and had the highest shear modulus at the 1-minute time point. Conversely, the measurement in the tibialis posterior (deep posterior compartment) was the last compartment and had the lowest increase in shear modulus at the 1-minute time point. Therefore, it is possible that all the compartments had a rapid decrease of ICP during the first minute after exercise, causing the decreasing trend in the shear modulus at the 1-minute measurement post exercise. Additionally, it is known that the rate decreased in blood pressure after exercise changes across individuals [155], which may have caused the large variability in the obtained data. It is worth noticing that the variability at the 1-minute point is not exclusive of the proposed method. Needle manometry also takes a few seconds for each compartment to be tested. Therefore, the variability at the 1-minute mark may be one of the factors affecting the accuracy of needle manometry.

The time from cessation of exercise to return to resting shear modulus ($T_{\text{Rest}}$) may also serve as a diagnostic parameter for CECS. Barrera-Ochoa et al. [33] carried out a retrospective cohort study on patients with suspected forearm CECS. They introduced $T_{\text{Rest}}$ (time to return to resting pressure) as a superior and more accurate technique than direct values of ICP based on the difference in accuracy between the $T_{\text{Rest}}$ and needle manometry values. They reported lower sensitivity and specificity values for needle manometry compared to the $T_{\text{Rest}}$ method, i.e., 73.5% and 84.2% versus 100% and 94.7%, respectively. The specificity increased to 100% when a cut off for $T_{\text{Rest}}$ was chosen to be more than 15 minutes for CECS
patients as opposed to the conventional 5 minutes measurement for needle manometry. Due to the positive direct correlation between ICP and shear modulus of TA and PL muscles reported by Sadeghi et al. [1], it is reasonable to expect that the time that elapses between maximum shear modulus and returns to baseline shear modulus would be similar to \( T_{\text{Rest}} \) for pressure. The decreasing trend of variability in shear modulus of PL and TA muscles over time (Fig. 6-5) indicates that the \( T_{\text{Rest}} \) for shear modulus may be a robust diagnostic method for CECS. Future studies will focus on evaluating specificity and sensitivity of \( T_{\text{Rest}} \) of shear modulus as a diagnostic parameter for CECS.

Our protocol offers several advantages over other conventional methods for the diagnosis of CECS. First, it holds promise as a non-invasive diagnostic tool for CECS. Needle manometry can cause pain since the medical practitioner must insert the needle into the compartments to take pressure readings several times. Besides, the trend in ICP over time cannot be calculated using needle manometry. Our protocol is capable of quantifying temporal changes in shear modulus of the lower-leg compartment muscles after a running exercise. Therefore, it should be beneficial for sports physicians and orthopedic surgeons who examine patients at risk of CECS. The positive direct relationship between ICP and the median shear modulus of TA and PL muscles (Spearman's correlation coefficient = 0.99) [1] also opens the possibility of diagnosing acute compartment syndrome. The non-invasive and safe nature of shear wave elastography may allow continuous monitoring of compartment pressures.

This study has some limitations. First, the gender effects were not investigated, which may have influenced the variability of the data. It has been indicated that the distribution of fiber type may be different between men and women [156]. Häkkinen [157] suggested different neuromuscular activation between men and women, evidenced by the significant decreases in the maximum voluntary electromyography signal in men compared to women. Therefore, there may be differences between men and women in the adaptation of the neuromuscular system to various muscle activation, which may ultimately influence muscle shear modulus. Additionally, SWE variability may be caused by misalignment of the muscle fibers direction.
relative to the transducer when trying to measure all the compartments as quickly as possible. Ultrasound SWE measurements in muscles are also dependent upon the muscles depth and good coupling between the transducer and skin. Since shear modulus measured by ultrasound SWE at different depths may be different, the measurement depth was kept 3 cm in all participants.

6.5 Conclusion

In conclusion, this chapter showed initial evidence of the temporal changes in shear modulus of lower-leg muscles after a running exercise using SWE. The highest shear modulus was obtained immediately after the cessation of running. Shear modulus of all compartments in both legs returned to their approximate pre-running values about 10 minutes after the cessation of exercise. This protocol can potentially be used for the diagnosis of CECS and to the effectiveness of surgical interventions. Additionally, our novel protocol for measuring shear modulus in lower-leg compartments may lead to a better understanding of the pathophysiology of CECS. Further research is warranted to investigate changes in shear modulus at a shorter scale (seconds) after exercise. These findings may help the development of lower-leg CECS ultrasound diagnostic criteria. Furthermore, our novel protocol of evaluating lower leg compartments’ shear modulus may lead to a better understanding of the pathophysiology of CECS. Further research is warranted to validate these findings to develop lower leg CECS ultrasound diagnostic criteria.
Chapter 7
Measurement of the Shear Modulus in Muscle Fascia of Lower Leg As a Prognostic Indicator of Compartment Syndrome

The content of this Chapter has been submitted to the Journal of Mechanical Behavior of Biomedical Materials.
Mechanical characterization of thin-layered tissues has broad applications ranging from the diagnosis of several pathologies to tissue engineering. Ultrasound SWE measures the mechanical properties of tissues by calculating the propagation speed of shear waves. However, the shear wave speed in thin-layered tissues such as muscle fascia of the lower leg is affected by the thickness and properties of surrounding tissues. The objective of this study was to introduce a method that combines numerical simulations and shear modulus measurements to evaluate the actual shear modulus of muscle fascia of the lower leg from measurements of the apparent shear modulus. First, an introduction about developed models for evaluating the actual shear modulus of layered tissues will be provided, followed by the Methods section, which gives the details of the proposed simulation method and validation of it. Experiments and results of numerical simulation and elastography measurement on healthy individuals are presented, followed by a discussion of the results. The rationale for this study is that the SWE-based simulation model presented in this study can provide accurate measurements of shear modulus in muscle fascia of lower leg compartments as a prognostic indicator of CS. Finally, an SWE-based simulation model will be used to measure the actual shear modulus in the fascia of the lower leg in a patient with a history of CECS.

7.1 Introduction

Altered mechanical properties of layered musculoskeletal tissues, such as fascia and aponeurosis, are a consequence or the cause of several pathologies. For example, increased stiffness muscle fascia of the lower leg has been associated with compartment syndrome [42]; decreased plantar aponeurosis elasticity may be a consequence of the plantar fasciitis, the most common cause of foot pain [158, 159]; and the strain of Achilles tendon aponeurosis increases in athletes with tendinopathy [160]. Therefore, measuring the mechanical properties of layered tissues may help the diagnosis or evaluation of several pathologies. SWE is a non-invasive method to quantify the mechanical properties of soft tissues [43-46]. Shear modulus obtained via SWE are based on bulk wave theory assuming that the wavelength of the shear wave is much smaller than the dimension of the medium. However, the thickness of some thin-layered tissues is
comparable to or smaller than the wavelength of the shear waves. Therefore, the thickness and the properties of the surrounding tissues have an important influence on the wave speed of layered tissues [47].

Guided wave elastography (GWE) have also been proposed to assess the shear modulus of layered tissues in several previous studies [47, 161, 162]. GWE has been used to characterize the mechanical properties of heart walls [51], arterial walls [163, 164], bladder walls [165], and corneas [166]. Acoustic radiation force (ARF) is used to generate the multimode guided waves in the thin-walled soft tissues, and then the two-dimensional fast Fourier transformation is performed in the propagated multimode signals to obtain the dispersion relation. The elastic properties of the layered tissue can then be quantified by fitting the dispersion curve within a theoretical model. However, the guided waves in most of these models are assumed to be nonleaky, i.e., the mechanical energy of the wave is confined to the layer. In many applications, the energy and motion in the layer are transferred to surrounding tissues or media, which have a significant effect on the wave speed. For instance, a recent study reported that the shear wave propagation speed in tendons immersed in a saline bath was 22% lower than that of the tendon tested in the air [48]. Therefore, the presence of adjacent tissues causes the wave propagating in the layer to have an 'apparent' wave speed that is different from 'actual' wave speed associated with its mechanical properties. Therefore, in order to accurately measure the shear modulus of layered tissues, it is necessary to consider the thickness and the properties of surrounding media or tissues.

Numerical simulation of shear wave propagation in SWE layered tissues may be a useful tool for evaluating the shear modulus of layered tissues. Several numerical transient SWE models have been proposed to measure the shear wave group velocity in soft tissues. Palmeri et al. [167] simulated the shear wave propagation in different elastic homogeneous media with ARF excitations and compared them with the reference values, reporting the largest overall error of 1.13%. Prieur et al. [168] simulated the ARF and subsequent shear wave displacement in a homogeneous and isotropic medium and compared the results with the analytical solutions, reporting that software “K-Wave” is an accurate and efficient tool for
simulation of shear wave propagation in a medium. These studies, however, were limited to the homogeneous media. Obtaining the relationship between the actual and apparent shear modulus of layered tissues using SWE simulation may be useful to estimate the actual shear modulus of layered using only SWE measurements.

The objective of this study was to introduce a method that combines numerical simulations and shear modulus measurements to evaluate the actual shear modulus of muscle fascia in the fascia of tibialis anterior (TA) muscle from measurements of the apparent shear modulus. The acoustic radiation force and shear wave propagation were modeled in the muscle fascia in lower leg compartments, and the shear modulus was calculated. The simulation results were validated on layered constructs of agarose gels with different concentrations. Additionally, the SWE measurements were also performed in vivo to measure the apparent shear modulus in the fascia of TA muscle and to use the proposed method to estimate their actual shear speed (or modulus).

7.2 Overview

7.2.1 Numerical Simulation of SWE

A simulation software package “K-Wave” running under Matlab (Mathworks, Natick, MA) was used for the simulation of SWE [169]. This software package allows simulation of the compressional and shear waves propagation created by ultrasound transducers. K-Wave works based on a pseudo-spectral time domain method for computing spatial derivatives through fast Fourier transformation, which makes it computationally efficient [170].

The simulation of SWE in k-Wave was performed in two steps. In the first step, the propagation of compressional waves emitted by the transducer was simulated using the function kspaceFirstOrder3D to
compute the ARF. The obtained pressure and velocity profiles were used in the second-order approximation formulation (Eq. 1) to compute the ARF [168].

\[
\sigma_{ij}^{\text{ARF}} = \frac{1}{2\rho} <p>^2 \delta_{ij} - \frac{1}{2\rho} <|v|^2> \delta_{ij} + \rho <v_i v_j>
\]  

(Eq. 1)

Where \( \sigma^{\text{ARF}} \), \( \rho \), \( v \), \( p \), and \( c \) are the stress tensor, density, particle velocity, acoustic pressure, and sound speed respectively, and \( <> \) designates the time average over a wave period. In the second step, \( \sigma^{\text{ARF}} \) was used as input stress for computing the propagation of shear waves in the thin layer using the function pstdElastic2D. The simulation of shear wave propagation was performed only in dimensions \( x \) and \( y \) to reduce the computation time. Therefore, only three components of the stress tensor \( (\sigma_x, \sigma_y, \sigma_{xy}) \) needed to be computed.

The layer and the substrates were considered linear, isotropic, and heterogeneous with different propagation speed, density, and attenuation coefficient at each layer, similar to the properties of human tissues (Figure 7-1). The domain dimensions in the \( x \), \( y \), and \( z \) directions were 256, 160, and 64 points, respectively. The perfectly matched layer (PML) was also considered in the model to ensure that there is no reflected signal from the domain boundaries. The PML dimensions in the \( x \), \( y \), and \( z \) directions were 20, 30, and 10 grid points, respectively. The spatial step size in \( x \) (depth), \( y \) (azimuth), and \( z \) (elevation) directions was 0.16 mm, 0.94, and 0.31, respectively, equivalent to the domain dimensions of 40 mm (\( x \)), by 150 mm (\( y \)), by 20 mm (\( z \)). The thin layer was located at a depth of 20 mm bounded by two substrates.
Figure 7-1. Schematic image of a thin layer with the thickness H bounded between two substrates used for the simulation of shear wave propagation.

An L7-4 transducer (Verasonics Inc., Redmond, WA) with 128 elements, the center frequency of 5.2080 MHz, and the width of 38-mm was modeled. The transducer was modeled at $x = 0$ and was centered in the $y$ and $z$ plane. The signal transmitted by the transducer was a 500 cycle sinusoidal pushing beam. The supersonic imaging technique developed by Bercoff et al. [43] used for the experimental measurements was simulated. Specifically, 32 elements of the transducer emitted focused ultrasound pushing beam successively at five increasing, equally spaced focal depths, ranging from 10 to 40 mm to create quasi-plane shear waves propagating through the tissue. The simulation propagated the signal for 19.2 µsec for each of five focal points, allowing it to travel through the specified domain. The acoustic pressure and the particle velocity were recorded over the whole domain and used to compute the stress tensor using Eq. (1).

The same properties, domain dimension, PML dimension, and spatial size, were used in the simulation of the shear wave propagation. The compressional waves speed were assumed 20 m/s. It should be noticed that this speed was taken artificially low to allow for larger time steps and reasonable computing time in the resolution of k-Wave [168]. It has been shown that such low compressional wave does not affect the
accuracy of the simulation of shear wave propagation [168]. The ARF was applied as a constant stress tensor for 96 µs, and the shear wave propagation modeling was simulated for 20 ms.

7.2.2 Simulation of SWE in agarose gels and validation

For the simulation of the shear wave propagation in layered constructs of agarose gels, the density, and the attenuation of the substrate were considered 1000 kg/m³, and 4.1 dB/MHz/cm, respectively. The measured shear modulus of the substrate using SWE was 3.28 ± 0.40 kPa and was used for the simulation. The simulations were run for several values of shear modulus ranging from 4 kPa to 256 kPa in the 1mm layer to obtain the corresponding apparent shear modulus.

Four agarose gel constructs were prepared with a thickness of 1 mm, an agarose concentration of 0.45% for the substrate, and agarose concentrations of 0.9%, 1.35%, 1.8%, and 2.7% for the thin layer. Agarose gels were mixed with a constant concentration of MgCO₃ (0.5% mass percentage) to produce ultrasound scatters. To measure the actual shear modulus of a stiffer thin layer, a homogeneous phantom was prepared with the same agarose concentration as each the layers. The ROI size = 7.39 × 7.39 mm² was chosen for measurement of shear modulus in the substrate and 7.39 mm × 1 mm for the layer. For each case, the mean shear modulus was calculated from the shear modulus map and used as the representative value for the sample. Measurements were repeated five times, and the mean and standard deviation (SD) values of the five trails were reported at each case.

7.3 In-vivo measurement

The Institutional Review Board of the Pennsylvania State University approved the study (STUDY00005848). All participants signed informed consent. Participants were excluded if they had a
history of cardiovascular conditions; any injury in their leg muscles; or a history of any neurological disease; were pregnant or not adult or had a terminal illness. The proposed protocol evaluates the shear modulus of the lower-leg muscles and fascia at lying down position.

A customized supersonic SWE method was used to measure the tissue shear modulus using an L7-4 transducer [1, 98, 171]. The region of interest (ROI) size = 7.39 × 7.39 mm² was chosen for measurement of shear modulus in the lower leg muscles, fascia, and fat layer [171]. The ROI for the muscles and fat layer were put next to the fascia. For the fascia of the TA muscle, the ROI with the same width, but variable height to only cover the thin layer was considered.

Shear modulus measurement of the fascia of the TA muscle

Ten healthy participants (three males and seven females; Mean age ± SD, 23.5±4.45; Mean BMI ± SD, 21.48 ± 2.0) were recruited. Participants laid supine with the knee and hip in full extension with the ankle resting on a fixture made of foam to constrain ankle movement (Figure 7-2). Subjects were instructed to remain relaxed during the shear modulus measurement. Since the fascia of the TA muscle is close to the skin, a gel pad with the thickness of 10 mm was used to increase the distance of transducer from the skin. Scan images of the TA muscle were taken at a point 30% of the distance from the tip of the lateral malleolus to the head of the fibula. Since SWE results are sensitive to the degree of muscle contraction and transducer position [93, 172], it is crucial to control these parameters to produce reliable and precise results. Ultrasound SWE is a reliable method when the transducer is placed parallel to the fiber orientation [89, 173]. Therefore, the shear modulus for TA muscle, fat layer, and fascia of TA was measured by elastography measurement parallel to the fibers, using an adjustable fixture to minimize contact pressure. When the pennation of the fibers was identifiable on the screen, the elastography measurements were conducted to measure the shear modulus. The measurements were repeated five times for each tissue for each participant, and the mean value and the SD were reported. Additionally, the thickness of fascia of the TA muscle was measured during B-mode imaging. The fascia thickness, as well as the shear modulus of TA muscle, fat, and fascia measured
by SWE, were used in the simulation to estimate the actual shear modulus of the fascia. In the simulation, the density of the fat, fascia and muscle were considered 950, 1120, and 1000 kg/m$^3$, respectively; and the attenuation were considered 1.8, 5.9, and 4.1 dB/MHz/cm, respectively [174].

![Image](image.png)

Figure 7-2. The experimental setup for SWE measurement in TA muscle, fat layer, and fascia of the TA. A gel pad was used since the fascia is close to the skin.

Additionally, to evaluate between-days reliability of SWE measurements of the fascia, the shear modulus measurements were repeated in two different days. The between-days reliability of the proposed method was analyzed by calculating Intraclass correlation coefficient (ICC) (model 3,3) with 95% confidence intervals (CIs) using IBM SPSS statistics software (v24, IBM, Chicago, IL, USA).

7.4 Results

7.4.1 Simulation and validation results

Representative shear modulus maps of the substrate and the thin layer for one of the agarose constructs are shown in Figure 7-3. The average value of the substrate (0.45% agarose) shear modulus was $3.28 \pm 0.40$ kPa. The apparent shear modulus for the 0.9%, 1.35%, 1.8%, and 2.7% agarose thin layers was $5.57 \pm 0.38$ kPa, $8.35 \pm 0.17$ kPa, $13.54 \pm 1.15$ kPa, and $18.66 \pm 1.61$ kPa, respectively. The actual shear wave modulus
measured from the homogenous phantoms made with the same agarose concentrations were 12.89 ± 0.75 kPa, 29.81 ± 1.16 kPa, 76.91 ± 5.50 kPa, and 153.26 ± 5.34 kPa. Representative plots of the numerically-calculated pressure fields of the push pulses with different focal points used in the simulation of the supersonic method are shown in Figure 7-4. The corresponding simulated shear wave propagation at different time points is shown in Fig 7-5. The relationship between apparent and actual shear modulus for the agarose constructs obtained from numerical simulations is shown in Figure 7-6. The apparent and actual shear modulus of the simulations and measurements were found to be in good agreement with a maximum error of 7.22% (Figure 7-6).

Figure 7-3. The Representative shear modulus maps of the substrate and 1 mm layer for one of the agarose constructs measured by SWE.
Figure 7-4. The pressure field of the push pulses at the elevation plane \((z = 10 \text{ mm})\) at different focal depths. Five push pulses are successively focused from 10 to 40 mm (Figure 4 a-e). The 1 mm thin layer is located at a depth of 20 mm (marked with two horizontal black lines). The pressure amplitude was normalized by its maximum amplitude.

Figure 7-5. Shear wave propagation at different time points: 8.08 ms (left), 13.53 ms (center), and 19.67 ms (right) in a layered tissue. The 1 mm thin layer is located at a depth of 20 mm (marked with two horizontal black lines). The actual shear modulus of the layer and substrates was 16 kPa and 3.28 kPa, respectively. The displacement amplitude was normalized by its maximum displacement.
The relationship between the apparent and actual shear modulus for the 1 mm layered constructs of agarose gels obtained by simulations and SWE experiments. A power fit of the simulation results was performed for better visualization of the numerical data.

7.4.2 In-vivo results

The representative shear modulus maps of the fascia and adjacent tissues are shown in Figure 7-7. The average shear modulus (SD) for the fascia of the TA muscle, fat layer, and TA muscle are shown in Table 7-1. The apparent shear modulus of the fascia was found to be higher than the surrounding tissues. The between-days ICC (95% CI) for the shear modulus measurement of the fascia of the TA muscle was 0.90 (0.71-0.96), suggesting excellent reliability.
Figure 7-7. The shear modulus maps of the fat layer, TA muscle, and fascia of the TA muscle.

Table 7-1. Obtained shear modulus in SWE measurements in the fat, TA muscle, and fascia of TA (mean ± SD).

<table>
<thead>
<tr>
<th>Tissue type</th>
<th>Shear modulus (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>6.35 ± 2.21</td>
</tr>
<tr>
<td>TA muscle</td>
<td>8.70 ± 1.81</td>
</tr>
<tr>
<td>Fascia</td>
<td>16.12 ± 4.40</td>
</tr>
</tbody>
</table>

The thickness of the fascia in the participants (mean ± SD) were found to be 0.9 ± 0.15 mm. Therefore, the simulation results with the corresponding average thickness can be used to compare the actual and apparent shear modulus in the fascia. The average of measured shear modulus for the TA muscle and fat were 8.70 ± 1.81 kPa and 6.35 ± 2.21 kPa, respectively. The measured shear modulus was put in the numerical simulations to obtain the actual shear modulus of the fascia. The relationship between the actual and apparent shear modulus for the fascia from the numerical simulations are shown in Figure 7-8. These
findings have demonstrated that the actual shear modulus of thin layers was higher than that by measured apparent shear modulus. In addition, the relationship between the actual and apparent shear modulus of fascia was found to be non-linear. The apparent shear modulus of the fascia measured by SWE (16.12 ± 4.40 kPa) was put in the corresponding power regression equation shown in Fig. 7-8 to estimate the actual shear modulus. The actual shear modulus of the fascia was found to be 109.73 ± 68.70 kPa. The obtained actual shear modulus of fascia will serve as an actual shear modulus range for healthy individuals.

Figure 7-8. The simulation results showing the relationship between the apparent and actual shear modulus of the fascia of the TA muscle. A power fit of the simulation results was performed for better visualization of the numerical data. The average and SD values are marked with the solid black lines and the shaded grey area, respectively.
7.5 Discussion

This study introduced a method that combines numerical simulations and shear modulus measurements to evaluate the actual shear modulus of the fascia of layered tissues from measurements of the apparent shear modulus. Agarose gels measurements showed that the shear modulus obtained by the simulation model and measurements were very similar validating the proposed method. The results of the simulation model for the fascia of TA showed a non-linear increase of apparent shear modulus with increasing actual shear modulus. The results of SWE-based simulation model suggest that simulation of shear wave propagation in layered tissues could be an excellent tool to obtain the relationship between the actual and apparent shear modulus of layered tissues.

Several previous studies proposed different lamb wave analytical models for measurement of shear modulus in layered tissues. Urban et al. [161] employed the ultrasound-based guided wave elastography of a thin elastic gelatin layer bounded by an overlying fluid and a solid substrate, deriving an analytic lamb wave propagation model. Couade et al. [162] also evaluated the shear modulus of the carotid wall in a group of healthy participants, deriving an analytical formulation for the shear wave dispersion in the arterial wall. Recently, Li et al. [47] established an inverse analytical approach to characterize the mechanical properties of layered soft tissues by considering the energy leakage in the thin layer based on the dispersion relation. They reported an agreement between their model and the dispersion curve, when the phase velocity of the thin layer is higher than the shear wave speed of substrate, while there were limitations in the analysis of lower phase velocities. These analytical approaches, however, involve complex equations and therefore, may be difficult for daily use by clinicians. Recently, Mo et al. [175] proposed a new empirical formula for calculating the shear modulus of thin layer tissues with several thicknesses, without the need of using complicated mathematical models. They demonstrated that the change in actual shear modulus of thin layers is more significant at higher thicknesses, which is very similar to our results. This might be due to the significant effect of energy leakage at lower thicknesses, causing less change in apparent shear modulus.
The formula proposed by Mo et al. [175] for calculating the actual shear modulus of layered tissues depends upon the apparent shear modulus and thickness of the layer as well as the frequency of the excited shear waves in the layer. However, the frequency of shear waves in transient SWE methods in clinical ultrasound systems is challenging to measure since the generated shear wave has a broad frequency spectrum [98]. Therefore, the shear wave group velocity may be more useful for clinical applications to evaluate the actual shear modulus of layered tissues. One advantage of the simulation model in this study is that the shear modulus of layered tissues is calculated using the shear wave group velocities. Therefore, the results of this study could be comparable to the SWE methods using the time-of-flight algorithm for calculating shear wave group velocities. An important characteristic of our work is that we provided information about the relationship between apparent and actual shear modulus of thin-layered tissues using shear wave group velocity. Consequently, the apparent shear modulus measured by SWE may be used to quantify the actual shear modulus of thin-layered tissues using the simulation results.

Our proposed SWE-based simulation model may have several clinical applications. The actual shear modulus of the fascia of TA muscle may be helpful for clinicians to use it as a prognostic indicator of chronic exertional compartment syndrome (CECS). Hurschler et al. [42] evaluated the mechanical properties of tibial fascia specimens harvested from the standard compartment (lateral) and pathological side (anterior) of patients with CECS, reporting that the fascia in the pathological compartment is stiffer and thicker in the axial side compared to the fascia in the normal compartment. These results suggest that increased fascial shear modulus may contribute to the pathologies such as CECS. Therefore, measurement of the actual shear modulus of the fascia of the lower leg may serve as a non-invasive tool for the prognosis of CECS.

The information about the actual shear modulus of thin-layered tissues can also be useful for estimating the amount of force in tissues such as tendon. Martin et al. [176] reported that the speed of shear wave propagation in thin-layered tissues increases with the square root of axial stress by showing a correlation
between the squared shear wave speed and axial stress in the Achilles ($R^2 = 0.96$) and patellar ($R^2 = 0.98$) tendons during isometric exertions. This finding highlights the importance of shear wave speed as a sensor of loading and muscle force. However, the presence of adjacent tissues in layered tissues causes the shear wave speed in the layer to be different from 'actual' wave speed associated with the axial stress [48]. The simulation method, presented in this study, could be useful to obtain the actual shear modulus in the muscle-tendon unit and potentially use that information for estimating the amount of force. Estimating the force in thin-layered tissues could be of crucial help to guide rehabilitative interventions and assess tissue healing following treatment.

The simulation run-time is an important factor for translating the numerical SWE models to practical applications. The K-Wave software used in this study has shown to be efficient for SWE simulation in heterogeneous media by using a finite time difference method. However, the layered musculoskeletal tissues with different thicknesses surrounded by tissues with different shear modulus may have different relationships. Therefore, a separate numerical analysis may be needed to obtain the relationship between the actual and apparent shear modulus for each specific layered tissue. Employing a predictive regression model using machine-learning algorithm may help find the dependency of the actual shear modulus of the layer to the apparent shear modulus and the thickness of the layer, as well as the shear modulus of substrates. This may eliminate the need for running multiple simulations to find the actual shear modulus of each specific layered tissue. Future studies will focus on developing a regression model based on machine learning algorithms for SWE simulations in layered tissues.

There are several limitations associated with this SWE-based simulation model. First, a linear elastic isotropic material model was used for the simulations, while the layered tissues such as fascia are anisotropic tissues, which may have viscoelastic behavior. However, the wave propagation is limited to one of the anisotropy planes. Therefore, only one of the anisotropic shear moduli plays a role in the simulation; such behavior can be represented by an isotropic material. The shear deformations are very small justifying
the use of linear elastic material. Additionally, previous SWE simulations showed a good agreement between the analytical solution and numerical simulation of shear wave propagation in tissues by assuming linear elastic isotropic properties [167, 168]. The tissue viscosity may contribute to the shear wave attenuation in human tissues [177, 178], but this was not considered in the simulation. The function pstdElastic2D in K-Wave does not include the viscosity of the materials for shear wave propagation modeling. However, Shih et al. [179] showed that the estimated elasticities of agar phantoms by ignoring viscosity were almost identical to those obtained by considering viscosity.

7.6 Conclusion

In conclusion, this study demonstrated the relationship between actual and apparent shear modulus of muscle fascia of lower leg using an SWE-based simulation model. The SWE measurements in layered constructs of agarose gels with different concentrations showed that the SWE-based simulation model could provide accurate measurement of actual shear modulus in layered tissues. The apparent shear modulus was found to increase with increasing the actual shear modulus at different thicknesses. The relationship found between the actual and apparent shear modulus of the fascia of TA muscle can be used in SWE measurements to estimate the actual shear modulus of these tissues. Overall, the preliminary results presented could be a useful clinical tool for more accurate diagnosis of several pathologies related to thin-layered tissues such as CECS and tennis leg.
Chapter 8
Summary and Future Work
In this thesis, the supersonic shear wave imaging (SSI) sequencing code was implemented for C5-2 and L7-4 transducers in Verasonics research scanner and shear wave elastography (SWE)-based protocol was developed to potentially use as a diagnostic/prognostic tool for compartment syndrome (CS) and multifidus muscle function evaluation in patients with lower back pain.

Chapter 1 presented a general overview of the background information about the muscle function evaluation and application of SWE.

Chapter 2 presented the development of a customized SSI code implemented in the Verasonic research scanner. The safety measurements were conducted to ensure the acoustic output parameters satisfy the FDA regulatory limits. The reliability of the developed codes was measured using commercially available elasticity phantom, and the obtained mean values at different depths were within 10% of the nominal values.

Chapter 3 proposed a new protocol to quantify localized force production capability in multifidus muscle using ultrasound SWE in healthy individuals. The postures of the subjects were influential on the multifidus shear modulus. The highest multifidus shear modulus was obtained during the sitting up position with the right arm lifted. The change in median shear modulus from lying prone to sitting up with the right arm lifted was more pronounced compared to changes in the other postures.

Chapter 4 proposed a new protocol to quantify dysfunction of the multifidus after radiofrequency neurotomy (RFN) and posterior lumbar fusion (PLF) surgery. Multifidus had higher shear modulus in matched healthy controls compared with the patients with a history of PLF and RFN in all three postures. The preliminary results presented in this study suggest that lumbar multifidus muscle force production after RFN and PLF procedures decreases. Therefore, SWE has the potential of enhancing clinical assessment of the paraspinal muscles after RFN and PLF. Future research in multifidus function evaluation could be evaluating the effect of BMI in healthy individuals and patients with low back pain on the function of the
multifidus muscle based on ultrasound SWE. Additionally, the relationship between the shear modulus changes after RFN/PLF and symptoms could be further investigated.

Chapter 5 described a protocol to quantify changes in lower-leg compartment stiffness induced by variation of ICP using ultrasound SWE in healthy individuals. The ICP level was influential on the TA and PL shear modulus. The lowest TA and PL shear modulus were obtained during the leg elevated position. The change in median shear modulus from zero to the 120 mmHg pressure point was more pronounced compared to changes in the other pressure points for TA and PL muscles. Future studies could be extended to other musculoskeletal muscles to evaluate muscle's shear modulus elicited by different models of elevated ICP. Overall, the preliminary results presented in this study suggest that SWE has the potential to quantify shear modulus changes of muscles under various blood pressure levels and help provide the background information needed to more accurately interpret the clinical results.

Chapter 6 proposed a new SWE-based protocol to quantify changes in lower-leg compartment shear modulus after running in CS patients and healthy individuals for diagnosis of CS. This study showed initial evidence of the temporal changes in shear modulus of lower-leg muscles after a running exercise using SWE. The highest shear modulus was obtained immediately after the cessation of running. Shear modulus of all compartments in both legs of healthy participants returned to their approximate pre-running values about 10 minutes after the cessation of exercise. This protocol can potentially be used for the diagnosis of chronic exertional compartment syndrome (CECS) and to the effectiveness of surgical interventions. Further research is warranted to investigate changes in shear modulus at a shorter scale (seconds) after exercise. These findings may help the development of lower-leg CECS ultrasound diagnostic criteria. Future research in the compartment syndrome study could be validating these findings in a larger sample size with the aim of developing lower leg CECS ultrasound diagnostic criteria. Additionally, measuring shear modulus in healthy individuals and CECS patients continuously (every 1 or 2 seconds) during the first two minutes to determine how fast the ICP changes after cessation of exercise. This can also be verified.
by changing the order of data collection in the protocol for manual measurements of all compartments. This data will help define a better timing sequence for the treadmill-running protocol. Future studies could also focus on evaluating specificity and sensitivity of $T_{\text{Rest}}$ of shear modulus as a diagnostic parameter for CECS.

Another potential future study could be recording ICP at the same time that shear modulus is measured in order to evaluate their relationship. This paired measurement (ICP and SWE) on CECS patients may lead to a better understanding of the pathophysiology of CECS.

Chapter 7 demonstrated the relationship between actual and apparent shear modulus of muscle fascia of lower leg using an SWE-based simulation model. The results showed that the SWE-based simulation model could provide an accurate measurement of actual shear modulus in layered tissues. The apparent shear modulus of the fascia of TA muscle was found to increase with increasing the actual shear modulus. The relationship found between the actual and apparent shear modulus of the fascia of TA muscle can be used in SWE measurements to estimate the actual shear modulus of the fascia. Overall, the preliminary results presented could be a useful clinical tool for more accurate diagnosis/prognosis of CS. Future studies could focus on developing a regression model based on machine learning algorithms for SWE simulations in layered tissues. This may eliminate the need for running multiple simulations to find the actual shear modulus of each specific layered tissue. Therefore, employing a predictive regression model using machine-learning algorithm may help find the dependency of the actual shear modulus of the layer to the apparent shear modulus and the thickness of the layer, as well as the shear modulus of substrates.
Bibliography


[73] Nordenfur, T., 2013, "Comparison of pushing sequences for shear wave elastography."


Appendix A: SSI sequencing in Verasonics Scanner using L7-4 transducer

%clear all
clear
% System parameters
filename = ('L7-4_SSI');

na = 120; % Set na = number of detect acquisitions.
SWIFrames = 10;
BmodeFrames = 20;
SWI_FR = 10000; % frame rate for shear wave imaging
swv = [];
% shear wave velocity map

SSI.NumPushes = 7; % number of focal points for pushing
SSI.ZOverPush = 1.0; % push line will be done ZOverPush times the SWIROI height

SSI.LatOffset = 10; % the push line will be located LatOffset wavelengths to the left of the SWIROI
SSI.PushRate = 5000; % number of push pulses per second, to define time between push pulses

nang = 3; % Set na = number of flash angles for 2D.
if (nang > 1)
dtheta2D = (8*pi/180)/(nang-1);
startAngle = -4*pi/180/2;
else
dtheta2D = 0;
startAngle=0;
end % set dtheta2D to range over +/- 15 degrees.

powermax = 250; % scaling of the display function
pushCycle = 50;
maxVoltage = 65;

% Define system parameters.
Resource.Parameters.numTransmit = 128; % number of transmit channels.
Resource.Parameters.numRcvChannels = 128; % number of receive channels.
Resource.Parameters.speedOfSound = 1540; % set speed of sound in m/sec before calling computeTrans
Resource.Parameters.verbose = 2;
Resource.Parameters.initializeOnly = 0;
Resource.Parameters.simulateMode = 0;
% Specify Trans structure array.
Trans.name = 'L7-4';
Trans.units = 'mm';
Trans = computeTrans(Trans); % L11-4v transducer is 'known' transducer
so we can use computeTrans.
Trans.maxHighVoltage = maxVoltage; % set maximum high voltage limit
for pulser supply.

% Define ROI for IQ processing, can be changed in GUI
SWIROI.CenterX = 0;
SWIROI.CenterZ = 50;
SWIROI.width = 30;
SWIROI.height = 30; % [width and height in wavelength]

%SWIFocusZ = SWIROI.CenterZ;
%SWIFocusX = SWIROI.CenterX;

%% Imaging parameters
P.startDepth = 5; % Acquisition depth in wavelengths
P.endDepth = 190; % This should preferably be a multiple of 128
samples.

% Specify PData(1) structure array for Bmode Imaging
PData(1).PDelta = [Trans.spacing,0,0.5];
PData(1).Size(1) = ceil((P.endDepth-P.startDepth)/PData(1).PDelta(3));
% startDepth, endDepth and pdelta set PData(1).Size.
PData(1).Size(2) = ceil((Trans.numelements*Trans.spacing)/PData(1).PDelta(1)); % single image page
PData(1).Origin = [-Trans.spacing*(Trans.numelements-1)/2,0,P.startDepth]; % x,y,z of upper lft crnr.

% Specify PData(2) structure array for Shearwave visualization
PData(2) = PData(1);
PData(2).PDelta = [0.5,0,0.25];
PData(2).Size(1) = ceil((P.endDepth-P.startDepth)/PData(2).PDelta(3));
% startDepth, endDepth and pdelta set PData(1).Size.
PData(2).Size(2) = ceil((Trans.numelements*Trans.spacing)/PData(2).PDelta(1));
PData(2).Region.Shape = struct(...
    'Name', 'Rectangle', ...
    'Position', [SWIROI.CenterX,0,SWIROI.CenterZ-SWIROI.height/2], ...
    'width', SWIROI.width,...
    'height', SWIROI.height);
PData(2).Region = computeRegions(PData(2));

%% Specify Resources.
% RcvBuffer for all raw data
Resource.RcvBuffer(1).datatype = 'int16';
Resource.RcvBuffer(1).rowsPerFrame = 2048*4;
Resource.RcvBuffer(1).colsPerFrame = 128;
Resource.RcvBuffer(1).numFrames = BmodeFrames;
% RcvBuffer for all raw data
Resource.RcvBuffer(2).datatype = 'int16';
Resource.RcvBuffer(2).rowsPerFrame = na*2048*4;
Resource.RcvBuffer(2).colsPerFrame = 128;
Resource.RcvBuffer(2).numFrames = SWIFrames;

% InterBuffer for Bmode (not required)
Resource.InterBuffer(1).numFrames = 1;

% InterBuffer for SWI visualization, colsPerFrame must be large enough
Resource.InterBuffer(2).numFrames = 1;
Resource.InterBuffer(2).pagesPerFrame = na;

% ImageBuffer for reference Bmode image
Resource.ImageBuffer(1).datatype = 'double';
Resource.ImageBuffer(1).numFrames = BmodeFrames;

Resource.DisplayWindow(1).Title = filename;
Resource.DisplayWindow(1).pdelta = 0.35;
ScrnSize = get(0,'ScreenSize');
DwWidth =
ceil(PData(1).Size(2)*PData(1).PDelta(1)/Resource.DisplayWindow(1).pdelta);
DwHeight =
ceil(PData(1).Size(1)*PData(1).PDelta(3)/Resource.DisplayWindow(1).pdelta);
Resource.DisplayWindow(1).Position = [250,(ScrnSize(4)-
(DwHeight+150))/2,
   DwWidth, DwHeight]; % lower left corner position
Resource.DisplayWindow(1).ReferencePt =
   [PData(1).Origin(1),PData(1).Origin(3)]; % 2D imaging is in the X,Z plane
Resource.DisplayWindow(1).Colormap = gray(256);
Resource.DisplayWindow(1).numFrames = BmodeFrames;

% Transmit parameters
% Specify Transmit waveform structure.
% - detect waveform
TW(1).type = 'parametric';
TW(1).Parameters = [Trans.frequency,0.67,2,1]; % A, B, C, D

% - Push waveform.
TW(2).type = 'parametric';
TW(2).Parameters = [Trans.frequency,1,pushCycle*2,1]; %

% Set TPC profile 5 high voltage limit.
TPC(5).maxHighVoltage = Trans.maxHighVoltage;

% Specify TX structure array.
TX = repmat(struct('waveform', 1, ...
   'Origin', [0.0,0.0,0.0,0.0], ...
   'focus', 0.0, ...%'
   'Steer', [0.0,0.0], ...
   'Apod', ones(1,Trans.numelements), ...%'
   'Delay', zeros(1,Trans.numelements)), 1, nang+SSI.NumPushes);

for ii = 1:nang
   TX(ii).Steer = [(startAngle+(ii-1)*dtheta2D),0.0];
TX(ii).Delay = computeTXDelays(TX(ii));
end

% - Set event specific TX attributes for push.
SSI.DistBetweenPushes = SWIROI.height*SSI.ZOverPush/(SSI.NumPushes-1);
% distance between focal points of the different push pulses
SSI.OldDistBetweenPushes = SSI.DistBetweenPushes;
SWIFocusZ = zeros(1,SSI.NumPushes);
for ii = 1:SSI.NumPushes
    SWIFocusZ(ii) = SWIROI.CenterZ + (SSI.DistBetweenPushes*(ii-
    ceil(SSI.NumPushes/2)));  % array with focal distances for the SSI push pulses
end

for ii = 1:SSI.NumPushes
    TX(nang+ii).waveform = 2;
    TX(nang+ii).focus = SWIFocusZ(ii);  % wavelength, can be changed in the GUI
    TX(nang+ii).focusX = 0;  % can be changed in the GUI
    TX(nang+ii).pushElements = 64;  % can be changed in the GUI
    TX(nang+ii).sysExtendBL = 1;
    TX(nang+ii).Apod = zeros(1,Trans.numelements);
    TX(nang+ii).Apod(64-TX(nang+1).pushElements/2 +1 : 64+TX(nang+1).pushElements/2) = 1;
    TX(nang+ii).Delay = computeTXDelays(TX(nang+1));
end

%% Receive parameters
% Specify Receive structure arrays.
% -- Receive will be changed after SWI on.
% -- Compute the maximum receive path length, using the law of cosines.
maxAcqLength = ceil(sqrt(P.endDepth^2 + ((Trans.numelements-1)*Trans.spacing)^2));
wlsPer128 = 128/(4*2);  % wavelengths in 128 samples for 4 samplesPerWave

% Total (na+1)*RcvBuffer.numFrames  (1 regular flash and na detections)
Receive = repmat(struct('Apod', ones(1,Trans.numelements), ...
    'startDepth', P.startDepth, ...
    'endDepth', P.startDepth + wlsPer128*ceil(maxAcqLength/wlsPer128), ...
    'TGC', 1, ...
    'bufnum', 1, ...
    'framenum', 1, ...
    'acqNum', 1, ...
    'sampleMode', 'NS200BW', ...
    'mode', 0, ...
    'callMediaFunc', 0), 1, BmodeFrames+na*SWIFrames);

% % Receive(1) to Receive(20) are regular flash imaging
for i = 1:BmodeFrames
    % -- Acquisition for full frame.
Receive(i).callMediaFunc = 1; % make media move per frame
Receive(i).framenum = i;
end

d = i; % detection starts from i+1, here is 21;

% - Set event specific Receive attributes for each frame.
for i = 1:SWIFrames
    for j = 1:na % na acquisitions per frame
        Receive(na*(i-1)+j+d).callMediaFunc = 1; % make media move per frame
        Receive(na*(i-1)+j+d).bufnum = 2;
        Receive(na*(i-1)+j+d).framenum = i;
        Receive(na*(i-1)+j+d).acqNum = j;
    end
end

% Specify TGC Waveform structure.
TGC.CntrlPts = [500, 590, 650, 710, 770, 800, 850, 950];
TGC.rangeMax = P.endDepth;
TGC.Waveform = computeTGCWaveform(TGC);

%% Reconstruction parameters
% Specify Recon structure arrays.
% - We need a Recon structure for the 2D image which will be used for each frame.
% Recon(1) is used in regular flash imaging
% Recon(2) is used in Bmode imaging after SWI on
% - 10 IQData frames will be stored after Recon(3) to Recon(12)
Recon = repmat(struct(...
    'senscutoff', 0.6, ...
    'pdatanum', 1, ...
    'rcvBufFrame', -1, ...
    'IntBufDest', [2,1], ...
    'ImgBufDest', [0,0], ...
    'RINums', 1), 1, 3);
Recon(1).IntBufDest = [1,1];
Recon(1).ImgBufDest = [1,-1];
Recon(1).RINums = 1;
Recon(2).IntBufDest = [0,0];
Recon(2).ImgBufDest = [1,-1];
Recon(2).RINums = 2;
Recon(3).pdatanum = 2;
Recon(3).IntBufDest = [2,1];
Recon(3).ImgBufDest = [0,0];
Recon(3).RINums = 3:na+2;

% Define ReconInfo structures.
% - ReconInfo for 2D frame.
ReconInfo(1) = struct('mode', 'replaceIntensity', ... % intensity output.
'txnum',1, ...
'rcvnum',1, ...
'regionnum',1);

ReconInfo(2) = struct('mode','replaceIntensity', ... % intensity output.
'txnum',1, ...
'rcvnum',d+1, ...  % the first Acq will be shown in the display window
'regionnum',1);

k = 2;  % k keeps track of index of last ReconInfo defined
% We need na ReconInfo structures for IQ reconstructions.

ReconInfo((k+1):(k+na)) = repmat(struct('mode', 'replaceIQ', ... % IQ output
'txnum', 1, ...
'recnum', 1, ...
'regionnum', 1), 1, na);

% - Set specific ReconInfo attributes.
for j = 1:na  % For each row in the column
    ReconInfo(k+j).txnum = mod(j-1,nang)+1;
    ReconInfo(k+j).rcnum = j+d;
    ReconInfo(k+j).pagenum = j;
end

%% Process parameters
% Specify Process structure array. (1) is used for B-mode imaging
% Specify Process structure array.
pers = 20;
Process(1).classname = 'Image';
Process(1).method = 'imageDisplay';
Process(1).Parameters = {
    'imgbufnum',1,... % number of buffer to process.
    'framenum',-1,... % (-1 => lastFrame)
    'pdatanum',1,... % number of PData structure to use
    'pgain',1.0,...  % pgain is image processing gain
    'reject',2,...   % reject level
    'persistMethod', 'simple',...
    'persistLevel', pers,...
    'interpMethod', '4pt',... % method of interp.
    (1=4pt)
    'grainRemoval', 'none',...
    'processMethod', 'none',...
    'averageMethod', 'none',...
    'compressMethod', 'power',...
    'compressFactor', 40,...
    'display', 1,...  % display image after processing
    'displayWindow',1};
% EF1 is external function for UI control
Process(2).classname = 'External';
Process(2).method = 'UIControl';
Process(2).Parameters = {'srcbuffer', 'none'};

% EF2 is external function for shearwave visualization
Process(3).classname = 'External';
Process(3).method = 'processIQ';
Process(3).Parameters = {'srcbuffer', 'inter', ... % name of buffer to process.
'srcbufnum', 2, ...
'srcframenum', 1, ...
'dstbuffer', 'none'};

%% SeqControl and Events for shearwave generation

% - Change to Profile 1 (low power)
SeqControl(1).command = 'setTPCProfile';
SeqControl(1).condition = 'immediate';
SeqControl(1).argument = 1;
% - Change to Profile 5 (high power)
SeqControl(2).command = 'setTPCProfile';
SeqControl(2).condition = 'immediate';
SeqControl(2).argument = 5;
% - Noop to allow time for charging external cap.
SeqControl(3).command = 'noop';
SeqControl(3).argument = 500000; % wait 100 msec.

% - time between regular flash imaging
SeqControl(4).command = 'timeToNextAcq';
SeqControl(4).argument = 10000; % 10 ms

% - time between push and detect acquisitions
SeqControl(5).command = 'timeToNextAcq';
SeqControl(5).argument = 250; % 500usec afterpush = 5;

% - time between detect acquisitions
SeqControl(6).command = 'timeToNextAcq';
SeqControl(6).argument = 1/SWI_FR*1e6; % 100usec PRF=6;

% - time between frames
SeqControl(7).command = 'timeToNextEB'; % set time between extended bursts
SeqControl(7).argument = 500000; % 500000usec = 500msec (~ 2 fps)

TTNEB=7;

% - Return to Matlab
SeqControl(8).command = 'returnToMatlab';

% - Trigger out
SeqControl(9).command = 'triggerOut';
SeqControl(10).command = 'timeToNextEB';
SeqControl(10).argument = 1/SSI.PushRate*1e6;    % time between push pulses (us)
TBPP = 10;                                      % time between push pulses

% - Jump back to start will be defined in the event due to the conditional
% event coding

nsc = length(SeqControl)+1;

% Specify Event structure arrays.
n = 1;

Event(n).info = 'ext func for UI control';
Event(n).tx = 0;                 % no transmit
Event(n).rcv = 0;                % no rcv
Event(n).recon = 0;              % reconstruction
Event(n).process = 2;            % process
Event(n).seqControl = 0;
n = n+1;

%% Regular flash imaging starts from event(nStartFlash)
nStartFlash = n;

% Switch to TPC profile 1 (low power) for flash imaging
Event(n).info = 'Switch to profile 1.';
Event(n).tx = 0;
Event(n).rcv = 0;
Event(n).recon = 0;
Event(n).process = 0;
Event(n).seqControl = 1;
n = n+1;

nStartAcqFlash = n;

for i = 1:BmodeFrames
    % Event(n).info = 'trigger out'; % Only needed for verifying push waveform with scope.
    % Event(n).tx = 0;
    % Event(n).rcv = 0;
    % Event(n).recon = 0;
    % Event(n).process = 0;
    % Event(n).seqControl = 9;
    % n = n+1;

    Event(n).info = 'Full aperture.';
    Event(n).tx = 1;                 % use 1st TX structure.
    Event(n).rcv = i;                % use ith Rcv structure for the ith frame.
    Event(n).recon = 0;              % no reconstruction.
    Event(n).process = 0;            % no processing
    Event(n).seqControl = [4,nsc];   % time between frames, SeqControl struct defined below.

    SeqControl(nsc).command = 'transferToHost';

end
nsc = nsc + 1;
n = n+1;

Event(n).info = 'recon and process';
Event(n).tx = 0;  % no transmit
Event(n).rcv = 0;  % no rcv
Event(n).recon = 1;  % reconstruction
Event(n).process = 1;  % process
Event(n).seqControl = 8;
n = n+1;
end

Event(n).info = 'Jump back';
Event(n).tx = 0;  % no TX
Event(n).rcv = 0;  % no Rcv
Event(n).recon = 0;  % no Recon
Event(n).process = 0;
Event(n).seqControl = nsc;
SeqControl(nsc).command = 'jump';
SeqControl(nsc).argument = nStartAcqFlash;

n = n+1;
nsc = nsc+1;

%% Shear Wave Imaging starts from event(nStartPush)
nStartPush = n;

% Switch to TPC profile 5 (high power) and allow time for charging ext. cap.
Event(n).info = 'Switch to profile 5.';
Event(n).tx = 0;
Event(n).rcv = 0;
Event(n).recon = 0;
Event(n).process = 0;
Event(n).seqControl = 2;
n = n+1;

Event(n).info = 'noop for charging ext. cap.';
Event(n).tx = 0;
Event(n).rcv = 0;
Event(n).recon = 0;
Event(n).process = 0;
Event(n).seqControl = 3;
n = n+1;

nStartAcqPush = n;

for i = 1:SWIFrames
    % Event(n).info = 'trigger out'; % Only needed for verifying push waveform with scope.
    % Event(n).tx = 0;
    % Event(n).rcv = 0;
    % Event(n).recon = 0;
    % Event(n).process = 0;
    % Event(n).seqControl = 9;
n = n+1;

% Push transmit
for jj = 1:SSI.NumPushes
    Event(n).info = 'Push transmit';
    Event(n).tx = nang+jj;
    Event(n).rcv = 0;
    Event(n).recon = 0;
    Event(n).process = 0;
    if jj < SSI.NumPushes
        if jj == 1
            Event(n).seqControl = [TBPP 9];
        else
            Event(n).seqControl = TBPP;
        end
    else
        Event(n).seqControl = [afterpush, TTNEB];
    end
    n = n+1;
end

for j = 1:na
    % Acquire frame
    Event(n).info = 'Acquire data';
    Event(n).tx = mod(j-1,nang)+1;
    Event(n).rcv = na*(i-1)+j+d;
    Event(n).recon = 0; % no reconstruction.
    Event(n).process = 0; % no processing
    Event(n).seqControl = PRF; %
    n = n+1;
end

Event(n-1).seqControl = 0; % do not want a TTNA here, since the
next transmit is a EB

Event(n).info = 'transfer data to Host';
Event(n).tx = 0; % no transmit
Event(n).rcv = 0; % no rcv
Event(n).recon = 0; % no reconstruction
Event(n).process = 0; % no process
Event(n).seqControl = nsc;
SeqControl(nsc).command = 'transferToHost'; % transfer frame to
host buffer
nsc = nsc+1;

Event(n).info = 'recon and process for SWI';
Event(n).tx = 0; % no transmit
Event(n).rcv = 0; % no rcv
Event(n).recon = [2,3]; % reconstruction
Event(n).process = 1; % process
Event(n).seqControl = 0;

n = n+1;

Event(n).info = 'ext func to process IQ';
Event(n).tx = 0; % no transmit
Event(n).rcv = 0; % no rcv
Event(n).recon = 0; % reconstruction
Event(n).process = 3; % process
Event(n).seqControl = 8;
n = n+1;
end

Event(n).info = 'Jump back';
Event(n).tx = 0; % no TX
Event(n).rcv = 0; % no Rcv
Event(n).recon = 0; % no Recon
Event(n).process = 0;
Event(n).seqControl = nsc;
SeqControl(nsc).command = 'jump';
SeqControl(nsc).argument = nStartAcqPush;
Appendix B: SWE-based Protocol for the lumbar multifidus elastography

1- Participants are first asked to change into shorts and wear hospital gown. Then, they are asked to sit up on the bed, sit upright in a normal resting posture, while their feet are not supported on the floor. The C5-2 transducer is placed in long-axis just lateral to the spinous processes and angled medially (10-15 degrees) according to the figure below:
2- In order to minimize the contact pressure, use an adjustable fixture to hold the transducer.

3- After adjusting the transducer, run the matlab code. To do this, go to the Verasonics folder and run the code. This window will then appear:

![Image of the window]

4- Change the High Voltage P1 to “40” and see the B-mode image. The transducer should be further adjusted at this stage. The L4-5 facet joint and the fibers of multifidus on top of the fascia must be clearly identifiable in the brightness-mode (B-mode) image (similar to Figure below).
5- After the transducer adjustment, click on “SWI on” to activate the elastography mode. This window will appear:
6- Change the “push cycles” to 500.

7- Change the “High Voltage P5” to 40.

8- Change the “region of interest (ROI) Width” to 40. Then move the ROI by clicking on “move ROI” to put it inside the multifidus muscle. Adjust the ROI height by clicking on the “ROI Height”. Locate the ROI 2 mm above the facet joint and cover the entire multifidus muscle.

9- Click on “move Focus” in the control panel shown above and click once in the B-mode image in the right side of the ROI as shown below:

10- Then click on “Calc All Frames”. The data will be collected for several frames (the number of frames can be modified by changing the “SWIFrames” in the code) and it automatically will go into freeze mode after a few seconds. Click on “Calculate” to calculate the average shear wave speed inside the ROI.

11- Click on “Freeze” again to let the code run again. Repeat this process 5 times and report the average of the five shear wave speeds.

12- Then, ask the participants to lift their arms horizontally similar to the figure below and repeat the step 3 to 11 again.
13- Lastly, ask the participants to lay prone in a fully relaxed position and repeat the step 3 to 11 again.

During the entire SWE measurements, ensure there is no noticeable body movement by monitoring the real time B-mode ultrasound images.
Appendix C: SWE-based Protocol for the running on treadmill study

a) Protocol to evaluate temporal changes in shear modulus in Tibialis Anterior (TA) and Peroneus Longus (PL) muscle after running

1- Participants are first asked to change into shorts and rest for 15 minutes before the test.

2- The L7-4 transducer is placed on the TA muscle. Place the transducer at a point 30% of the distance from the tip of the lateral malleolus to the head of the fibula. Mark the ultrasound transducer location on the skin for accurate placement of the transducer after running.

3- In order to minimize the contact pressure, use an adjustable fixture to hold the transducer.

4- After adjusting the transducer, the matlab code will be run. To do this, go to the Documents folder (C:\Users\PSUID\Documents)

5- Double click on “Vantage-3.4.0-1711281030” in the workspace

6- Type “activate” in the command window

7- Open the code “SetUpL7_4_SSIfocusedelements” from the address below and run the code:

C:\Users\SUS653\Documents\Vantage-3.4.0-1711281030\Examples_Biomedical\Specialty_Applications\RadiationForce_ShearWaveVisualization
8- The window below appears:

![Diagram of a window with various settings and options]

9- Change the High Voltage P1 to “40” and see the B-mode image. The transducer should be further adjusted at this stage. The pennation of the fibers should be clearly identifiable on the screen. Figure below shows the B-mode image of TA (left) and PL muscle (right).
10- After transducer adjustment, click on “SWI on” to activate the elastography mode.

11- Change the “push cycles” to 500.

12- Change the “High Voltage P5” to 40.

13- Change the “region of interest (ROI) Width” and “ROI Height” to 30 and 30, respectively. Then move the ROI by clicking on “move ROI” to keep the distance from the transducer surface to the bottom of the ROI 3 cm.

14- Click on “move Focus” in the control panel and click once in the B-mode image in the right side of the ROI as shown below:
15- After observing the shear wave propagation inside the ROI, click on “Freeze” and then click on “Calculate” to calculate the average shear wave speed inside the ROI.
16- Click on “Freeze” again to let the code run again.

17- Repeat this process 5 times and report the average of the five shear wave speeds.

18- Ask the participants to run for 10 minutes on the treadmill with the speed of 6.5~7 miles per hour.

19- Within ten seconds of cessation of exercise, ask the participants to get on the table and put the transducer at the marked location.
20- Repeat elastography measurements (step 15) at the same location in the muscle every minute for ten minutes.

21- After ten minutes of resting, repeat the same protocol for the PL muscle (step 7 to 20) to quantify the shear modulus changes of the lateral compartment after cessation of running.

b) **Protocol to quantify changes in shear modulus of all lower leg compartments**

In this protocol, the transducer was hand-held instead of using an adjustable fixture.

1- Ask the subjects to rest for 15 minutes before testing or muscles.

2- Repeat steps 7 to 9 in the last protocol and mark the location of all four compartments (Figure below).

3- Repeat steps 10 to 17 (in the protocol(a)) for all four compartments, including the TA, PL, soleus and tibialis posterior muscles, bilaterally.
4- Click on “SWI off” to go back to the B-mode image and click on “Freeze”.

5- Then, ask the participants to run for 10 minutes on the treadmill with the speed of 6.5~7 miles per hour.
6- After cessation of treadmill running, ask the participants to get on the table and put the hand-held transducer at the marked locations. Repeat the steps 10 to 16 (in the protocol (a)) on four compartments bilaterally 1- and 5-minutes after running, respectively. The order of shear modulus measurement at each time point is as follows: TA, PL, Soleus and Tibialis posterior muscles, respectively.
Vita

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