Universitätsklinik Tübingen - Neurologie

Reference values in shear wave elastography of skeletal muscles

Inaugural-Dissertation
zur Erlangung des Doktorgrades
der Medizin

der Medizinischen Fakultät
der Eberhard Karls Universität
zu Tübingen

vorgelegt von
Romano, Alyssa Katherine

2021

Dekan: Professor Dr. rer. nat. Bernd Pichler

1. Berichterstatter: Prof. Dr. Alexander Grimm
2. Berichterstatter: Prof. Dr. Marius Stefan Horger
(ggf. 3. Berichterstatter:)

Tag der Disputation: 13.04.2022

Dedication

For Justus, Kathrin, Lukas and Liv.

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1 Introduction

1.1 Physical background Shear-Wave-Elastography

Shear wave elastography (SWE) was created as an extension of ultrasonography in order to measure the elasticity, or rather simply spoken, stiffness, of various bodily tissues. SWE in this context, ultrasound SWE, is one elastographic method among many others (e.g. strain elastography); the scope of this thesis is not sufficient for a detailed description of all elastographic methods, which is why only SWE is discussed.

Shear waves, also referred as a synonym for transverse waves, are ultrasound waves that propagate perpendicular to longitudinal waves (Gennisson et al., 2013). The longitudinal waves are originally generated from an ultrasound transducer and shear waves are generated simultaneously in a 90°-angle alongside the longitudinal waves (**Figure 1**). The shear in shear waves describes the fact that there is a change in the substance layer without a change in the volume itself (Taljanovic et al., 2017).

Elasticity of a tissue (e.g. muscle) can be quantified, considering a linear elastic behavior, using the equation for shear elastic modulus (μ), where $\mu=\rho V_s^2$ (Bouillard et al., 2011). In this equation, ρ represents the density of the examined tissue (as an example, ρ for muscle is assumed to be 1000 kg/m^3) and V_s denotes the shear wave velocity (SWV). Through this equation, it becomes clear that an increase in shear elastic modulus, or stiffness, is positively correlated with an increase in SWV (Leong et al., 2013). Shear elastic modulus is typically depicted in kPa, whereas SWV is represented in m/s. The higher the velocity of the shear waves traveling between two known points of interest within the examined tissue, the higher the stiffness of the tissue is believed to be and the more elastic it is (Alfuraih et al., 2017).

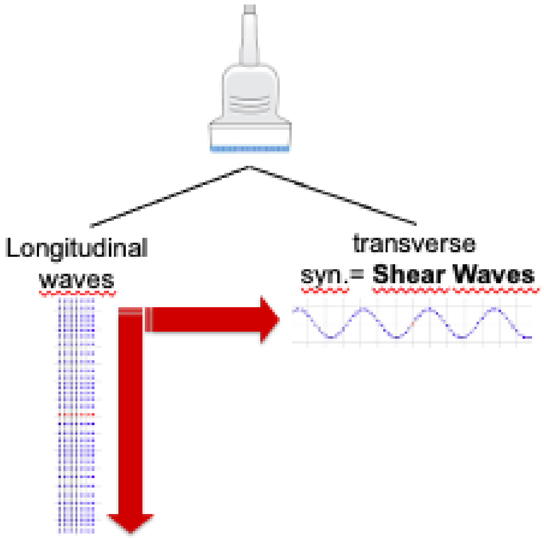

Figure 1. Longitudinal waves and shear waves *(Own representation*): Longitudinal waves (left) sent from an ultrasound transducer result in the simultaneous formation of shear waves (right) that travel perpendicularly, in the transverse plane.

The shape and speed of shear waves can be depicted using Standard B-Mode Pictures of special ultrasound devices allowing for the reconstruction of organs in morphological images (**Figure 2**). The speed in which these waves travel through tissue depends on the tissue composition and the corresponding elasticity (Gennisson et al., 2013). Shear waves are particular in that they are only generated at low frequencies and propagate slower, at a speed of less than 50 m/s through human tissue. This is important to note since the speed of longitudinal waves through soft tissue is around 1540 m/s (Wilhjelm et al., 2016). In this respect, shear waves are more readily visualizable through ultrasound because of their slower speed.

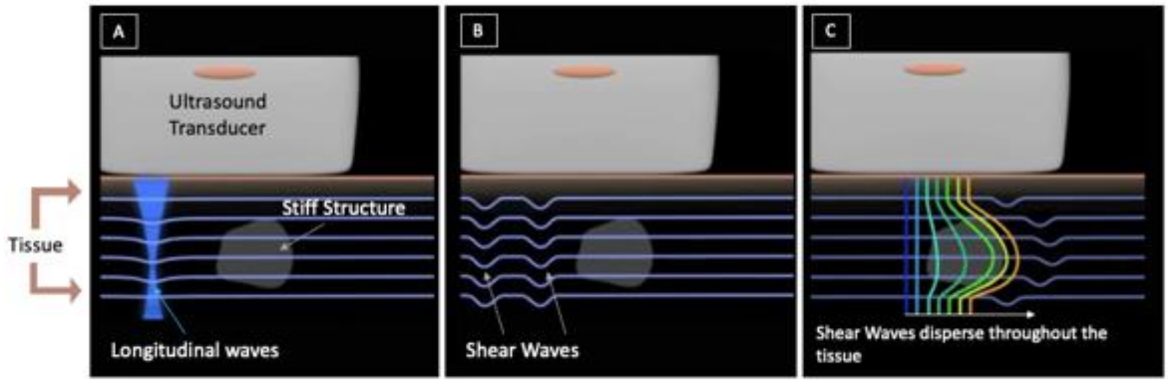

Figure 2. Simplistic representation of the principles of SWE *(figure modified from Kaafarini, 2018*): (**A**) Longitudinal waves are generated from an ultrasound transducer and travel from superficial to deeper lying tissues. (**B**) Concurrently, shear waves travel perpendicular in reference to the longitudinal waves in the transverse plane through bodily tissues. (**C**) When shear waves come into contact with structures of different stiffnesses (here light grey), they disperse throughout the tissue with different velocities that are represented with distinctive colors. In propagation mode of SWE, tracking starks from a dark blue color and progresses to warmer colors. Shear waves travel slower in softer areas of tissue and appear blue, whereas faster shear waves, that travel through contracted or stiff tissue, appear red (Kaafarini, 2018, Akagi et al., 2015; Taljanovic et al., 2017). Therefore, the relationship between the speed of the shear waves can be interpreted based on the color presented in the system imaging. A slow speed of 2-6 m/s appears blue, medium speeds of 6-8 m/s appear green and speeds above 10 m/s show a color range of orange to red.

1.2 Applications in daily clinical routine

So far, SWE has become a well established non-invasive method in the diagnosis and control of various tumors or change in tissue, such as in Breast, Liver, Thyroid and Prostate tissue (Cosgrove et al., 2012; Barr et al., 2015; Ferraioli et al., 2015; Gennisson et al., 2013). As an example, through the process of hepatic fibrosis, the liver becomes stiffer through the proliferation of fibroblast-like cells (Ferraioli et al., 2015). Through SWE, cut-off values for the different stages of liver fibrosis and liver cirrhosis have been established. Hereby, SWE has been useful in the assessment and diagnosis of liver fibrosis by potentially eliminating the need for a biopsy. Furthermore, SWE has also been shown to be exceedingly accurate in characterizing breast lesions, specifically in the differentiation between benign and malignant lesions (Barr et al., 2015) (**Figure 3**). Above a certain stiffness value, breast lesions should be biopsied as there is a higher chance that they are malignant (Barr et al., 2015). In these many areas of medicine, SWE has shown to be an important tool in partially eliminating the need for invasive biopsies, by creating a greater certainty about the characteristics of the lesion through imaging.

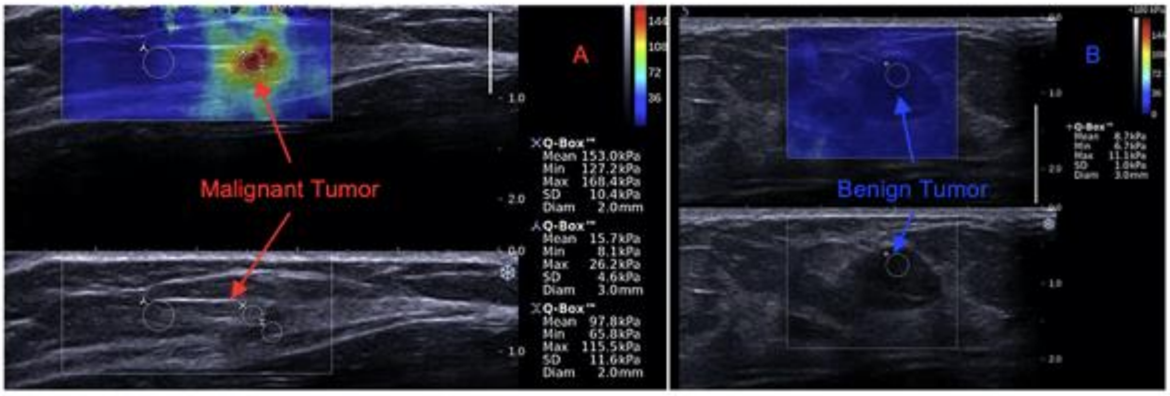

Figure 3. SWE in breast tumor diagnostic. (*figure modified from Barr et al., 2015):* (**A**) Upper: A SWE image of breast tissue from a 50-year-old patient with a mass in her left breast. This lesion had a high shear wave speed (153 kPa) as designated by the warm colors (red and orange) in the middle of the tumor in the SWE image. After biopsy, the tumor was confirmed to be an invasive ductal carcinoma, a malignant tumor. Lower: B-mode image. (**B**) Upper: A SWE image of breast tissue from a 48-year-old woman with a mass in her left breast. The lesion had a lower low shear wave speed (8.7 kPa), represented by the blue color in SWE. After biopsy, the lesion was confirmed to be a fibroadenoma, a benign tumor. Lower: B-mode image. SWE is ideal for breast tumor diagnostic since malignant tumor masses typically have a greater amount of tumor cells in a smaller area of tissue, making the tissue denser and therefore stiffer. This increase in stiffness in comparison to normal breast tissue or benign tumors, can be detected with SWE.

1.3 Current state of SWE in muscle diagnostic

Muscle is another form of tissue that can be readily visualized and analyzed with SWE. For this reason, SWE has been increasingly important in evaluating muscle stiffness in various musculoskeletal disorders or muscular involvement of neurological diseases.

As an example, Duchenne muscular dystrophy (DMD) is an X-linked recessive disorder of the muscle (Yiu & Kornberg, 2015). This disease is associated with muscle weakness, due to a deletion in the Dystrophin gene that results in a functionless Dystrophin protein. Without the right Dystrophin protein, muscle cells die off and are then replaced with fat and connective tissue. This results in a change of composition inside the muscle and a change in muscle density. These changes can be detected with SWE. DMD patients have been shown to have statistically significantly higher SWE values ($p < 0.033$) or greater muscle stiffness in the gastrocnemius medialis, tibialis anterior, vastus lateralis, biceps brachii and triceps brachii muscle (Lacourpaille et al., 2015). There also seems to be a positive correlation between the progression of the disease and age. Approximately half of DMD patients over the age of 9 years have joint contractions (McDonald et al., 2015), leading to greater stiffness within the muscles contracting the joint. These findings suggest that SWE can be an additional, sensitive, non-invasive diagnostic tool in muscle diagnostics for DMD.

Another area in which SWE has shown to be useful is in the diagnostic of patients with myotonia (**Figure 4**). Myotonia is a disease associated with delayed muscle relaxation, meaning that the muscle remains contracted for a longer period of time due to an autosomal inherited mutation in ion channels, such as chloride or sodium channels (Barchi, 1988). This results in either impaired repolarization of muscle cells or a hyperexcitability (mutations of chloride channels), or the inactivation of the channel is delayed at the end of the depolarization phase (mutations of sodium channels). In both cases, these defective channels affect the amount of time it takes for the muscle to return to its relaxed state after contraction. For these reasons, patients typically present with cramping. This is routinely clinically tested with fist-clenching test. This test is however subjective, in which clinicians approximate how long it takes for the underarm muscles of the forearm to relax after clenching their fists. With SWE, the amount of time it takes for the muscle to fully relax after contraction can be objectively quantified (Kronlage et al., 2021). This is useful in measuring how well membrane-stabilizing anticonvulsives work by patients with myotonia. The time it takes for the muscle to relax can be measured with SWE before and after the use of these anticonvulsive drugs, such as lamotrigrine or carbamazapine. The amount of time it takes for the muscle of patients with myotonia to relax has been demonstrated to be significantly longer ($p < 0.05$) than in those without (Kronlage et al., 2021). This illustrates the ability of SWE to be used as biomarker and non-invasive tool in myotonia diagnostic.

Another example is Parkinson's disease. This neurological disease is associated with the secondary development of rigor, or an increase in muscle stiffness, that progresses over time with the advancement of the disease. It has been demonstrated that Parkinson patients had a significantly higher shear muscle modulus ($p < 0.05$) in the biceps brachii muscle in comparison to healthy individuals (Du et al., 2016). SWE of the biceps brachii can be used as a potential method for the evaluation of muscle stiffness in patients with Parkinson.

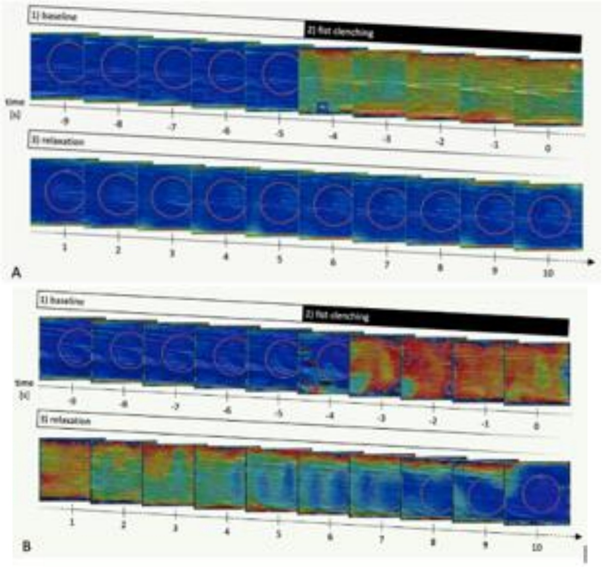

Figure 4. <u>SWE of the superficial flexor digitorum muscle of the forearm in (**A**) healthy volunteers and patients with (**B**) myotonia</u> (f*rom Kronlage et al., 2021*): *1) Baseline*: SWE images of the superficial flexor digitorum muscle of the forearm were recorded in a relaxed state. Each image represents one second. *2) Fist clenching*: Participants were then asked to actively clench their fists for 5 seconds resulting in 5 images. These images showed warmer colors, demonstrating that the shear waves were traveling faster through contracted muscle. *3) Relaxation:* Participants relaxed their

forearm again and 10 images were recorded. The warmer colors – red and orange – remain visible for a longer period of time during the relaxation phase in patients with myotonia in comparison to the healthy volunteers. This indicates that the muscle remains contracted or stiffer for longer after an active muscle contraction, a classic characteristic of myotonia.

Because of the newness in the method SWE, it is not yet generally categorized as an routine diagnostic tool in the domain of neuromuscular disease or to evaluate disease progression in this field for several reasons, which will be discussed below (Alfuraih et al., 2018). However, SWE holds promise as an alternative for non-invasive diagnostic, biomarkers and disease monitoring. Along with its promise to be helpful in the diagnostic of DMD, myotonia and Parkinson's disease as described above, SWE has the potential to be used in monitoring age related changes including sarcopenia and clinical frailty syndrome (Taljanovic et al., 2017) or in the quantitative assessment of disease activity in patients with *GNE*-related myopathy (*GNE* = Glucosamine (UDP-N-Acetyl)-2-Epimerase/N-Acetylmannosamine Kinase) and other genetic muscle disorders such as cerebral palsy (Carpenter et al., 2015; Ryu & Jeong, 2017). Other areas include muscle spasticity after stroke or spinal cord injury (Carpenter et al., 2015). The advantages of SWE in the area of muscle diagnostic include that this methodology is non-invasive, unlike other techniques such myographies or muscle biopsies for the characterization of many hereditary musculoskeletal disorders (Ryu & Jeong, 2017; Kronlage et al., 2021).

1.4 SWE and age

Besides using SWE for the diagnostic of pathologically altered muscle tissue, SWE has recently come to be an increasingly important tool in evaluating the basic morphology of healthy skeletal muscle tissue (Cortez et al., 2016). One of the main areas in this field is the changes in muscles that are associated with healthy aging individuals (Alfuraih et al., 2019), such as an increase in fatty stores, decrease in extracellular water within the muscle and overall muscle degeneration, which might influence elasticity (Cortez et al., 2016). Because of these changes, the natural density of the muscle also changes (Forsberg et al., 1991). Muscle density can be defined as the relation of the amount of muscle fiber tissue to adipose tissue within a muscle compartment (Cawthon et al., 2009). Muscles with more fat tissue are less dense. There seems to be a difference in muscle density between young and elderly individuals, which can affect the age-related difference in muscle shear modulus (Akagi et al., 2015).

The effect of age has been demonstrated to play a significant role in SWE in various muscles, but there are conflicting findings as to whether SWE increases or decreases with age. It has been demonstrated that shear modulus values in the rectus femoris muscle and lateral head of the gastrocnemius muscle were significantly ($p < 0.05$) higher in a younger population (n=16, average age 22 years) than in elderly participants (n=26, average age 71 years) (Akagi et al., 2015). The average shear modulus in the rectus femoris muscle in younger men was 3439 Pa. In the elderly men, the average was 2843 Pa. For the lateral head of the gastrocnemius muscle, the average shear muscle modulus was 3134 Pa compared to 2343 Pa in the elderly men. In this study, no difference in the shear modulus value of the soleus muscle between younger men (3561 Pa) and elderly men (3270 Pa) was found (Akagi et al., 2015). This study demonstrates that (1) shear modulus values of muscle may decrease with age, but (2) not in all muscles. The reason why shear modulus does not significantly change in all muscles is an open point of discussion.

Other studies regarding SWE and age are scarce. In one study using a vibro-ultrasound system, there was no significant difference ($p > 0.1$) in the average shear modulus of the vastus intermedius muscle between young (n=10, average age 27.6 years) and elderly women (n=10, average age 56.7 years) in a relaxed state (Wang et al., 2014). On the other hand, ultrasound SWE values for the biceps brachii muscle were significantly higher ($p < 0.05$) in elderly individuals (n=32, age > 60 years) than in a younger population (n=111, age < 60 years), when the arm was fully extended at the elbow (Eby et al., 2015). Although different muscles were analyzed in each of these studies, it cannot yet be agreed upon if shear modulus increases or decreases with age. In an related method, magnetic resonance elastography (MRE) was used to measure the shear modulus of the tibialis anterior muscle in participants between the ages of 50 and 70 years (Domire et al., 2009). No significant correlation ($p > 0.05$) between age and shear muscle modulus was found. This must be taken into careful consideration since MRE is a different methodology compared to SWE, therefore the results are not necessarily transposable.

However, the general trend as to whether SWE generally increases or decreases with age has not been clearly demonstrated. Further research is required to solidify the differences, or lack there of, in shear modulus of muscles among younger and elderly populations. If there proves to be a significant trend between age and shear modulus, then SWE could be a useful tool for evaluating muscles of elderly individuals, such as in the screening of older patients at risk of sarcopenia and frailty, and in predicting earlier muscle changes associated with aging (Taljanovic et al., 2017).

1.5 Limitations of SWE

The method of SWE is limited in its validity by several factors, as there are important external (e.g. device, operator) and internal (e.g. selected muscle, relaxation of the patient) influences that can only be partially controlled when performing SWE (**Figure 5**): One of which is the operator (Alfuraih et al., 2018; Carpenter et al., 2015; Gennisson et al., 2013; Kaafarini, 2018). The amount of pressure, or pre-load, applied by the operator to the probe has been shown to significantly affect SWE values. An increase in SWV has been associated with increased pre-load (Carpenter et al., 2015), since excess pressure on the probe compresses the tissue underneath and increases elasticity values (Kaafarini, 2018). For example, the average SWV with preload on the rectus femoris muscle was 4.85 m/s, compared to 3.70 m/s without preload (Carpenter et al., 2015). However, it is important to note that in this study, there was no significant difference ($p > 0.1$) in mean SWV values for the gastrocnemius muscle (5.88 m/s with preload, 6.20 m/s without preload) and vastus lateralis muscle (5.81 m/s with preload, 5.32 m/s without preload). The lack of ability to standardize the amount of pressure applied by the operator to the probe is therefore considered the main limitation of this technique (Gennisson et al., 2013). Studies have conferred that transducer compression should be minimized so that the subcutaneous layer of tissue is not deformed during measurement (Alfuraih et al., 2018; Correas et al., 2013; Cortez et al., 2016; Hall et al., 2013).

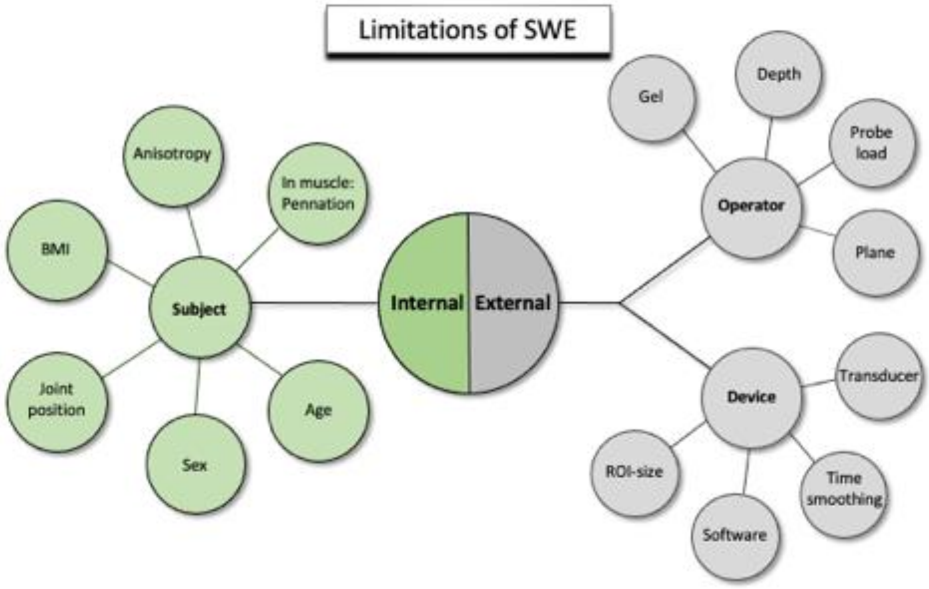

Figure 5. Limitations of SWE (*Own representation*): The limitations of SWE can be divided into internal (green) and external factors (grey). The external factors include the broader categories of the operator and the device. In reference to the operator, the amount of gel, the depth at which SWE values are acquired, the probe load or pressure in which the operator applies to the tissue and the plane of the transducer in relation to the tissue, all influence the SWE values. In regard to the device, there are many different ultrasound systems that can be used for SWE. Within the device and software, different settings can be changed while acquiring SWE values, such as ROI (region of interest) size and time smoothing (Alfuraih et al. 2017; Ewertsen et al. 2016). Time smoothing describes time averaging of recordings. The greater the time smoothing, the more the images and values are averaged together. Additionally, there are different transducers that are equipped for different fields of view and depths. The internal limiting factors of SWE are centered around the subject itself. SWE depends on muscle pennation angle or the angle in which the muscle fibers run in relation to the longitudinal axis of the muscle itself, because shear waves travel best parallel to muscle fibers. Muscles with greater pennation angles prevent the shear waves from travelling as parallel along the entire muscle fiber as possible. The natural anisotropy of the muscle affects SWE values because shear waves travel at different speeds through different mediums. BMI and age play a role in SWE acquisition since a higher BMI is typically associated with a thicker layer of subcutaneous fat, which increases the depth of acquisition. Additionally, the entire body composition, especially within the muscle,

changes with aging. Lastly, joint position changes the degree in which the muscle fibers are stretched or compressed, changing the density of the medium in which the shear waves travel, affecting SWE values.

The amount of gel used by the operator has also been a topic of discussion in the domain of SWE, since it has been shown to affect measurements in breast and thyroid tissue (Barr and Zhang 2012; Lam et al. 2016). Standoff gel describes the spreading of approximately 5mm of ultrasound gel on the skin, which is not deformed when the transducer comes in contact with the gel during measurement (Alfuraih et al. 2018). The technique described as normal probe load is when the transducer comes in direct contact with the skin using a minimal layer of gel, causing flattening of the gel, but not deformation of the superficial epimysium layer of muscle. It has been shown the SWV values of muscle decreases with standoff gel, but negligibly (Alfuraih et al. 2018). However, it is important to note that standoff gel decreased the intraclass correlation coefficients (ICC) in this study, correlating to reduced reliability. Microbubbles in the gel layer of standoff gel may affect the quality of the push pulse from the transducer, resulting in larger variance and lower reliability. Variance can be defined as the degree of deviation from the actual value. The greater the variance, the greater the uncertainty, as there is a larger range or distribution in which the exact value can be found, making the measurement less precise. On the other hand, others support that errors can be avoided by using copious amounts of gel with light pressure (Cortez et al. 2016) or standoff gel when evaluating muscle at shallow depths (Taljanovic et al. 2017). A generalized conclusion of how much gel should be used in SWE has therefore not yet been conclusively reached.

SWE is also limited by depth. Variance in SWV has been shown to increase proportionally to depth, limiting SWE’s ability to effectively analyze the characteristics of deeper lying muscles (Alfuraih et al. 2018) (**Figure 6**). However, there are conflicting views as to whether SWV values themselves increase or decrease with depth (Carpenter et al. 2015; Ewertsen et al. 2016), but it is still considered an important limitation of SWE (Taljanovic et al. 2017). In general, the reliability of SWE values is higher for muscles that lie superficial in comparison to muscles that are deeper

(Alfuraih et al. 2018). There is, however, no known cut-off point for the acceptable variability in SWE, but guidelines for Thyroid SWE recommend that acquisitions should not be taken at depths deeper than 4-5 cm (Cosgrove et al. 2012). This guideline can be carried over for SWE of muscle (Alfuraih et al. 2018) .

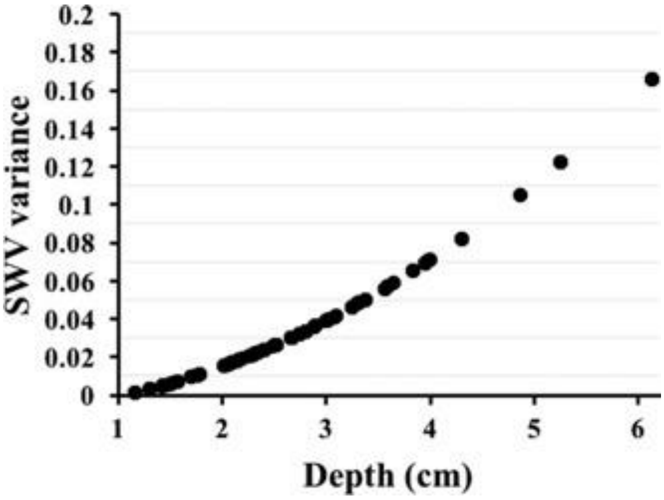

Figure 6. The effect of depth on SWE variance *(figure from (Alfuraih et al. 2018)*: The variance in SWE values acquired from the vastus lateralis muscle increased proportionally with increasing depth, reaching 0.07 (+/- 0.53 m/s) at 4 cm and 0.17 (+/- 0.82 m/s) at 6 cm. From this data, it has been postulated that the maximum depth of acceptable variability in SWE of muscle lies at 4 cm.

The anisotropic nature of muscle itself presents another important limiting factor to consider when collecting SWE values. Anisotropy describes the presence of various substances, such as muscle fibers, veins, arteries, nerve tissue, fatty tissue and connective tissue within the muscle, which all contribute to its heterogeneity. Since there are so many kinds of tissues found in muscle, the ultrasound waves have different speeds, depending on what they encounter. Tendons, for example, have much different SWV than muscle fibers themselves (Kaafarini, 2018). The waves also do not propagate in non-viscous liquids and SWE values may be affected by calcifications (Kaafarini, 2018). In general, the greater the anisotropy of the tissue, the greater the increase in SWV and the less reliable the values are (Alfuraih et al. 2017). The anisotropy of the selected field may be partially controlled by placing the transducer in the longitudinal plane in relation to the muscle so the shear waves travel parallel to the muscle fibers

(Alfuraih et al. 2017; Eby et al. 2013; Gennisson et al. 2013). Therefore, it is important to line up the transducer parallel to the muscle fibers in view, not the muscle itself or body part, for the most reliable SWE values (Alfuraih et al. 2017) and the reason why muscles with variable pennation angles (the angles at which muscle fibers run in relation to the longitudinal axis of the muscle itself) such as in the gastrocnemius muscle, produce less reliable SWE values (Brandenburg et al. 2014; Drakonaki 2012).

Another factor which affects SWE values is joint position. The shear modulus of the biceps brachii muscle at full extension was significantly higher ($p < 0.0001$) than when the elbow was bent at 90° (Eby et al. 2015). In both positions, the muscle was not actively contracted, but at rest. This suggests that passive stretch in the muscle can influence SWE values. Additionally, shear modulus values were significantly higher ($p < 0.01$) for the semimembranosus and semitendinosus muscle when the hip was bent at 90° compared to when the hip at 0° (Berrigan et al. 2020), regardless of knee position. Shear modulus values were also higher in both muscles when the knee was fully extended compared to bent at 90° for both muscles, when the hip was likewise bent at 90°. There was, however, no significant difference when the hip was positioned at 0°. These studies demonstrate that designating standard positions for each muscle, where the muscle is neither stretched nor contracted, is crucial for the implementation of SWE in muscle diagnostic.

Inter- and intraoperator reliability in SWE has been tested and shown to have validity in several studies (Chino et al. 2014; Cortez et al. 2016). Reliability, or the ability to produce the same or similar values at different times, is typically calculated using ICC (intraclass correlation coefficient). ICC can measure the reliability index in test-retest situations among one operator (intraoperator reliability) or between different operators (interoperator reliability). The higher the ICC value, the greater the reliability (Koo and Li 2016). ICC values less than 0.5 are considered poor, between 0.5 and 0.75 are moderate, between 0.75 and 0.9 are good and greater than 0.90 are excellent.

On one hand, inter- and intraoperator reliability in SWE values decreases, for example, when the muscles are evaluated in the transverse plane (Cortez et al. 2016). This is because shear waves travel perpendicularly to the muscle fibers, instead of parallel along them. Other factors that may contribute to this phenomenon include amount of the preload exerted by the operator on the probe (in the case of interoperator reliability) and the specific location of the muscle selected to examine and the muscle's natural anisotropy (in the case of intraoperator reliability). SWV has also been demonstrated to be higher in the transverse plane. Therefore, it has been agreed among many researchers that SWE of muscle should be performed longitudinally, so that the shear waves travel as close to parallel through the muscle fibers as possible (Alfuraih et al. 2018; Eby et al. 2015; Gennisson et al. 2013; Taljanovic et al. 2017), since the precision of SWE measurements has been shown to be higher in the longitudinal plane (Cortez et al. 2016). This has been instilled because shear waves propagate most readily parallel to the muscle fibers in the longitudinal plane of the muscle.

1.6 The need for reference values in SWE

Currently, reference SWE values for skeletal muscles have not yet been fully investigated. A few reference values for the anterior tibialis muscle, gastrocnemius muscle and soleus muscle have been published (Ryu and Jeong 2017). However, reference values for other important muscles, such as the triceps brachii and biceps femoris muscle, that are optimal for SWE, have not yet been well documented. The greatest issue in collecting reference values is that that SWE values may vary depending on sex, age and BMI. Other factors that contribute could be muscle thickness or different isotropy depending on sex and/or general athletic ability (Alfuraih et al. 2017; Fujiwara et al. 2010; Janssen et al. 2000; Ryu and Jeong 2017).

In order to use SWE as a reliable diagnostic tool, reference values must to be collected from a healthy population of individuals, both female and male, in various age ranges with various BMIs. The establishment of baseline SWE values of various muscles is important for the conceptualization of reference values that may be used as cut-offs in the diagnostic of various musculoskeletal diseases.

2 Aims and hypotheses

The general aim of this study was to acquire reference SWE values for skeletal muscles, as the compilation of these values is important for the establishment of reference ranges to eventually be used in clinical muscle diagnostic.

The specific aims of this study were to investigate if there is a difference in SWE between men and women, as well as between younger (< 35 years) and elderly individuals (> 55 years). This study also considered the possible effect of BMI on SWE and investigated which muscles were most reliably measurable, i.e., without high variance in measured values. Among these muscles, joint position was assessed to investigate which are most appropriate for SWE. Lastly, the inter- and intraoperator reliability of SWE was tested. In concrete terms the following hypotheses were tested:

(1) If not explicitly considering the joint position, SWE and its variance will be significantly higher than when controlling the joint position

(2) Elderly individuals will have higher SWE, most likely due to the stiffening of muscle that comes with aging – i.e. the reduction of extracellular water and the atrophy of muscle and replacement with connective tissue.

(3) Higher BMIs will have lower SWE since the density of fat (0.9 g/cm^3) is less than the density of muscle (1.06 g/cm^3) .

(4) Deeper muscles will exhibit greater variance in SWE and are therefore less suitable to measure, since here the method of SWE might be limited.

(5) SWE in muscles may be significantly different between men and women and that men will have higher SWE, since it is known that men exhibit generally higher muscle density.

3 Methods

3.1 Participants

All healthy volunteers recruited had no reported history or clinical signs of neuromusclar disease. All participants were asked to refrain from doing any athletic, strenuous activity before the examination to ensure the muscles were in an optimal, relaxed state. Only muscles on the right side of the body were investigated. All participants were over the age of 18 and provided informed consent through written documentation. The ethics committee of the University of Tübingen approved the study and the examinations were carried out abiding by the Declaration of Helsinki.

Healthy volunteers for this study were recruited in 3 groups. In the first group, an initial protocol, Protocol 1 (no joint control), was carried out. Details of the characteristics of this cohort are found in **Table 1**. In this protocol, only 2 different body positions were used. However, more muscles were studied. In Protocol 1 (no joint control), 25 healthy volunteers (17 males and 8 females) were recruited through contacts of the Neurology department in Tübingen. The mean age [standard deviation (SD)] of the participants was 33.0 (13.3) years and the mean body mass index (BMI) was 23.1 (2.5).

Protocol 1 (no joint control)					
Participant	Age (years)	Height (cm)	Weight (kg)	BMI	Sex
H1	27	164	60	22.3	f
H2	28	185	68	19.9	m
H3	32	180	72	22.2	m
H4	27	176	65	21.0	f
H5	26	178	81	25.6	m
H6	29	185	82	24.0	m
H7	29	163	53	19.9	f
H8	30	178	82	25.9	m
H9	23	171	62	21.2	f
H10	33	173	63	21.0	m
H11	33	158	59	23.6	f
H12	25	186	85	24.6	m
H13	28	164	57	21.2	f
H14	37	171	62	21.2	m
H15	68	172	85	28.7	f
H16	67	190	96	26.6	m
H17	25	185	83	24.3	m
H18	25	190	90	24.9	m
H19	35	180	69	21.3	m
H20	30	175	66	21.6	m
H21	23	172	64	21.6	f
H22	24	188	73	20.7	m
H23	22	181	76	23.2	m
H24	33	185	75	21.9	m
H25	65	175	89	29.1	m

Table 1. A summary of the characteristics in the subjects examined in Protocol 1 (no joint control).

After refinement of the protocol to create Protocol 2 (joint control), 27 participants (15 males and 12 females) under the age of 60 years old were recruited through contacts within the department. The development of this protocol came through further research within the literature as well as individual testing with the ultrasound device. Under this cohort, the mean age was 26.5 (3.1) years and the average BMI was 21.7 (2.2). Further descriptions of this group are found in **Table 2.**

Protocol 2 (joint control, young)					
Participant	Age (years)	Height (cm)	Weight (kg)	BMI	Sex
H26	30	186	61	17.6	m
H27	24	175	75	24.5	m
H28	23	165	58	21.3	f
H29	29	189	75	21.0	m
H30	25	188	83	23.5	m
H31	24	170	53	18.3	f
H32	24	175	63	20.6	m
H33	28	177	72	23.0	m
H34	25	170	56	19.4	f
H35	24	172	63	21.3	f
H36	28	185	68	19.9	f
H37	25	160	54	21.1	f
H38	29	168	54	19.1	f
H39	21	181	76	23.2	m
H40	25	178	73	23.0	m
H41	31	166	52	18.9	f
H42	32	180	67	20.7	m
H43	29	174	64	21.1	f
H44	28	179	82	25.6	m
H45	23	173	68	22.9	f
H46	26	186	85	24.6	m
H47	26	178	80	25.2	m
H48	27	176	64	20.7	f
H49	22	182	82	24.8	m
H50	33	180	73	22.5	m
H51	26	164	60	22.3	f
H52	28	174	59	19.5	m

Table 2. A summary of the characteristics in the younger subjects examined in Protocol 2 (joint control, young).

Lastly, 10 participants (7 male and 3 female) over the age of 55 years were recruited for the study through contacts within the Neurology Department in Tübingen as part of Protocol 2 (joint control, elderly). This group was examined using the same protocol as in Protocol 2 (joint control, young). In this group, the average age was 64.0 (8.5) years and the average BMI was 26.4 (2.6). A summary of the characteristic in this group are found in **Table 3.**

Protocol 2 (joint control, elderly)					
Participant	Age (years)	Height (cm)	Weight (kg)	BMI	Sex
H53	63	183	80	23.9	m
H54	68	172	85	28.7	f
H55	67	190	96	26.6	m
H56	57	185	80	23.4	m
H57	56	173	74	24.7	m
H58	83	174	87	28.7	m
H59	67	192	94	25.5	m
H60	55	172	72	24.3	f
H61	57	194	100	26.6	m
H62	67	178	100	31.6	f

Table 3. A summary of the characteristics in the elderly subjects examined in Protocol 2 (joint control, elderly).

3.2 Ultrasound imaging protocol

Ultrasound imaging of skeletal muscle was performed using a Canon Aplio i800 system. The superficial probe, PLI 1205 BX / i18Lx5, was the selected transducer. The square shear wave box depicting the measured area measured an area of 2x2cm. The box was typically placed in the most superficial. homogenous area of the muscle found. In propagation mode, the shear waves were visualized using colored bands. The beginning of the tracking was presented in a cooler, blue color and the end of the area in a warmer color, such as orange or red. A color-coded map was also displayed showing the general homogeneity of the selected tissue. Ideally, this color-coded map was a relatively homogenous blue color and was used to select a uniform area of muscle tissue to measure. In these areas, the muscle fibers ran parallel to one another and few. if not any blood vessels were in the frame of reference. To prevent probe-induced stiffness, the minimal sufficient pressure was applied to the probe without deforming the subcutaneous tissue and ultrasound gel was used to enhance contrast. A copious amount of gel was used to enhance imaging. The majority of the presets of this system for muscle in SWE Imaging were used. However, the following were altered: region of interest size (ROI): 4, time smoothing: 0 (without time averaging of the SWE values between each picture recording), frame rate: 2 (one picture recorded per second), map type: speed (shear wave velocity in meters per second).

3.3 Body and muscle positioning

In Protocol 1 (no joint control), SWE of various muscles in the upper extremity, lower extremity and back were measured. A detailed description of the body positions used in Protocol 1 (no joint control) are described in **Table 4**. 3 SWE measurements and the according standard deviation of each measurement were recorded. For the muscles of the upper extremity. volunteers were asked to lay on their back with their arm stretched out laying next to their side on a basic examination table. When measuring the SWE of the back muscles. participants were asked to lay on their stomach with their hands at their side. For the lower extremity muscles, volunteers were asked to lay on their stomach or back depending on if the muscle was on the ventral or dorsal side of the body.

The protocol was then adjusted, creating Protocol 2 (joint control), optimizing the position of the muscles so that they were not strained in terms of stretch or contraction, but in the most optimally relaxed state (**Table 5**). It was also decided to narrow down the number of muscles that were investigated, focusing on the muscles that were the most optimal to measure – meaning larger, more superficial lying muscles. The measurement of these muscles have a higher reliability in comparison to deeper muscles. This was decided based on the analysis of the results from Protocol 1 (no joint control) and from experimenting with different body positions for each muscle.

Protocol 1 (no joint control)			
Location	**Position Description**	**Muscles Examined**	**Further Specifications**
Upper Extremity	Supine, arms and legs stretched out, resting on examination table	Deltoid muscle (DE)	Pars acromialis
		Biceps brachii muscle (BB)	5cm proximal of the distal insertion tendon
		Extensor carpi radialis muscle (ECR)	
		Flexor digitorum profundus muscle (FDP)	
		Triceps brachii muscle (TR)	5cm proximal of the distal insertion tendon
Back	Prone, arms stretched with hands at their sides	Multifidius muscle C8 (MU C8)	
		Erector spinae muscle Th10 (ES Th10)	
		Erector spinae muscle L3 (ES L3)	
Lower Extremity	Prone, arms and legs stretched out, resting on examination table	Vastus lateralis muscle (VA)	5cm proximal of the distal insertion tendon
		Tibialis anterior muscle (TA)	5cm distal of the proximal insertion tendon
	Prone, arms stretched with hands at their sides	Biceps femoris muscle, caput longum (BF)	
		Gastrocnemius muscle, caput mediale (GCM)	

Table 4. A summary of joint position and SWE acquisition location for each muscle examined in Protocol 1 (no joint control).

Protocol 2 (joint control)			
Location	**Position Description**	**Muscles Examined**	**Further Specifications**
Upper Extremity	Supine, elbow resting on a pillow. arm bent at the elbow 90°	Deltoid muscle (DE)	Pars acromialis
		Biceps brachii muscle (BB)	5cm proximal of the distal insertion tendon
		Extensor carpi radialis muscle (ECR)	
	Supine, arm stretched out	Flexor digitorum profundus muscle (FDP)	
	Left lateral recumbent, arm stretched out, resting on hip	Triceps brachii muscle (TR)	5cm proximal of the distal insertion tendon
Lower Extremity	Prone, legs almost completely stretched out with a small pillow under the knees	Vastus lateralis muscle (VA)	5cm proximal of the distal insertion tendon
			5cm distal of the proximal insertion tendon
	Sitting, feet flat on the floor	Biceps femoris muscle, caput longum (BF)	
	Sitting, lower leg free hanging	Tibialis anterior muscle (TA)	5cm distal of the proximal insertion tendon
		Gastrocnemius muscle, caput mediale (GCM)	5cm proximal of the distal insertion tendon

Table 5. A summary of joint position and SWE acquisition location for each muscle examined in Protocol 2 (joint control).

3.4 Statistical analysis

The statistical analysis was performed using SPSS 27.0 Software (IBM, Armonk, New York, USA). For each protocol, descriptive statistics were computed – including the mean, median, range, standard deviation and variance. Normal distribution was tested using descriptive statistics. Due to the smaller sample sizes, the majority of the data was not normally distributed. Therefore, Mann Whitney U Tests were used to test for significant differences in SWE between groups such as age (young vs. elderly), sex (men vs. women) and BMI (normal weight vs. overweight), Additionally differentiation in the variance between Protocol 1(no joint control) and Protocol 2 (joint control, young) was tested for using the Wilcoxon Signed Rank test. For all tests, the significance level was set to $p < 0.05$. All graphs were created using SPSS.

4 Results

4.1 Average SWE Results

The average SWE for the muscles examined in Protocol 1 (no joint control) were consistently higher across all muscles than in Protocol 2 (joint control) (**Figure 7, Figure 8, Table 6**). When not controlling for joint position (Protocol 1), the standard deviation of the measurements were also consistently higher than when controlling specifically for joint position (Protocol 2 – Groups 1 & 2). The muscles in the back - MU (C8), ES (Th10) and ES (L3) - exhibited some of the highest SWE averages and variance in Protocol 1 (no joint control). These muscles were particularly small, lay deep and were particularly difficult to examine. Due to the impracticality of these muscles for SWE, they were not further researched in Protocol 2 (joint control). Sample SWE pictures from Protocol 2 (joint control) can be found in **Figures 9 and 10**.

Muscle	**Protocol 1** *(no joint control)* *n=25*	**Protocol 2** *(joint control, young)* *n=27*	**Protocol 2** *(joint control, elderly)* *n=10*	**Protocol 2** *(joint control, young & elderly)* *n=37*
	SWE m/s (SD)	SWE m/s (SD)	SWE m/s (SD)	SWE m/s (SD)
DE	3.35 (0.88)	2.26 (0.49)	2.32 (0.42)	2.27 (0.47)
BB	4.14 (0.97)	1.95 (0.30)	1.99 (0.45)	1.96 (0.34)
ECR	3.28 (0.94)	2.51 (0.37)	2.58 (0.86)	2.53 (0.62)
FDP	3.07 (1.57)	2.30 (0.37)	4.26 (2.27)	2.82 (1.49)
TR	3.22 (1.19)	1.81 (0.42)	2.11 (0.51)	1.88 (0.46)
MU (C8)	4.51 (1.31)			
ES (Th10)	3.96 (1.28)			
ES (L3)	4.75 (1.74)			
VA	1.96 (0.86)	1.68 (0.34)	1.64 (0.38)	1.66 (0.35)
BF	3.24 (1.40)	2.31 (0.38)	2.74 (1.40)	2.43 (0.81)
TA	3.21 (0.81)	2.56 (0.32)	2.73 (0.85)	2.61 (0.52)
GCM	3.00 (0.94)	2.18 (0.43)	2.20 (0.40)	2.20 (0.40)

Table 6. Summary of the average SWE and standard deviation for each muscle examined in all protocols and groups of this study.

DE = deltoideus. BB = biceps brachii. ECR = extensor carpi radialis. FDP = flexor digitorum profundus. TR = triceps brachii. MU (C8) = multifidius (C8). ES (Th10) = Erector spinae (Th10). ES (L3) = Erector spinae (L3). VA = vastus lateralis. BF = biceps femoris (caput longum). TA = tibialis anterior. GCM = gastrocnemius (caput mediale).

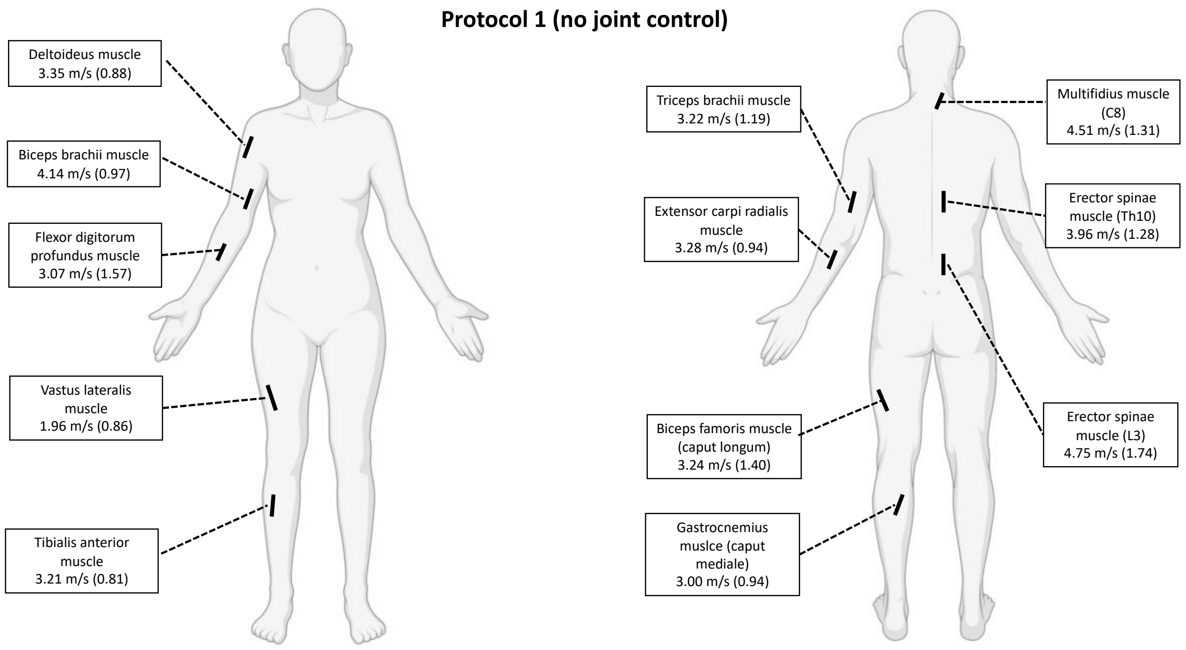

Figure 7. Simplified representation of the muscle measurement locations and SWE averages in Protocol 1 (no joint control). (*Own representation*): The average SWE in m/s (standard deviation) for each muscle examined in Protocol 1 (no joint control) is shown. Additionally, the approximate location and orientation of the ultrasound probe in which each muscle was measured is depicted by the solid black lines.

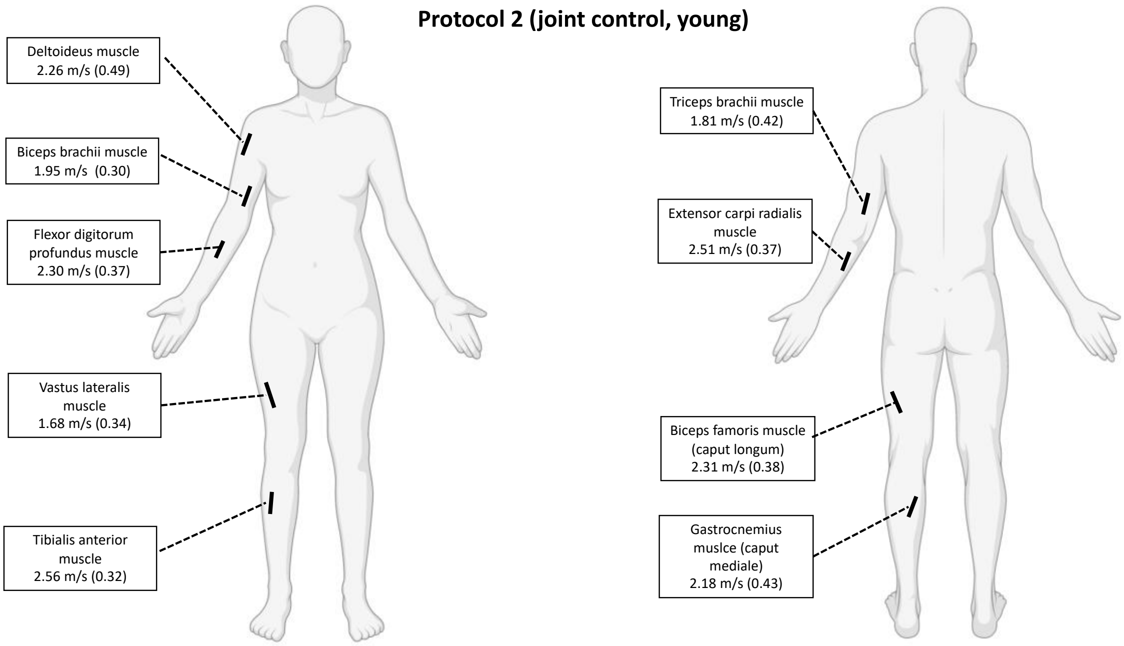

Figure 8. Simplified representation of the muscle measurement locations and SWE averages in Protocol 2 (joint control, young). (*Own representation*): The average SWE in m/s (standard deviation) for each muscle examined in Protocol 2 – Group 1 (joint control, young) is shown. Additionally, the approximate location and orientation of the ultrasound probe in which each muscle was measured is depicted by the solid black lines.

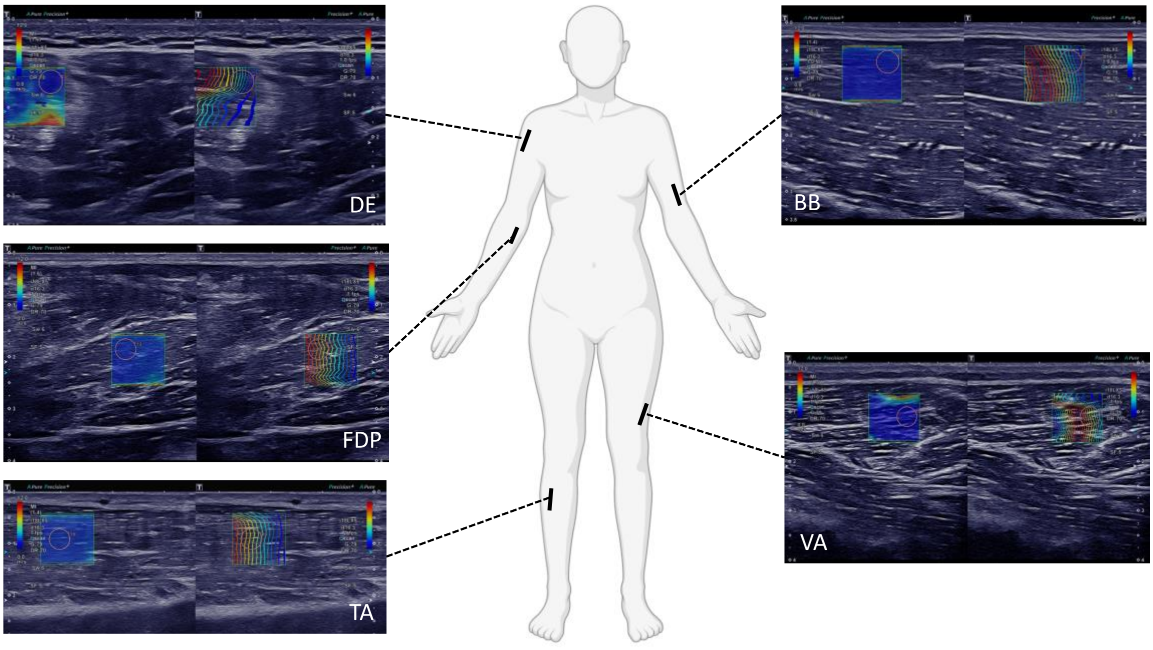

Figure 9. SWE pictures of DE, FDP, TA, BB and VA during the examination of a 32 year old male (H42) in Protocol 2 (joint control, young). SWE pictures of the deltoid (DE), flexor digitorum profundus (FDP), tibialis anterior (TA), biceps brachii (BB) and vastus lateralis (VA) muscle are shown. On the left side of each picture, the B-mode images in gray scale are overlaid with SWV data in color. The cooler colors, such as the blue in these pictures, depict slower shear wave speeds, typically ranging from 0-6 m/s. As predicted in Protocol 2 (joint control), the muscles were positioned in an optimally relaxed states, to avoid strain, which was demonstrated by the consistent blue coloring within the SWE pictures. On the right side of each picture, the shape of the shear waves is displayed with lines. The blue lines represent the origin of the shear waves and the red lines represent the change in the shear waves as they propogate accordingly through the muscle. In these pictures, the greater depth of acquisition required for FDP and DE can be seen. The layer of subcutaneous fat above the DE was typically thick than for TA, BB and VA. Additionally, the greater pennation angle of DE is illustrated in that the muscle fibers could not be completely optimally displayed to parallel in the longitudinal plane. Alternatively, the path of the muscle fibers of FDP, TA, BB and VA could be displayed well in the longitudinal plane.

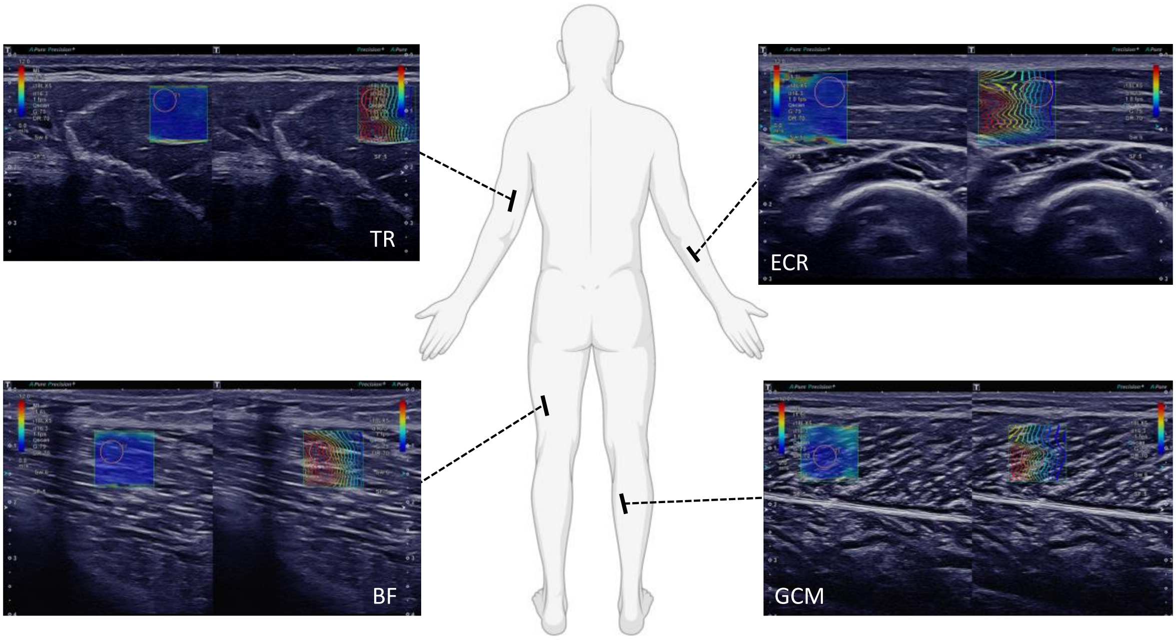

Figure 10. SWE pictures of TR, BF, ECR and GCM acquired during the examination of a 32 year old male (H42) in Protocol 2 (joint control, young). SWE pictures of the triceps brachii (TR), biceps femoris caput longum (BF), extensor carpi radialis (ECR) and gastrocnemius caput mediale (GCM) muscle are shown. On the left side of each picture, the B-mode images in gray scale are overlaid with SWV data in color. The cooler colors, such as the blue in these pictures, depict slower shear wave speeds, typically ranging from 0-6 m/s. As predicted in Protocol 2 (joint control), the muscles were positioned in an optimally relaxed states, to avoid strain, which was demonstrated by the consistent blue coloring within the SWE pictures. On the right side of each picture, the shape of the shear waves are depicted by lines within the green outlined box. The blue lines represent the origin of the shear waves and the red lines represent the change in the shear waves as they propogate accordingly through the muscle. In these pictures, the greater depth of acquisition required for TR and BF compared to ECR and GCM can be seen. The layer of subcutaneous fat above the TR and BF was typically thicker than for ECR and GCM. Additionally, the greater pennation angles in TR and GCM are illustrated in which the muscle fibers could not be completely optimally displayed to parallel in the longitudinal plane. Alternatively, the path of the muscle fibers in ECR and BF could be displayed well in the longitudinal plane.

4.2 Variance

As hypothesized, SWE variance was significantly higher (p = 0.008) in Protocol 1 (no joint control) in comparison to precise joint position in Protocol 2 (joint control, young) (**Figure 11**), where the muscles were positioned an optimal rested state. The averages and standard deviations for each group are depicted in **Figures 7 and 8**. Variance was tested using the Related-Samples Wilcoxon Rank Test. The variance in these two groups were optimal to compare based on the similar number of subjects [n=25 in Protocol 1 (no joint control), n=27 in Protocol 2 – Group 1 (joint control, young)] and average ages [Protocol 1 (no joint control) - 33 years, Protocol 2 – Group 1 (joint control, young) - 26.5 years].

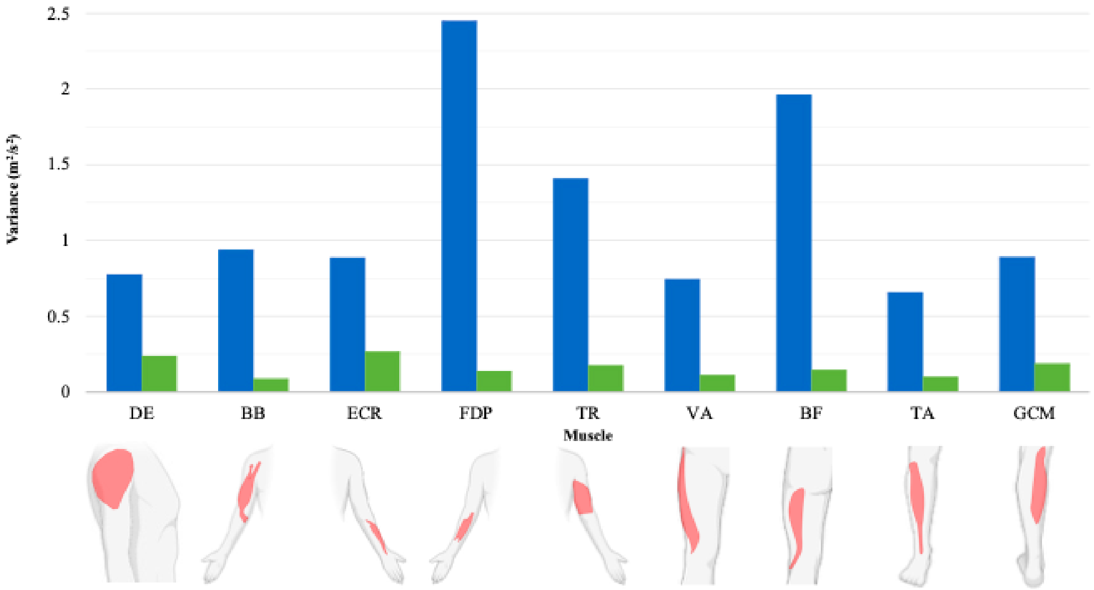

Figure 11. Variance in SWE depending on joint control (*Own representation)*: Variance in Protocol 1 (no joint control) (blue) was significantly higher than in Protocol 2 (joint control) (green) when comparing 9 muscles evaluated in this study. The variances all remained under 0.3 m^2/s^2 in Protocol 2 (joint control), whereas the variance in Protocol 1 (no joint control) was consistently higher than 0.6 m^2/s^2 and could range up to almost 2.5 m^2/s^2.

BB = biceps brachii. BF = biceps femoris (caput longum). DE = deltoid. ECR = extensor carpi radialis. FDP = flexor digitorum profundus. GCM = gastrocnemius (caput mediale). TA = tibialis anterior. TR = triceps brachii. VA = vastus lateralis.

4.3 Depth

As predicted, the variance was particularly high for deeper lying muscles such as in BF (1.963) and FDP (2.450) in Protocol 1 (no joint control) (**Table 7**). In Protocol 2 - Group 1 (joint control, young), the variance was the highest among ECR (0.266) and DE (0.239). However, in Protocol 2 – Group 2 (joint control, elderly), the variance was once again highest in FDP (5.137) and BF (1.946), similar to Protocol 1 (no joint control).

Muscle	**Variance** **Protocol 1** *(no joint control)* *n=25*	**Variance** **Protocol 2 – Group 1** *(joint control, young)* *n=27*	**Variance** **Protocol 2 – Group 2** *(joint control, elderly)* *n=10*	**Variance** **Protocol 2 – Groups 1 & 2** *(joint control, young & elderly)* *n=37*
DE	0.776	0.239	0.176	0.221
BB	0.941	0.089	0.203	0.119
ECR	0.887	0.266	0.737	0.389
FDP	2.450	0.140	5.137	2.226
TR	1.410	0.177	0.263	0.216
VA	0.745	0.114	0.147	0.122
BF	1.963	0.147	1.946	0.658
TA	0.656	0.101	0.738	0.273
GCM	0.892	0.189	0.158	0.179

Table 7. Variances in muscle SWE in all protocols and groups of this study.

DE = deltoideus muscle. BB = biceps brachii. ECR = extensor carpi radialis. FDP = flexor digitorum profundus. TR = triceps brachii. VA = vastus lateralis. BF = biceps femoris (caput longum). TA = tibialis anterior. GCM = gastrocnemius (caput mediale).

4.4 Age (Young vs. Elderly)

Elderly individuals exhibited higher SWE all muscles except VA (**Table 8, Figure 12**). When comparing the cohort of Protocol 2 – Group 1 (joint control, young) to Protocol 2 – Group 2 (joint control, elderly), there was a significant difference in the SWE of FDP ($p < 0.001$) and TR ($p = 0.006$) (**Figure 13**). The average SWE in FDP of the younger group was 2.30 m/s, compared to 4.26 m/s for the elderly group. Similarly, the average SWE among the younger population was slower at 1.81 m/s in TR, compared to 2.11 m/s in the elderly.

Muscle	**Protocol 2 – Group 1** *(joint control, young)* *n=27*	**Protocol 2 – Group 2** *(joint control, elderly)* *n=10*
	SWE m/s (SD)	SWE m/s (SD)
DE	2.26 (0.49)	2.32 (0.42)
BB	1.95 (0.30)	1.99 (0.45)
ECR	2.51 (0.37)	2.58 (0.86)
FDP*	2.30 (0.37)	4.26 (2.27)
TR*	1.81 (0.42)	2.11 (0.51)
VA	1.68 (0.34)	1.64 (0.38)
BF	2.31 (0.38)	2.74 (1.40)
TA	2.56 (0.32)	2.73 (0.86)
GCM	2.18 (0.43)	2.20 (0.40)

Table 8. Comparison of average muscle SWE between young and elderly. When comparing the SWE based on age, there was a significant difference was observed in FDP (*p* < 0.001) and TR (*p* = 0.006) among the young and elderly, as designated by *. The average SWE was lower in the younger population in all muscles except for VA. DE = deltoideus. BB = biceps brachii. ECR = extensor carpi radialis. FDP = flexor digitorum profundus. TR = triceps brachii. VA = vastus lateralis. BF = biceps femoris (caput longum). TA = tibialis anterior. GCM = gastrocnemius (caput mediale).

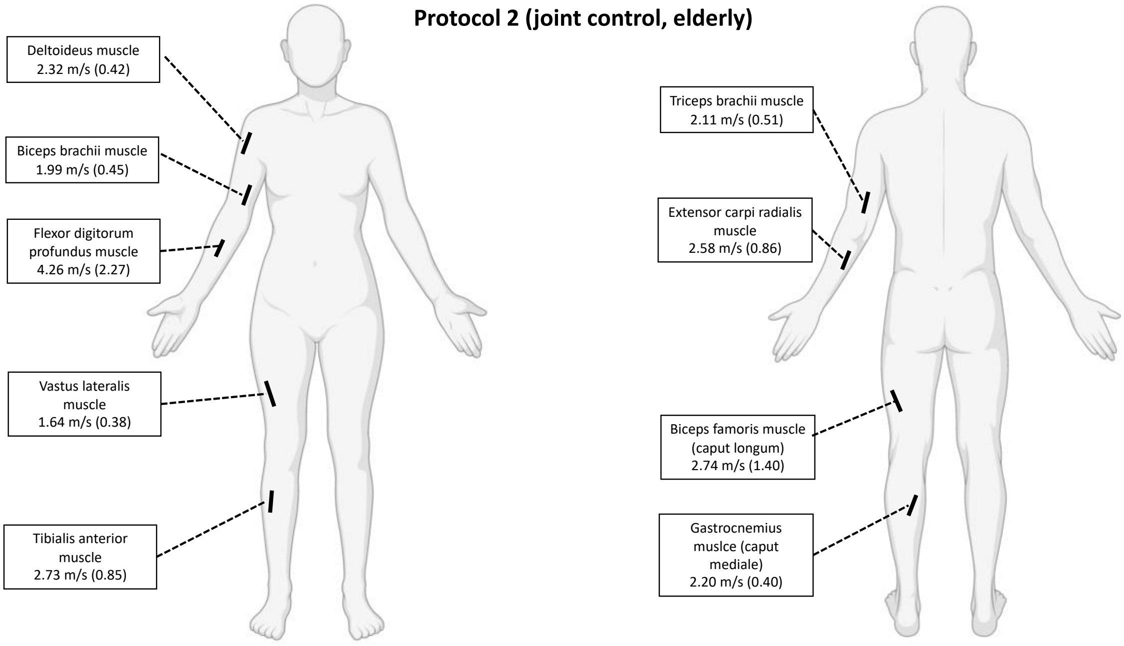

Figure 12. Simplified representation of the muscle measurement locations and SWE averages for Protocol 2 – Group 2 (joint control, elderly). (*Own Representation*): The average SWE in m/s (standard deviation) for each muscle examined in Protocol 2 (joint control, elderly) is shown. Additionally, the approximate location and orientation of the ultrasound probe in which each muscle was measured is depicted by the solid black lines.

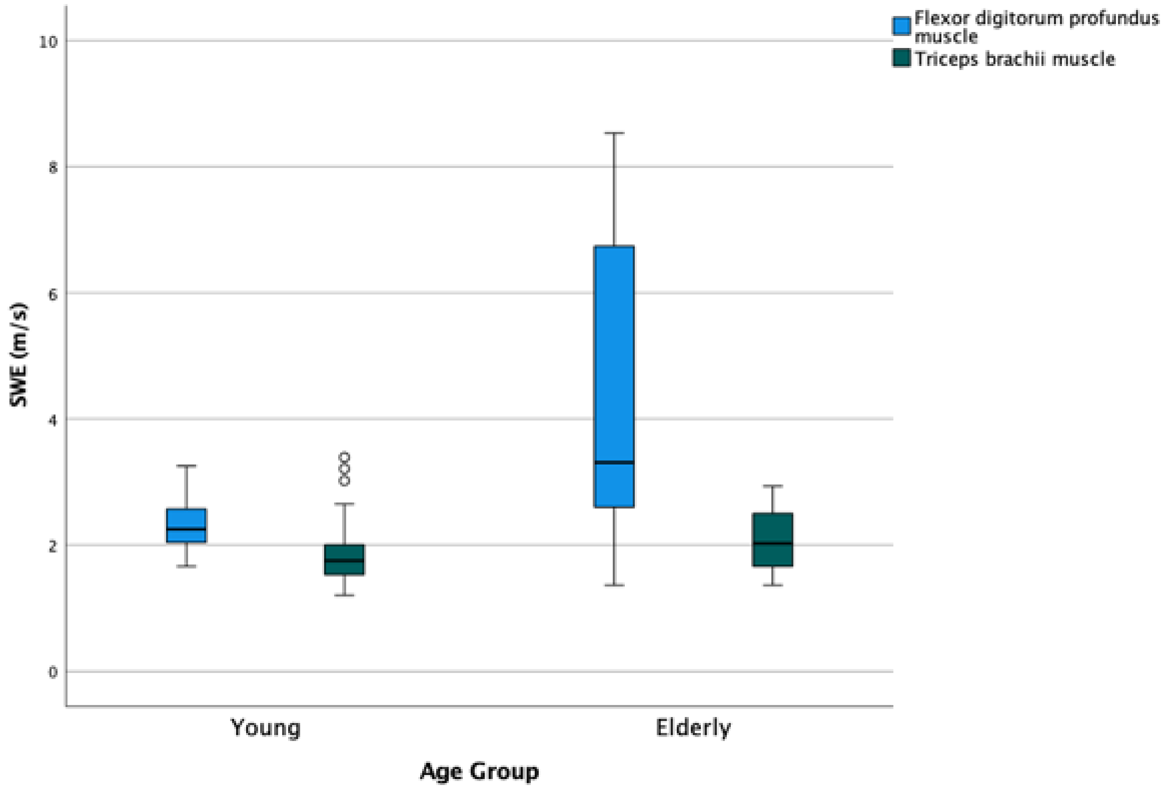

Figure 13. Significant differences in muscle SWE depending on age group. There was a significant difference in the SWE of the flexor digitorum profundus muscle (*p* < 0.001) and the triceps brachii (*p* = 0.006) muscle depending on age group. The young group (blue) represents SWE from participants ≤ 35 years old and the elderly group (green) designates the SWE from participants ≥ 55 years old. For the flexor digitorum profundus muscle, the young had an average SWE of 2.30 m/s and the elderly had an average of 4.26 m/s. In the triceps brachii muscle, the averages were 1.81 m/s and 2.11 m/s respectively.

4.5 Sex (Men vs. Women)

The average SWE was higher in men in DE, BB, FDP and BF (**Table 9**). In DE and FDP, this difference was significant (*p* = 0.04 and *p* = 0.002, respectively). For DE, men had an average SWE of 2.35 m/s and the women exhibited an average of 2.15 m/s (**Figure 14**). In FDP, these averages were 3.13 m/s and 2.39 m/s, respectively. However, women exhibited higher SWE averages in ECR, TR, VA, TA and GCM. The SWE of the muscles in females was significantly higher in TR (*p* = 0.01) and VA (*p* =

0.005). For TR, the averages were 2.06 m/s for women and 1.77 m/s for men. In VA, the means were 1.77 m/s and and 1.60 m/s, respectively.

Muscle	**Protocol 2 – Groups 1 & 2** *(joint control)* **Men** *n=22*	**Protocol 2 – Groups 1 & 2** *(joint control)* **Women** *n=15*
	SWE m/s (SD)	SWE m/s (SD)
DE*	2.35 (0.48)	2.16 (0.44)
BB	2.03 (0.41)	1.85 (0.17)
ECR	2.48 (0.52)	2.59 (0.75)
FDP*	3.13 (1.78)	2.39 (0.73)
TR°	1.77 (0.36)	2.06 (0.55)
VA°	1.60 (0.31)	1.77 (0.38)
BF	2.50 (1.01)	2.32 (0.36)
TA	2.55 (0.29)	2.70 (0.74)
GCM	2.17 (0.49)	2.21 (0.29)

Table 9. Comparison of average muscle SWE between men and women. The average SWE was higher in men in DE, BB, FDP, BF. In DE and FDP, this difference was significant, as signified by *.The women exhibited higher SWE in ECR, TR, VA, TA and GCM. The SWE was significantly higher in women in TR and VA, as designated by °.

DE = deltoideus muscle. BB = biceps brachii. ECR = extensor carpi radialis. FDP = flexor digitorum profundus. TR = triceps brachii. VA = vastus lateralis. BF = biceps femoris (caput longum). TA = tibialis anterior. GCM = gastrocnemius (caput mediale).

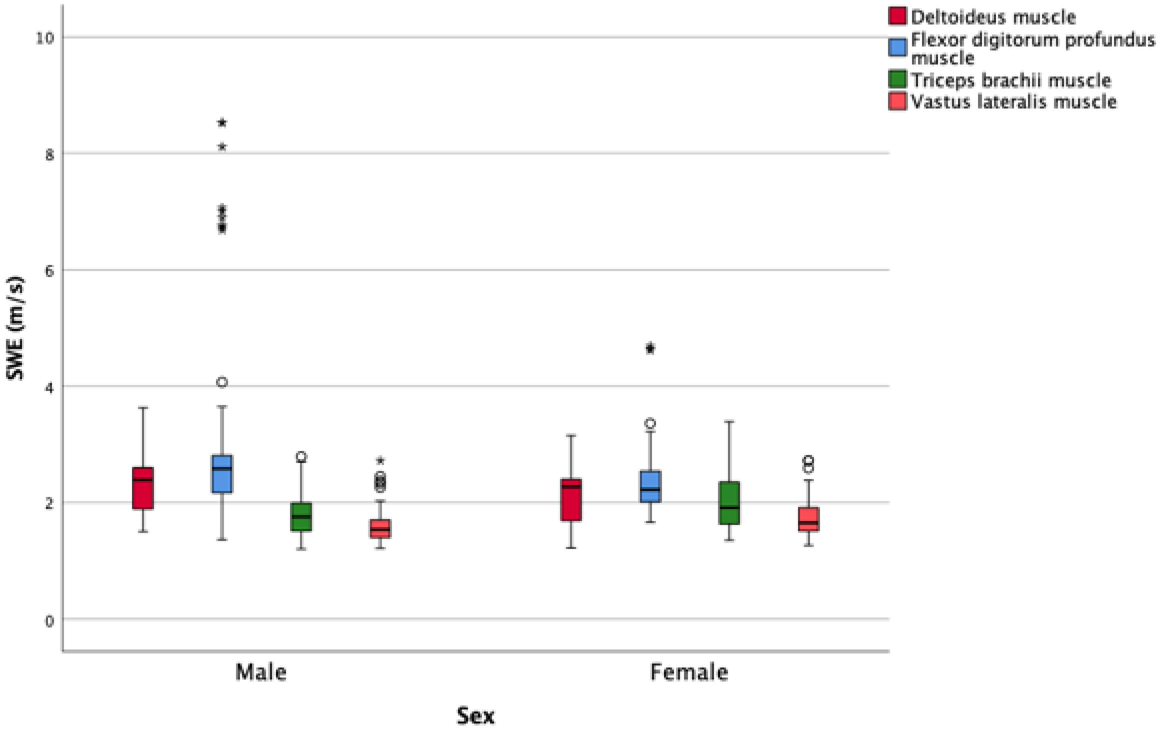

Figure 14. Significant differences in SWE in selective muscles depending on sex. A significant difference ($p < 0.05$) in the SWE of the deltoid (red), flexor digitorum profundus (blue), triceps brachii (green) and vastus lateralis muscle (orange) were observed. The average SWE was significantly higher in males for the deltoid (2.35 m/s for men, 2.16 m/s for women) and flexor digitorum profundus muscle (3.13 m/s for men, 2.39 m/s for women). However, mean SWE values were significantly higher in women for the triceps brachii (2.06 m/s for women, 1.77 m/s for men) and vastus lateralis muscle (1.77 m/s for women, 1.60 m/s for men).

4.6 BMI

Contrasting with the original prediction that individuals with higher BMIs (BMI $\geq$ 25 kg/m^2) would have lower SWEs, the average SWE was regularly higher for overweight individuals in all muscles except for ECR and GCM (**Table 10**). There was a significant difference ($p < 0.001$) between the SWE values of normal weight and overweight individuals in ECR (2.52 m/s for normal weight, 2.35 m/s for overweight) and FDP (2.49 m/s for normal weight, 4.07 m/s for overweight) (**Figure 15)**.

Muscle	**Protocol 2 – Groups 1 & 2** *(joint control)* **Normal Weight** *n=27*	**Protocol 2 – Groups 1 & 2** *(joint control)* **Overweight** *n=8*
	SWE m/s (SD)	SWE m/s (SD)
DE	2.23 (0.50)	2.39 (0.41)
BB	1.90 (0.21)	2.07 (0.55)
ECR*	2.52 (0.46)	2.35 (0.95)
FDP*	2.49 (0.96)	4.07 (2.31)
TR	1.85 (0.45)	2.02 (0.50)
VA	1.62 (0.32)	1.66 (0.32)
BF	2.31 (0.35)	2.94 (1.52)
TA	2.56 (0.29)	2.72 (0.96)
GCM	2.24 (0.40)	2.11 (0.42)

Table 10. Comparison of average muscle SWE between normal weight and overweight individuals. In general, the mean SWE was higher for overweight individuals in all muscles except for ECR and GCM. Significant differences ($p < 0.001$) in SWE values between normal weight and overweight individuals are designated by * and were found in ECR and FDP.

DE = deltoideus muscle. BB = biceps brachii. ECR = extensor carpi radialis. FDP = flexor digitorum profundus. TR = triceps brachii. VA = vastus lateralis. BF = biceps femoris (caput longum). TA = tibialis anterior. GCM = gastrocnemius (caput mediale).

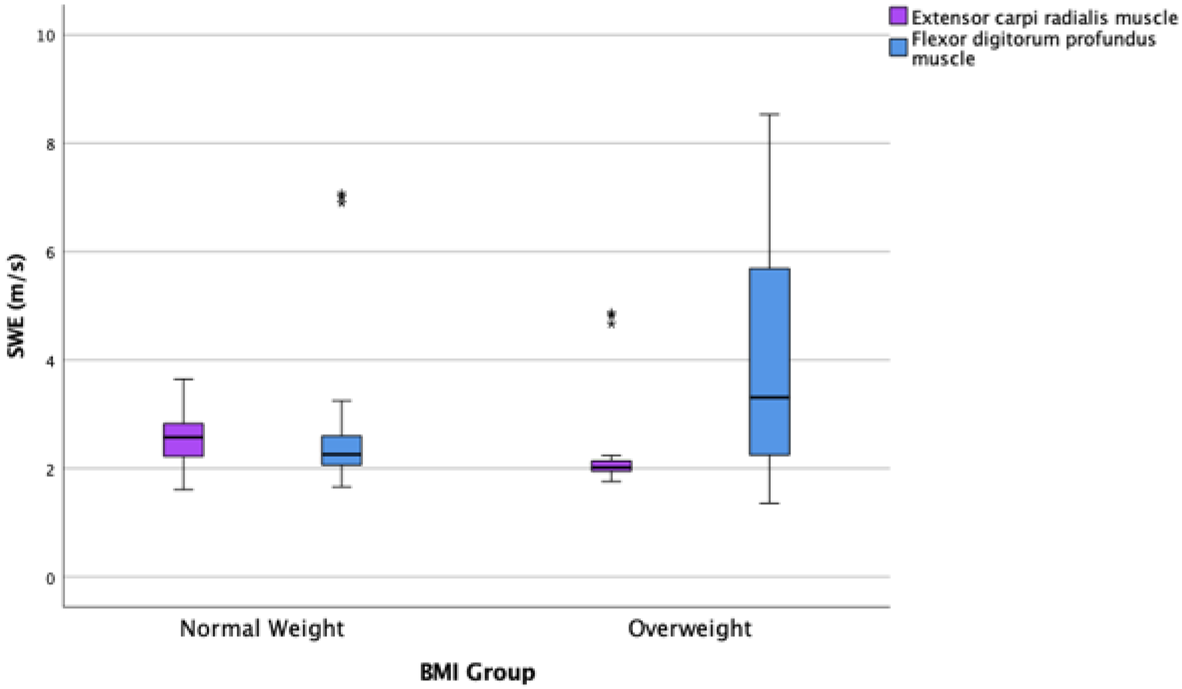

Figure 15. Significant differences in SWE in selective muscles depending on BMI. The mean SWE of the flexor digitorum profundus muscle (blue) was significantly higher ($p < 0.001$) in overweight (BMI $\geq$ 25 kg/m^2) individuals (4.07 m/s) than in normal weight (BMI 18.5 - 25 kg/m^2) individuals (2.49 m/s). Alternatively, the average SWE for the extensor carpi radialis muscle (purple) was significantly higher ($p < 0.001$) in normal weight individuals (2.52 m/s) than in overweight individuals (2.35 m/s).

5 Discussion

5.1 Average SWE Results

This study examined the SWE of various skeletal muscles in order to contribute reference values for muscle SWE, so that SWE can eventually be used in the realm of diagnostic for neuromuscular disease. A novelty is, that SWE was performed on muscles that otherwise have not been examined or SWE has not been reported in m/s instead of kPa. For example, the findings in DE, ECR, FDP, TR, MU (C8), ER (Th10) and ER (L3) represent newly examined muscles with SWE. Additionally, findings from previously reported studies could be reproduced, such as SWE in BB, VA, BF, TA and GCM. This current study tested for differences in SWE between men and women, young and elderly, as well as different BMI categories. Furthermore, this study demonstrates that variance within SWE can be considerably decreased when controlling for joint position. Consequently, it can be concluded that joint position in SWE must become standardized for each muscle in order to generate a precise range of baseline values.

5.1.1 Comparison to the literature

When comparing the average SWE results in this study to those that have already been reported by other authors, the findings often fall into the ranges or are close in value of what has already been described (an overview of the previous published findings is shown in **Table 11**. It must be noted and taken into consideration that each study used different devices, probes, probe orientation and joint positions, therefore resulting in different findings, since all of which may have a meaningful effect on SWE, as previously described. A further description of the joint positions used in each protocol are found in **Table 12**. Furthermore, many studies have reported SWE in pascal or kPa. In this discussion, the focus will be comparing the findings of the current study to those studies, which also reported in m/s, to avoid error that comes through conversion factors, except for that of Akagi et al. 2015, as this paper was also important in the beginnings of the research for this study.

When comparing the SWE for the biceps brachii (BB) muscle in this work with others previously reported, we found the same average of 1.95 m/s as described by Alfuraih et al. 2019. Other values of 1.76 m/s and 2.22 m/s have also been described (Alfuraih et al. 2018; Ewertsen et al. 2018). Here, the similar positioning for the examination of the muscle as described in Alfuraih et al. 2018 and 2019 was used, where the elbow was bent at 90° and resting on the torso. In Protocol 2 (joint control), the hand was pronated to prevent contraction, whereas the participants in other studies had their hand supinated ((Alfuraih et al. 2018; 2019). In another study from Ewertson et al., the muscles were examined in the transverse plane, which led to higher SWE and variance than in the longitudinal plane, due to the greater anisotropy of the muscle in the transverse plane. In all other studies mentioned, as within protocols of this study, SWE was performed in the longitudinal plane, as this is the recommended orientation (Alfuraih et al. 2017; Eby et al. 2013; Gennisson et al. 2013). In the longitudinal plane, the limiting factor of anisotropy is partially controlled for. Here, the shear waves travel more readily parallel along the natural direction of the muscle fibers instead of perpendicular through them.

Similarly in the vastus lateralis (VA) muscle, the reported average of 1.68 m/s is similar to those of other studies, such as 1.76 m/s and 1.77 m/s (Alfuraih et al. 2018; 2019). Here, the positioning in this study was distinctively different in that the legs were supported by a cushion underneath the knee in a supine position, so the legs were not at full extension. In other studies, the legs were typically fully extended (Alfuraih et al. 2018; 2019) or the participants laid prone on the examination table (Carpenter et al. 2015). Additionally, Carpenter et al. obtained values from the midpoint of the muscle, where in the current study the muscles were routinely examined 5cm from the distal or proximal tendon, depending on the muscle. The SWE of VA reported by Carpenter et al. 2015 are typically higher than in other studies. This is most likely due to the positioning and the fact that only 5 participants were examined. The small sample size is critical since the data from 5 subjects is often not representative of the general population. Also, when lying prone, VA may be deformed due to the compression of the adjacent rectus femoris muscle on the examination table, which may have an effect on SWE.

Another important muscle in which SWE can be compared is the gastrocnemius muscle (GC). Few studies have examined the gastrocnemius caput laterale (GCL) (Akagi, Yamashita, and Ueyasu 2015; Carpenter et al. 2015), whereas others have investigated at the gastrocnemius caput mediale (GCM) (Cortez et al. 2016) and in others, both heads were examined (Ewertsen et al. 2016). The average of 2.18 m/s for GCM in this study falls within the range as described by Cortez et al., in which a junior and senior clinician analyzed GCM and reported values ranging from 1.89 m/s to 2.38 m/s. The protocol described by Cortez et al. was similar the protocol in this study, in which the knee was bent at approximately 90°. However, in this study, the participants were sitting on the edge of the examination table, whereas in the participants in Cortez et al. were lying supine on the examination table with the leg externally rotated at the hip and the knee bent. The SWE found by Carpenter et al. for GCL was higher than what has been reported in the GCM, being 4.34 m/s. Contrarily, the SWE described by Akagi et al. for GCL was 1.63 m/s, distinctly lower than that of Carpenter et al. and also in this study.

The differences in SWE of GC found by Carpenter et al., Akagi et al. and the current study vary most likely due to the various body and joint positioning used when examining GC. The positioning might be important as signified by greater divergence among findings. Less divergence between findings can be found when the body and joint positions are similar to one another. For example, as described earlier for BB, where the average SWE of 1.95 m/s was found in this study and in Alfuraih et al. 2019 - the joint position (elbow bent at 90°) and general body positioning (supine, lying on the examination table, hand resting on torso) were the same. When comparing the known values for GC, very different body and joint positions were used in each study. This may have led to the greater disparities in the findings. For example, Carpenter et. al and Akagi et al. examined GCL in the prone position, whereas GCM in this study was examined in a sitting position. Akagi et al. also examined GCL with 20° ankle flexion plantar, whereas Carpenter et al. supported the ankle with a cushion underneath the ankle joint and the precise ankle joint angle was not described. All of these different body positions affect the position and state of the muscle examined, which in turn has an effect on SWE, as it has been previously demonstrated that when muscle is passively stretched or actively contracted, higher SWE is observed (Eby et al. 2015; Kronlage et al. 2021). This may be the case for the greater variance among known SWE in GC.

Another important similarity found was that the SWE of deeper lying muscles exhibit higher SWE. This phenomenon may be connected to known tendency of variance in SWE to increase proportionately with increasing depth (Alfuraih et al. 2018). This trend is supported by the findings of Carpenter et al., in which SWE increased in the gastrocnemius, rectus femoris and vastus lateralis muscle with increasing depth of acquisition (Carpenter et al. 2015). In this study, ECR, FDP and BF all exhibited average SWEs above or equal to 2.30 m/s, which were greater than the SWE of more superficial lying muscles such as BB, GCM or VA, all of which had SWEs under 2.20 m/s. However, this does not explain why the SWE of TA, a superficial lying muscle, often covered by a smaller layer of subcutaneous fat in comparison to BF and other muscles for example, exhibited a relatively high average SWE of 2.56 m/s. The high SWE in TA may be partially contributed to a suboptimal joint position chosen in Protocol 2 (joint control). The foot was free hanging, which may have induced a passive stretch of the muscle as the foot was in a plantar flexion position due to the force of gravity. This once again emphasizes the need to develop standardized optimal joint positions for each muscle in SWE.

- **The SWE values obtained from this study are within the range of those already reported. However, noticeably, the more similar the joint positions between the two studies were, the more similar the values were.**

Author	**This study**	**Alfuraih et al. 2018**	**Alfuraih et al. 2019**	**Ewertson et al. 2016**	**Carpenter et al. 2015***	**Akagi et al. 2015***	**Cortez et al. 2017**
Cohort	*n* = 27	*n* = 20	*n* = 26	*n* = 10	*n* = 5	*n* = 31	*n* = 16
Average Age in years (SD)	26.5 (3.1)	36.7 (11.8)	28.1 (4.1)	Median: 32.5	Range: 27–33	22 (1)	25 (12)
Average BMI in kg/m^2 (SD)	21.7 (2.2)	23.0 (3.1)	24.5 (5.3)	All < 31	Not given	21.5	23.2 (2.97)
Device and Probe	Canon Aplio i800, , PLI 1205 BX / i18Lx5 probe	General Electric LOGIQ-E9 System, linear 9- to 5-MHz probe	Two-dimensional Aixplorer, SuperLinearTM SL10–2 MHz probe.	Acuson S3000 Helx, linear array probe (9L4) or low frequency, curved array probe (4C1)	Siemens S3000 unit, 9-MHz linear transducer	ACUSON S2000, electronic linear array probe (9L4 Transducer) 4–9 MHz	Supersonic Shear Imaging module with SL15-4 high frequency linear probe
Muscle	SWE m/s (SD)	SWE m/s (SD)	SWE m/s (SD)	SWE m/s (SD)	SWE m/s (SD)	SWE m/s (SD)	SWE m/s (SD)
DE	2.26 (0.49)						
BB	1.95 (0.30)	1.76 (0.10)	1.95 (0.22)	2.22 (0.64)			
ECR	2.51 (0.37)						
FDP	2.30 (0.37)						
TR	3.22 (1.19)						
VA	1.68 (0.34)	1.76 (0.10)	1.77 (0.15)		4.52 (1.49)		
BF	2.31 (0.38)	1.54 (0.12)	1.73 (0.12)				
TA	2.56 (0.32)						3.49 (0.58) - 3.86 (0.46)
GCM* or GCL^	2.18* (0.43)			1.77*^ (0.79)	4.34^ (1.56)	1.63^ (0.99)	1.89* (0.32) - 2.38* (0.58)

Table 11. Summary of reported SWE measurements for certain muscles in m/s by author. When comparing the known values of SWE for certain muscles, it is important to take into consideration the different cohort sizes, ultrasound devices, probes, probe orientation and joint position, as these may all have an effect on SWE. The SWE results found in this study align with those previously reported.

[*] Carpenter et al. 2015 used the probe in a transverse orientation, where as all other studies listed in the table positioned the ultrasound probe longitudinally in relation to the muscle fibers.

[#] The SWE from Akagi et al. was converted from Pascal to m/s using the equation of $G = \rho c_S{}^2$, where G = shear modulus (kPa), ρ = density of muscle (assumed to be 1.06 kg/m^3 here). c_S = shear wave speed (m/s) (Sigrist et al. 2017).

Joint Position						
Author **Muscle**	**This study**	**Alfuraih et al. 2018 & 2019**	**Ewertson et al. 2016***	**Carpenter et al. 2015***	**Akagi et al. 2015***	**Cortez et al. 2017**
DE	Supine, elbow resting on a pillow. arm bent at the elbow 90°					
BB		Supine, elbow resting on a pillow. arm bent at the elbow 90°	Sitting, forearm resting, supinated underarm			
ECR						
FDP	Supine, arm stretched out					
TR	Left lateral recumbent					
VA	Supine, legs almost completely stretched out with a small pillow under the knees	Supine, knees fully extended and feet slightly everted		Prone, lower extremity fully supported		
BF	Sitting, feet flat on the floor	Prone, bent knees (90º), legs rested against a wall				
TA	Sitting, lower leg free hanging					Supine, leg extended and heel on the examination table
GCM or GCL			Prone, feet relaxed, hanging from bed	Prone, lower extremity fully supported	Prone, hip and knee at ~ 0°, ankle 20° plantar flexion	Supine, knee flexed and hip in external rotation

Table 12. A summary of joint position for specific muscles used in various SWE protocols by author. When comparing SWE results of particular muscles from different studies, it is important to consider the joint position, as this may have a noteworthy effect on the reported SWE.

5.2 Joint control

5.2.1 Joint control and SWE

As predicted, when not controlling for joint position (Protocol 1), SWE was consistently higher across all muscles in comparison to the SWE obtained in Protocol 2 (joint control). This may be contributed to the fact that the muscle position in Protocol 1 (no joint control) often positioned the muscle in a passively stretched state. Stretching increases the length of the individual muscle fibers, consequently inducing strain and therefore making the muscle stiffer, leading to higher SWE (Eby et al. 2015). For example, the arm was fully extended in Protocol 1 (no joint control), here BB is passively stretched, which led to a higher average SWE of 3.35 m/s, compared to 2.27 m/s in Protocol 2 (joint control), where the arm was bent 90° at the elbow and the hand rested prone on the torso. Eby et al. found a similar trend, where the SWE of BB was consistently higher when the arm was fully extended compared to when the arm was bent at 90° in the elbow joint. Similarly in VA, when the leg was fully extended in Protocol 1 (no joint control), the SWE was 1.96 m/s, higher than in Protocol 2 (joint control), where the average was 1.66 m/s. In Protocol 2 (joint control), the knee was supported by a cushion so that the quadriceps muscle was not fully stretched.

- **Not controlling for joint position led to higher SWE.**

5.2.2 Joint control and variance

The variance was consistently significantly higher in Protocol 1 (no joint control) compared to Protocol 2 (joint control) for all muscles. This aligns with the original hypothesis in that variance would be higher when not controlling for joint position. The variances in Protocol 2 - Group 1 (joint control, young) consistently remained under 0.3, whereas the variance in Protocol 1 (no joint control) was higher than 0.6 for each muscle, and could range up to almost 2.5. These results may stem from the positioning used in Protocol 2 (joint control), where the muscles were positioned in an optimal rested state, in which the muscles were ideally neither contracted nor passively stretched. It is important that the muscles are not passively stretched since muscle elasticity of each individual is dependent upon their flexibility, or the ability of the muscle fiber to stretch. The same amount of muscle stretch in distance can lead to different amounts of strain on the muscle, depending on its elasticity (Gleim and McHugh 1997). Some people are considered more flexible than others, meaning that a greater change in muscle length can take place before there is a strain on the muscle fibers. Therefore, in Protocol 2 (joint control), the uncontrollable factor of individual muscle elasticity depending on the person is partially controlled for, which may lead to reduced variance within the measurements of SWE.

- **There was a significant increase in variance when not controlling for joint position.**

5.2.3 The need for joint control to establish reference SWE values

The previous points demonstrate the need of precise joint position in order to generate a range of reference values for SWE that can be used in clinical diagnostic. As predicted, when controlling for joint position, the lesser the variance within the results and also the lower the SWE. With joint control, the results are more precise, which is necessary for the establishment of a range of baseline values. Then, SWE reports can be more accurately categorized as normal or pathologic. Also, joint position must be standardized for each muscle in order to the values to be completely objectively comparable to one another. When the joint protocol in this study was similar to those of other studies, the more similar the SWE measurements were. As described previously, the average SWE for BB reported by Alfuraih et al. of 1.95 m/s was also the average SWE for BB found in this study. The positioning of body and elbow joint in their study was identical to that in this study. On the other hand, the positioning used for GC ranges from being supine and sitting (in this study) to prone to laying on the exam table (Carpenter et al.) or prone, laying on the exam table with the knee and ankle bent at a specific angle (Akagi et al.). These various positions all led to greater degrees of variation in the SWE of GC being namely 2.18 m/s, 4.34 m/s and 1.63 m/s respectively. SWE results may fall into a smaller range of reported values when the joint position is specifically controlled for, making the creation of an accurate data bank of reference SWE values possible.

- **Joint position must be controlled for when performing SWE in order to establish reference values**

5.3 Depth

5.3.1 Effect of depth on variance

It is well documented that the variance of SWE increases proportionately with depth (**Figure 6**) (Alfuraih et al. 2018; Carpenter et al. 2015; Ewertsen et al. 2016). As hypothesized, a similar phenomenon was also observed in this study as FDP, ECR and BF exhibited the highest variances when examining both the young and elderly and controlling for joint position (Protocol 2). These muscles are embedded within others and/or lie substantially deeper underneath the skin and subcutaneous fat and connective tissue in comparison to the other muscles examined in this study. It must also be noted that ECR and FDP have relatively small muscle volumes, which may have limited the accuracy of SWE for these muscles. The constraint of depth and muscle size might be specific reasons as to why the muscles in the back, such as MU (C8), ES (Th10) and ES (L3) were not examined again in Protocol 2 (joint control). These muscles had some of the highest observed variances in Protocol 1 (no joint control) and were difficult to present in an optimal way using the ultrasound device due to their depth, consistently being over 3cm, since humans store more subcutaneous fat in the trunk compared to the extremities (Bredella 2017), it can be noted that muscles in the periphery may be more suitable for SWE, as they lie closer to the surface of the skin, making them more optimal for SWE.

- **Muscles that lie deeper underneath the skin (>3cm) are less optimal for SWE, since there is a positive correlation between increasing depth and variance**.

5.3.2 Effect of depth on SWE

As previously reported, not only does the variance in SWE increase with increasing depth, but also the SWE itself (Carpenter et al. 2015). Muscles that lie underneath other muscle, such as FDP, or are below a thicker layer of subcutaneous fat and connective tissue, like DE and BF, exhibited higher SWE than those of superficial lying muscles. Humans store greater amounts of fat underneath the skin near the trunk and in the legs compared to the arms (Bredella 2017), leading to greater depth between skin and muscle in these areas. This reason can be used to understand why SWE was higher in DE (2.27 m/s) and BF (2.43 m/s) than in other muscles such as BB (1.96 m/s) or VA (1.66 m/s). However, this does not explain why the SWE of ECR and TA, also superficial, distal lying muscles, covered by less subcutaneous fat than those muscles near the trunk, also had relatively high SWEs of 2.53 m/s and 2.61 m/s, respectively, in comparison to other muscles evaluated in this study. Other factors leading to higher SWEs in ECR and TA could be the smaller size of the muscle in the case of ECR and the location of measurement or position for TA. The leg was hanging free for the evaluation of TA, which may have induced a passive stretch in the muscle and the thickness of the muscle 5cm from the proximal insertion point might not have been the most optimal point to take the measurement, due to its thin muscle body here close to the tendon. The findings in this study seem to align with general tendency that depth may not be a significant factor in explaining an increase or decrease in SWE (Alfuraih et al. 2018).

- **Depth alone cannot explain significant increases or decreases in SWE, but may be a compounding factor along with muscle body size and joint position.**

5.4 Age

The prediction that elderly individuals would have higher SWEs was upheld for all muscles except for VA. The findings in the current study align with those described by Eby et al., in which SWE increased in BB with increasing age, when the elbow was bent at 90°. However, the increase here was not statistically significant. In this study, the SWE was significantly higher in FDP and TR in elderly individuals (FDP: 2.30 m/s in the younger population, 4.26 m/s for the elderly population) and (TR: 1.81 m/s in the younger group, 2.11 m/s for the elderly group). However, it must be noted that a total difference of 0.30 m/s in SWE between the younger and elderly group for TR is less than the SD for these groups (0.42 and 0.51 m/s, respectively). Depth and unpracticality (small muscle volume, pennation angle – the angle between muscle fibers and the longitudinal axis of the muscle itself) of FDP may have also contributed to the outcomes. This leads to the question of how large the absolute difference in SWE must be in order to be considered actually significantly different in the realm of diagnostic. Significant differences in terms of diagnostic may vary from significant differences in terms of statistics. Also, the population sizes of these groups were different. 27 young participants were recruited for the younger cohort and 10 elderly individuals elected to participate as part of the elderly group. Furthermore, not all participants were over the age of 65, the considered age in the field of geriatrics. The results of this study suggest that muscle stiffness increases with age, leading to higher SWE. However, these differences can be considered negligible in the grand scheme of SWE. With each measurement, there is a certain degree of uncertainty as demonstrated by the SD and variance. In this study, although a statistically significant difference in SWE between two groups was found, the SD was often greater than the absolute difference in SWE measurements between the two groups.

The possible reasons as to why SWE increased with age may be due to the processes of muscle atrophy and alteration within the muscle that naturally come with age. The decrease in intra- and extracellular water leads to the muscle fibers becoming more compacted and aligning closer to one another, which may contribute to higher SWE. For example, the satellite cells of the muscle appeared more densely compact in muscle biopsies of participants aged > 60 years (Tomonaga 1977). Furthermore, the amount of collagen and connective tissue inside of the muscle increases with age. In this aspect, the muscle body becomes more similar to the tendon. In this aspect, it is then expected that SWE also increases, since it has been demonstrated that shear modulus is higher in the tendon than in the muscle itself (Kaafarini 2018; Kot et al. 2012). SWE studies of the flexor digitorum longus and soleus muscle of rats demonstrated a positive correlation between an increase in connective tissue within the muscle that comes with aging rats and an increase in muscle stiffness (Alnaqeeb, Al Zaid, and Goldspink 1984). These principles can be caried over to build an understanding for why SWE increases, though maybe not drastically significantly, but noticeably with age.

Lastly, it is known that fat depots redistribute with aging (Kuk et al. 2009). The amount of fat stores decreases in subcutaneous tissues and relocates from being predominantly in the abdominal-visceral and gluteal-femoral region to other places, such as within muscle and underneath its fascia (Addison et al. 2014; Sepe et al. 2011; Tomonaga 1977). In this study, the elderly cohort had a notably higher average BMI of 26.4, compared to 21.7 of the younger group. As discussed in more detail later, SWE was also higher in individuals with higher BMIs in all muscles except for ECR and GCM. This leads to the question of which factors are the most important in determining greater muscle stiffness in the elderly, BMI or structural change within the muscle itself. This research question could be explored with a larger study of elderly individuals, meaning more than the 10 elderly subjects in this study, in order to examine if higher BMI has a heightening effect on increasing SWE in the elderly. Additionally, more participants with higher BMIs could be specifically recruited to more accurately analyze the effect of BMI on SWE, independent of age.

- **Muscle SWE may increase with age, however the difference may be considered negligble.**

5.5 Sex

The hypothetical trend that men would have generally higher SWE across all muscles due to the known phenomenon of men having greater muscle density was not to be observed in this study. The men demonstrated higher SWEs in 4 of the 9 observed muscles (DE, BB, FDP and BF) in Protocol 2 (joint control), while the remaining other 5 muscles had higher SWEs among the women. These findings align partially with those demonstrated by Akagi et al. in which shear modulus was consistently higher in men for the rectus femoris and soleus muscle. However, the findings in this study contrast with the those from Chen et al. and Eby et al., in which SWE of BB was shown to be tendentially higher in women.

In this study, the difference in SWE between men and women in DE and FDP was significant (2.35 m/s in men, 2.16 m/s in women for DE and 3.13 m/s in men, 2.39 m/s in women for FDP). Men typically store more adipose tissue within their trunk and abdomen, where as women accumulate more fatty tissue in their hips and thighs (Bredella 2017). This may explain why the SWE in DE was significantly higher in males and SWE was alternatively higher in VA in females, under the assumption that greater fat storage within the muscle correlates positively with higher SWE. However, this does not explain why SWE was statistically significantly higher in TR in females (2.06 m/s in women, 1.77 m/s in men). One explanation could be the observed tendency of women in this study to have more subcutaneous fat and less muscle mass in their arms compared to the men in this study.

Once again, the absolute differences in muscle SWE between men and women were consistently smaller than the observed SD for all measurements. For example, the statistically significant difference in SWE between males and females in DE (2.35 m/s in men, 2.16 m/s in women) must be carefully taken into consideration considering the SDs for all measurements within these groups were 0.48 m/s and 0.44 m/s, respectively. This tendency holds true for all other muscles analyzed in the comparison between men and women.

The results in the current study demonstrate the lack of correlation between sex and distinctively different SWE between men and women, no matter which muscles are evaluated, which deviates from the original hypothesis that men would have traditionally higher SWE. Fat distribution, BMI and athletic ability may have a more dominant effect on SWE rather than the factor of sex itself. When considering the relatively small absolute difference between average SWE values between men and women, this difference is often smaller than the SD for the measurements for each muscle. This leads to the preliminary conclusion that reference values for SWE may not need to be seperately documented for men and women.

- **There seems to be a lack of correlation between sex and SWE, other factors may play a more significant role such as BMI and athletic ability.**

5.6 BMI

It was predicted that individuals with higher BMIs would also have lower SWE – since muscle is more dense in mass than fat. However, the opposite trend was observed in this study. Individuals with higher BMIs (BMI $\geq$ 25 kg/m^2) exhibited higher average SWE in all muscles except for ECR and GCM. The difference was found to be statistically significant in ECR (2.52 m/s for normal weight and 2.35 m/s for overweight). Here it must be emphasized, that the absolute difference in the averages between these two groups was only 0.17 m/s - considerably lower than the SD of 0.46 m/s for the normal weight group and 0.95 m/s for the overweight group. This trend applies to all muscles in the analysis of the effect of BMI on SWE – meaning that the SD was consistently greater than the absolute difference in the average SWE between normal weight and overweight individuals. However, SWE was significantly higher in elderly individuals (4.07 m/s) compared to younger individuals (2.49 m/s). Here the high SD of 2.31 m/s among the elderly must be noted. These findings are also limited by the different group sizes. 27 individuals fell into the category of normal weight, whereas only 8 individuals were considered overweight. 2 individuals were excluded from this analysis, since they were considered underweight (BMI $\leq$ 18.5 kg/m^2).

Overweight individuals may have higher SWE measurements due to multiple factors. First, a higher BMI positively correlates with an increase of fat deposits within the muscle (Fonvig et al. 2012). This results in a greater anisotropy and thickness of the muscle. A greater thickness of muscle has been associated with greater stiffness, therefore leading to higher SWE measurements (Akagi et al. 2012; Kuo et al. 2013). Secondly, individuals with higher BMIs also have greater amounts of subcutaneous fat (Nadeem, Bacha, and Gilani 2018). This increases the depth of SWE acquisition, which may increase the SWE measurement itself, as well as the variance (Alfuraih et al. 2018; Carpenter et al. 2015). There have been reports in which there is a positive correlation between BMI and muscle stiffness using SWE, for example in the trapezius muscle of adults (Kuo et al. 2013) and the rectus femoris muscle of children (Berko et al. 2014). Alternatively, decreases in SWE of BB with increasing BMI in children have also been reported (Berko et al. 2014). Generally, there seems to be no significant effect of BMI on SWE (Alfuraih et al. 2019; Eby et al. 2015). This study also supports the negligible effect of BMI on SWE. The trend of higher SWE observed in overweight individuals may be due to the increase in depth of acquisition and/or the greater anisotropy of the muscle with more fat deposits. However, this increase in SWE seems to be relatively inconsequential when considering SD, variance and absolute difference in average SWE between the two groups.

- **Individuals with higher BMIs may exhibit higher SWE, however the difference may be considered negligble.**

5.7 Muscles suitable for SWE

The broader range of muscles examined in this study allowed for the analysis of which muscles are most appropriate for SWE. When comparing the variance and feasibility of investigating certain muscles, it becomes clear that BB is one of the best muscles to examine for SWE: The muscle lies superficial, the muscle fibers run very much parallel to one another and its variance was the lowest. Other well suitable muscles for SWE may be TA, VA and GCM, when considering the practicality (superficial lying, thick muscle body, easy to locate and present with SWE) and lower variance when analyzing the muscles of younger and elderly. TA and VA, like BB, have muscle fibers that run almost completely parallel to one another and lie directly underneath the skin, not covered by other muscles – removing the factors of depth and pennation angle that can lead to skewed SWE. These muscles can also be easily located with SWE, in comparison to ECR, FDP and BF in which the corresponding muscle was first found in the transverse plane and then the ultrasound probe was rotated to present it in the longitudinal plane. BB and TA also have relatively large muscle volumes, allowing for SWE even among less trained individuals. The SWE of smaller volumed muscles, such as FDP and ECR, was noticeably more difficult to accurately perform, particularly in women and the elderly, who may not typically train these underarm muscles. In conclusion, BB, VA and TA are muscles that are regularly used and crucial for movement in daily life and may therefore be some of the best muscles to examine in SWE.

- **Large, superficial lying muscles with low pennation angles may be most suitable for SWE - such as BB, VA and TA. These muscles were practical to examine and demonstrated low variance.**

6 Summary

SWE or shear wave elastography is a method used to evaluate the stiffness of bodily tissues, such as skeletal muscle, and has been suggested as an innovative tool for the future diagnostic of neuromuscular disease. In order to use SWE for diagnostic, reference values must be collected. The aim of this study was to contribute to the range of baseline SWE for certain skeletal muscles, as well as evaluate new muscles, that have not yet been previously examined with SWE. Additionally, this study focused on demonstrating the need for precise joint position in muscle SWE. Two protocols were to test for differences in SWE in the areas of joint position, sex, age and BMI. SWE pictures and the shear wave velocities (SWV) were acquired from twenty five volunteers using Protocol 1 (no joint control), in which various muscles of the upper extremity, back and lower extremity were examined. After preliminary analysis of

Protocol 1 (no joint control), the joint position of nine muscles was specifically controlled for in Protocol 2 (joint control), in which the muscles were placed in an optimally relaxed position, avoiding contraction or passive stretching. Thirty-seven volunteers were analyzed with Protocol 2 (joint control), in which twenty-seven were <35 years old and ten were >55 years old. Statistically significant differences in variance were found between Protocol 1 (no joint control) and Protocol 2 (joint control) ($p = 0.008$). SWE also varied significantly ($p < 0.05$) in certain muscles when selecting for sex, age or BMI. However, the absolute difference in SWE between these groups was often smaller than the standard deviation of the measurements, leading to the conclusion that the effect of sex, age and BMI on SWE may be negligible. The results in this study are within range of those already reported and may contribute to the compilation of reference values for a data bank used in SWE diagnostic, however joint position must be controlled in order for these reference values to be precise.

7 Deutsche Zusammenfassung

Scherwellenelastographie (SWE) ist eine Methode zur Messung der Steifigkeit von Körpergeweben, wie z. B. der Skelettmuskulatur, und wird gegenwärtig als neue, innovative und ergänzende Modalität für die Diagnostik von neuromuskulären Erkrankungen disktutiert. Um die SWE für eine etwaige Diagnostik nutzen zu können, müssen Normwerte erhoben werden. Das Ziel dieser Studie war es, einen Beitrag zum Bereich der Norm-SWE für bestimmte Skelettmuskeln zu leisten sowie Muskeln zu untersuchen, die bisher noch nicht mittels SWE untersucht wurden. Zusätzlich konzentrierte sich diese Studie darauf, die Notwendigkeit einer präzisen Gelenkstellung bei der Messung der Muskel-SWE zu demonstrieren. Mit zwei Protokollen sollten Unterschiede in der SWE in den Bereichen Gelenkstellung, Geschlecht, Alter und BMI verglichen werden. SWE-Bilder und die Scherwellengeschwindigkeiten (SWV) wurden von fünfundzwanzig Probanden mit Protokoll 1 (keine Kontrolle der Gelenkstellung) aufgenommen, wobei verschiedene Muskeln der oberen Extremität, des Rückens und der unteren Extremität untersucht wurden. Nach der vorläufigen Analyse von Protokoll 1 (keine Kontrolle der Gelenkstellung) wurde die Gelenkstellung von neun Muskeln in Protokoll 2 (Kontrolle der Gelenkstellung) gezielt kontrolliert, in dem die Muskeln in eine optimal entspannte Position gebracht wurden, wobei eine Kontraktion oder passive

Dehnung vermieden wurde. Siebenunddreißig Probanden wurden mit Protokoll 2 (Kontrolle der Gelenkstellung) analysiert, davon waren siebenundzwanzig <35 Jahre alt und zehn >55 Jahre alt. Es wurden statistisch signifikante Unterschiede in der Varianz zwischen Protokoll 1 (keine Kontrolle der Gelenkstellung) und Protokoll 2 (Kontrolle der Gelenkstellung) gefunden ($p = 0{,}008$). Die SWE variierte auch signifikant ($p < 0{,}05$) in bestimmten Muskeln, wenn Geschlecht, Alter oder BMI verglichen wurden. Der absolute Unterschied in der SWE zwischen diesen Gruppen war jedoch oft kleiner als die Standardabweichung der Messungen, was zu der Schlussfolgerung führt, dass der Einfluss von Geschlecht, Alter und BMI auf die SWE vernachlässigbar sein könnte. Die Ergebnisse dieser Studie war mit den Ergebnissen anderer Studien vergleichbar, bzw. konnte diese reproduzieren, kann einen Betrag zur Normwerte der Muskel-SWE leisten und zeigt wichtige Limitationen der Methode SWE auf.

8 Bibliography

Addison, O., Marcus, R. L., LaStayo, P. C., & Ryan, A. S. (2014). Intermuscular Fat: A Review of the Consequences and Causes. *International Journal of Endocrinology, 2014*, e309570. https://doi.org/10.1155/2014/309570

Akagi, R., Yamashita, Y., & Ueyasu, Y. (2015). Age-Related Differences in Muscle Shear Moduli in the Lower Extremity. *Ultrasound in Medicine & Biology, 41*(11), 2906–2912. https://doi.org/10.1016/j.ultrasmedbio.2015.07.011

Akagi, R., Chino, K., Dohi, M., & Takahashi, H. (2012). Relationships between muscle size and hardness of the medial gastrocnemius at different ankle joint angles in young men. *Acta Radiologica, 53*(3), 307–311. https://doi.org/10.1258/ar.2011.110481

Alfuraih, A. M., Tan, A. L., O'Connor, P., Emery, P., & Wakefield, R. J. (2019). The effect of ageing on shear wave elastography muscle stiffness in adults. *Aging Clinical and Experimental Research, 31*(12), 1755–1763. https://doi.org/10.1007/s40520-019-01139-0

Alfuraih, A. M., O'Connor, P., Hensor, E., Tan, A. L., Emery, P., & Wakefield, R. J. (2018). The effect of unit, depth, and probe load on the reliability of muscle shear wave elastography: Variables affecting reliability of SWE. *Journal of Clinical Ultrasound: JCU, 46*(2), 108–115. https://doi.org/10.1002/jcu.22534

Alfuraih, A. M., O'Connor, P., Tan, A. L., Hensor, E., Emery, P., & Wakefield, R. J. (2017). An investigation into the variability between different shear wave elastography systems in muscle. *Medical Ultrasonography, 19*(4), 392–400. https://doi.org/10.11152/mu-1113

Alnaqeeb, M. A., Al Zaid, N. S., & Goldspink, G. (1984). Connective tissue changes and physical properties of developing and ageing skeletal muscle. *Journal of Anatomy, 139 (Pt 4)*, 677–689.

Barchi, R. L. (1988). Myotonia. *Neurologic Clinics, 6*(3), 473–484.

Barr, R. G., Nakashima, K., Amy, D., Cosgrove, D., Farrokh, A., Schafer, F., Bamber, J. C., Castera, L., Choi, B. I., Chou, Y.-H., Dietrich, C. F., Ding, H., Ferraioli, G., Filice, C., Friedrich-Rust, M., Hall, T. J., Nightingale, K. R., Palmeri, M. L., Shiina, T., … Kudo, M. (2015). WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 2: breast. *Ultrasound in Medicine & Biology, 41*(5), 1148–1160. https://doi.org/10.1016/j.ultrasmedbio.2015.03.008

Barr, R. G., & Zhang, Z. (2012). Effects of precompression on elasticity imaging of the breast: Development of a clinically useful semiquantitative method of precompression assessment. *Journal of Ultrasound in Medicine: Official Journal of the American Institute of Ultrasound in Medicine, 31*(6), 895–902. https://doi.org/10.7863/jum.2012.31.6.895

Berko, N. S., FitzGerald, E. F., Amaral, T. D., Payares, M., & Levin, T. L. (2014). Ultrasound elastography in children: Establishing the normal range of muscle elasticity. *Pediatric Radiology, 44*(2), 158–163. https://doi.org/10.1007/s00247-013-2793-z

Berrigan, W. A., Wickstrom, J., Farrell, M., & Alter, K. (2020). Hip position influences shear wave elastography measurements of the hamstring muscles in healthy subjects. *Journal of Biomechanics*, *109*, 109930. https://doi.org/10.1016/j.jbiomech.2020.109930

Bouillard, K., Nordez, A., & Hug, F. (2011). Estimation of Individual Muscle Force Using Elastography. *PLOS ONE*, *6*(12), e29261. https://doi.org/10.1371/journal.pone.0029261

Brandenburg, J. E., Eby, S. F., Song, P., Zhao, H., Brault, J. S., Chen, S., & An, K.-N. (2014). Ultrasound elastography: The new frontier in direct measurement of muscle stiffness. *Archives of Physical Medicine and Rehabilitation*, *95*(11), 2207–2219. https://doi.org/10.1016/j.apmr.2014.07.007

Bredella, M. A. (2017). Sex Differences in Body Composition. *Sex and Gender Factors Affecting Metabolic Homeostasis, Diabetes and Obesity*, 9–27. https://doi.org/10.1007/978-3-319-70178-3_2

Carpenter, E. L., Lau, H. A., Kolodny, E. H., & Adler, R. S. (2015). Skeletal Muscle in Healthy Subjects versus Those with GNE-Related Myopathy: Evaluation with Shear-Wave US--A Pilot Study. *Radiology*, *277*(2), 546–554. https://doi.org/10.1148/radiol.2015142212

Cawthon, P. M., Fox, K. M., Gandra, Shravanthi. R., Delmonico, M. J., Chiou, C.-F., Anthony, M. S., Sewall, A., Goodpaster, B., Satterfield, S., Cummings, S. R., & Harris, T. B. (2009). Do muscle mass, muscle density, strength and physical function similarly influence risk of hospitalization in older adults? *Journal of the American Geriatrics Society*, *57*(8), 1411–1419. https://doi.org/10.1111/j.1532-5415.2009.02366.x

Chen, J., O’Dell, M., He, W., Du, L.-J., Li, P.-C., & Gao, J. (2017). Ultrasound shear wave elastography in the assessment of passive biceps brachii muscle stiffness: Influences of sex and elbow position. *Clinical Imaging*, *45*, 26–29. https://doi.org/10.1016/j.clinimag.2017.05.017

Chino, K., Akagi, R., Dohi, M., & Takahashi, H. (2014). Measurement of muscle architecture concurrently with muscle hardness using ultrasound strain elastography. *Acta Radiologica*, *55*(7), 833–839. https://doi.org/10.1177/0284185113507565

Correas, J. M., Drakonakis, E., Isidori, A. M., Hélénon, O., Pozza, C., Cantisani, V., Di Leo, N., Maghella, F., Rubini, A., Drudi, F. M., & D’ambrosio, F. (2013). Update on ultrasound elastography: Miscellanea. Prostate, testicle, musculo-skeletal. *European Journal of Radiology*, *82*(11), 1904–1912. https://doi.org/10.1016/j.ejrad.2013.05.031

Cortez, C. D., Hermitte, L., Ramain, A., Mesmann, C., Lefort, T., & Pialat, J. B. (2016). Ultrasound shear wave velocity in skeletal muscle: A reproducibility study. *Diagnostic and Interventional Imaging*, *97*(1), 71–79. https://doi.org/10.1016/j.diii.2015.05.010

Cosgrove, D. O., Berg, W. A., Doré, C. J., Skyba, D. M., Henry, J.-P., Gay, J., Cohen-Bacrie, C., & the BE1 Study Group. (2012). Shear wave elastography for breast masses is highly reproducible. *European Radiology*, *22*(5), 1023–1032. https://doi.org/10.1007/s00330-011-2340-y

Domire, Z. J., McCullough, M. B., Chen, Q., & An, K.-N. (2009). Feasibility of Using Magnetic Resonance Elastography to Study the Effect of Aging on Shear Modulus of

Skeletal Muscle. *Journal of Applied Biomechanics*, *25*(1), 93–97. https://doi.org/10.1123/jab.25.1.93

Drakonaki, E. (2012). Ultrasound elastography for imaging tendons and muscles. *Journal of Ultrasonography*, *12*(49), 214–225.

Du, L., He, W., Cheng, L., Li, S., Pan, Y., & Gao, J. (2016). Ultrasound shear wave elastography in assessment of muscle stiffness in patients with Parkinson's disease: A primary observation. *Clinical Imaging*, *40*(6), 1075–1080. https://doi.org/10.1016/j.clinimag.2016.05.008

Eby, S. F., Cloud, B. A., Brandenburg, J. E., Giambini, H., Song, P., Chen, S., LeBrasseur, N. K., & An, K.-N. (2015). Shear wave elastography of passive skeletal muscle stiffness: Influences of sex and age throughout adulthood. *Clinical Biomechanics (Bristol, Avon)*, *30*(1), 22–27. https://doi.org/10.1016/j.clinbiomech.2014.11.011

Eby, S. F., Song, P., Chen, S., Chen, Q., Greenleaf, J. F., & An, K.-N. (2013). Validation of shear wave elastography in skeletal muscle. *Journal of Biomechanics*, *46*(14), 2381–2387. https://doi.org/10.1016/j.jbiomech.2013.07.033

Ewertsen, C., Carlsen, J., Perveez, M. A., & Schytz, H. (2018). Reference Values for Shear Wave Elastography of Neck and Shoulder Muscles in Healthy Individuals. *Ultrasound International Open*, *4*(1), E23–E29. https://doi.org/10.1055/s-0044-102013

Ewertsen, C., Carlsen, J. F., Christiansen, I. R., Jensen, J. A., & Nielsen, M. B. (2016). Evaluation of healthy muscle tissue by strain and shear wave elastography—Dependency on depth and ROI position in relation to underlying bone. *Ultrasonics*, *71*, 127–133. https://doi.org/10.1016/j.ultras.2016.06.007

Ferraioli, G., Filice, C., Castera, L., Choi, B. I., Sporea, I., Wilson, S. R., Cosgrove, D., Dietrich, C. F., Amy, D., Bamber, J. C., Barr, R., Chou, Y.-H., Ding, H., Farrokh, A., Friedrich-Rust, M., Hall, T. J., Nakashima, K., Nightingale, K. R., Palmeri, M. L., … Kudo, M. (2015). WFUMB guidelines and recommendations for clinical use of ultrasound elastography: Part 3: liver. *Ultrasound in Medicine & Biology*, *41*(5), 1161–1179. https://doi.org/10.1016/j.ultrasmedbio.2015.03.007

Flatres, A., Aarab, Y., Nougaret, S., Garnier, F., Larcher, R., Amalric, M., Klouche, K., Etienne, P., Subra, G., Jaber, S., Molinari, N., Matecki, S., & Jung, B. (2020). Real-time shear wave ultrasound elastography: A new tool for the evaluation of diaphragm and limb muscle stiffness in critically ill patients. *Critical Care*, *24*(1), 34. https://doi.org/10.1186/s13054-020-2745-6

Fonvig, C. E., Bille, D. S., Chabanova, E., Nielsen, T. R. H., Thomsen, H. S., & Holm, J.-C. (2012). Muscle fat content and abdominal adipose tissue distribution investigated by magnetic resonance spectroscopy and imaging in obese children and youths. *Pediatric Reports*, *4*(1). https://doi.org/10.4081/pr.2012.e11

Forsberg, A. M., Nilsson, E., Werneman, J., Bergström, J., & Hultman, E. (1991). Muscle composition in relation to age and sex. *Clinical Science*, *81*(2), 249–256. https://doi.org/10.1042/cs0810249

Fujiwara, K., Asai, H., Toyama, H., Kunita, K., Yaguchi, C., Kiyota, N., Tomita, H., & Jacobs, J. V. (2010). Changes in muscle thickness of gastrocnemius and soleus associated with age and sex. *Aging Clinical and Experimental Research*, *22*(1), 24–30. https://doi.org/10.1007/BF03324811

Gennisson, J.-L., Deffieux, T., Fink, M., & Tanter, M. (2013). Ultrasound elastography: Principles and techniques. *Diagnostic and Interventional Imaging*, *94*(5), 487–495. https://doi.org/10.1016/j.diii.2013.01.022

Gleim, G. W., & McHugh, M. P. (1997). Flexibility and its effects on sports injury and performance. *Sports Medicine (Auckland, N.Z.)*, *24*(5), 289–299. https://doi.org/10.2165/00007256-199724050-00001

Hall, T. J., Milkowski, A., Garra, B., Carson, P., Palmeri, M., Nightingale, K., Lynch, T., Alturki, A., Andre, M., Audiere, S., Bamber, J., Barr, R., Bercoff, J., Bercoff, J., Bernal, M., Brum, J., Huan Wee Chan, Shigao Chen, Cohen-Bacrie, C., … Heng Zhao. (2013). RSNA/QIBA: Shear wave speed as a biomarker for liver fibrosis staging. *2013 IEEE International Ultrasonics Symposium (IUS)*, 397–400. https://doi.org/10.1109/ULTSYM.2013.0103

Janssen, I., Heymsfield, S. B., Wang, Z., & Ross, R. (2000). Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *Journal of Applied Physiology*, *89*(1), 81–88. https://doi.org/10.1152/jappl.2000.89.1.81

Kaafarini, N. (2018). *Shear Wave Elastography, Aplio i-series*.

Kimura, J. (n.d.). Types of Electromyographic Abnormalities. In *Electrodiagnosis in Diseases of Nerve and Muscle*. Oxford University Press. Retrieved 22 March 2021, from https://oxfordmedicine.com/view/10.1093/med/9780199738687.001.0001/med-9780199738687-chapter-14

Koo, T. K., & Li, M. Y. (2016). A Guideline of Selecting and Reporting Intraclass Correlation Coefficients for Reliability Research. *Journal of Chiropractic Medicine*, *15*(2), 155–163. https://doi.org/10.1016/j.jcm.2016.02.012

Kot, B. C. W., Zhang, Z. J., Lee, A. W. C., Leung, V. Y. F., & Fu, S. N. (2012). Elastic Modulus of Muscle and Tendon with Shear Wave Ultrasound Elastography: Variations with Different Technical Settings. *PLOS ONE*, *7*(8), e44348. https://doi.org/10.1371/journal.pone.0044348

Kronlage, C., Grimm, A., Romano, A., Stahl, J.-H., Martin, P., Winter, N., & Marquetand, J. (2021). Muscle Ultrasound Shear Wave Elastography as a Non-Invasive Biomarker in Myotonia. *Diagnostics (Basel, Switzerland)*, *11*(2). https://doi.org/10.3390/diagnostics11020163

Kuk, J. L., Saunders, T. J., Davidson, L. E., & Ross, R. (2009). Age-related changes in total and regional fat distribution. *Ageing Research Reviews*, *8*(4), 339–348. https://doi.org/10.1016/j.arr.2009.06.001

Kuo, W.-H., Jian, D.-W., Wang, T.-G., & Wang, Y.-C. (2013). Neck Muscle Stiffness Quantified by Sonoelastography is Correlated with Body Mass Index and Chronic Neck Pain Symptoms. *Ultrasound in Medicine & Biology*, *39*(8), 1356–1361. https://doi.org/10.1016/j.ultrasmedbio.2012.11.015

Lacourpaille, L., Hug, F., Guével, A., Péréon, Y., Magot, A., Hogrel, J.-Y., & Nordez, A. (2015). Non-invasive assessment of muscle stiffness in patients with Duchenne muscular dystrophy. *Muscle & Nerve*, *51*(2), 284–286. https://doi.org/10.1002/mus.24445

Lam, A. C. L., Pang, S. W. A., Ahuja, A. T., & Bhatia, K. S. S. (2016). The influence of precompression on elasticity of thyroid nodules estimated by ultrasound shear wave elastography. *European Radiology*, *26*(8), 2845–2852. https://doi.org/10.1007/s00330-015-4108-2

Leong, H.-T., Ng, G. Y., Leung, V. Y., & Fu, S. N. (2013). Quantitative Estimation of Muscle Shear Elastic Modulus of the Upper Trapezius with Supersonic Shear Imaging during Arm Positioning. *PLOS ONE*, *8*(6), e67199. https://doi.org/10.1371/journal.pone.0067199

McDonald C., Abresch R., Carter G., Fowler W. Jr, Johnson E., Kilmer D., & Sigford B.. Profiles of neuromuscular diseases. Duchenne muscular dystrophy. Am J Phys Med Rehabil. 1995 Sep-Oct;74(5 Suppl):S70-92. doi: 10.1097/00002060-199509001-00003. PMID: 7576424.

Nadeem, B., Bacha, R., & Gilani, S. A. (2018). Correlation of Subcutaneous Fat Measured on Ultrasound with Body Mass Index. *Journal of Medical Ultrasound*, *26*(4), 205–209. https://doi.org/10.4103/JMU.JMU_34_18

Ryu, J., & Jeong, W. K. (2017). Current status of musculoskeletal application of shear wave elastography. *Ultrasonography*, *36*(3), 185–197. https://doi.org/10.14366/usg.16053

Sepe, A., Tchkonia, T., Thomou, T., Zamboni, M., & Kirkland, J. L. (2011). Aging and regional differences in fat cell progenitors—A mini-review. *Gerontology*, *57*(1), 66–75. https://doi.org/10.1159/000279755

Sigrist, R. M. S., Liau, J., Kaffas, A. E., Chammas, M. C., & Willmann, J. K. (2017). Ultrasound Elastography: Review of Techniques and Clinical Applications. *Theranostics*, *7*(5), 1303–1329. https://doi.org/10.7150/thno.18650

Taljanovic, M. S., Gimber, L. H., Becker, G. W., Latt, L. D., Klauser, A. S., Melville, D. M., Gao, L., & Witte, R. S. (2017). Shear-Wave Elastography: Basic Physics and Musculoskeletal Applications. *Radiographics: A Review Publication of the Radiological Society of North America, Inc*, *37*(3), 855–870. https://doi.org/10.1148/rg.2017160116

Tomonaga, M. (1977). Histochemical and Ultrastructural Changes in Senile Human Skeletal Muscle. *Journal of the American Geriatrics Society*, *25*(3), 125–131. https://doi.org/10.1111/j.1532-5415.1977.tb00274.x

Wang, C.-Z., Li, T.-J., & Zheng, Y.-P. (2014). Shear Modulus Estimation on Vastus Intermedius of Elderly and Young Females over the Entire Range of Isometric Contraction. *PLOS ONE*, *9*(7), e101769. https://doi.org/10.1371/journal.pone.0101769

Wilhjelm, J. E., Illum, A., Kristensson, M., & Andersen, O. T. (2016). *Medical diagnostic ultrasound—Physical principles and imaging*. 21.

Yiu, E. M., & Kornberg, A. J. (2015). Duchenne muscular dystrophy: Duchenne muscular dystrophy. *Journal of Paediatrics and Child Health*, *51*(8), 759–764. https://doi.org/10.1111/jpc.12868

9 Declaration of own contribution

The work was carried out at the University Hospital Tübingen - Department of Neurology under the supervision of Alexander Grimm, MD, supervisor.

The conception of the study was done by / in collaboration with Dr. med. Justus Marquetand, mentor and Dr. med. Cornelius Kronlage, mentor.

The experiments were carried out by myself after training by Dr. med. Justus Marquetand.

The statistical analysis was performed independently by me.

I certify that I have written the manuscript independently and that I have not used any sources other than those indicated by me.

Tübingen, on 15.09.2021

[Alyssa Romano]

10 Acknowledgements

I would sincerely like to thank everyone who participated as a subject in this study. Thank you to these colleagues, friends and family of friends for selflessly dedicating their time and energy to make this thesis possible. Thank you to my supervisor, Justus, for not only guiding me not only through the entire process of carrying out a thesis, but taking the time to help me develop as a researcher, future doctor and person. Thank you to Cornelius for helping me learn the technique and including me in on other projects within the Neurology Department research as well. I would like to thank all the colleagues part of the EMG branch of the Neurology department in Tübingen, for their patience and giving me the time and space to use the ultrasound device. Lastly, I would like to thank my family for supporting me during the writing and researching process.