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RELIABILITY AND RESPONSIVENESS OF MUSCULOSKELETAL ULTRASOUND IN SUBJECTS WITH AND WITHOUT SPINAL CORD INJURY

Shauna Dudley-Javoroski, PT, PhD¹, Tara McMullen, BA, DPT¹, Michelle R. Borgwardt, BSE, DPT², Lauren M. Peranich, BS, DPT¹, and Richard K. Shields, PT, PhD¹

¹ Physical Therapy and Rehabilitation Science, Carver College of Medicine, The University of Iowa, Iowa City Iowa

² Rehabilitation Therapies, University of Iowa Health Care, Iowa City, Iowa

Abstract

Rehabilitation after spinal cord injury (SCI) aims to preserve the integrity of the paralyzed musculoskeletal system. The suitability of ultrasound (US) for delineating training-related muscle/tendon adaptations after SCI is unknown. The purpose of this study was to quantify within- and between-operator reliability for US and to determine its responsiveness to post-training muscle/tendon adaptations in SCI subjects. Two novice operators and one experienced operator obtained sonographic images of the vastus lateralis, patellar tendon, soleus, and Achilles tendon from 7 SCI subjects and 16 controls. For control subjects, within-operator concordance (ICC(3,1)) ranged from 0.58 to 0.95 for novice operators and exceeded 0.86 for the experienced operator. Between-operator concordance (ICC (2,1)) ranged from 0.62 to 0.74. Ultrasound detected muscle hypertrophy ($p < 0.05$) following electrical stimulation training in subjects with SCI (responsiveness), but did not detect differences in tendon thickness. These error estimates support the utility of US in future post-SCI training studies.

Keywords

ultrasonography; reliability; responsiveness; spinal cord injury; electrical muscle stimulation; training; hypertrophy

INTRODUCTION

Muscle atrophy and bone mineral loss are well-characterized sequelae of spinal cord injury (SCI) (Dudley-Javoroski and Shields 2008, Dudley-Javoroski and Shields 2008, Eser et al. 2005, Eser et al. 2004, Shields 2002, Shields and Dudley-Javoroski 2006, Shields et al. 2006). Preservation of muscle function and bone mineral density (BMD) may limit secondary complications of SCI (fractures, pressure ulcers) and may equip paralyzed limbs to withstand the rigors of ambulation, should a cure for SCI emerge. Electrical stimulation training is a

Corresponding author: Richard K. Shields, PhD, PT, Graduate Program in Physical Therapy and Rehabilitation Science, The University of Iowa, 1-252 Medical Education Building, Iowa City, IA 52242-1190; Office: 319-335-9791; Fax: 319-335-9707; richard-shields@uiowa.edu.

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promising strategy to preserve the function and integrity of the paralyzed musculoskeletal system (Dudley-Javoroski and Shields 2008, Dudley-Javoroski and Shields 2008, Shields and Dudley-Javoroski 2006, Shields et al. 2006). Recent studies support that muscle-derived mechanical loads attenuate the decline of bone mineral density after spinal cord injury (Dudley-Javoroski and Shields 2008, Shields and Dudley-Javoroski 2006).

As the linkage between the muscular and skeletal systems, tendon is a key player in muscle-bone loading scenarios. However, unlike muscle and bone, post-SCI tendon adaptations have heretofore received little attention. Only one study has documented post-SCI changes to tendon, suggesting that tendon cross-sectional area (CSA) may decline after SCI (Maganaris et al. 2006). Because reduced CSA may reflect loss of collagen, loss of non-collagenous matrix, or both, the implications of reduced CSA are not straightforward. If collagen fibrils are lost, then muscle forces applied over an atrophied tendon may lead to excessive strain (deformation) within the tendon. In this situation, the considerable muscle forces developed during electrical stimulation (Shields and Dudley-Javoroski 2006) could pose a risk to the integrity of the muscle-tendon unit.

The aforementioned study (Maganaris et al. 2006) did not examine measurement variation in ultrasound images of tendon. Establishing the reliability of ultrasound in this context is a key precursor to quantifying possible adaptations of tendon to SCI or to training. The principal drawback to ultrasonography is that the scan operator's probe placement and scanning technique may affect image quality because of operator-induced variability (Costa et al. 2009). For example, a tendon or muscle image captured slightly oblique to the structure's longitudinal axis may appear larger in diameter than a true axial-plane image. In longitudinal studies, variations in operator technique may yield image differences across repeated scans that could be misinterpreted as post-SCI adaptations. In studies which employ unilateral electrical stimulation training (a within-subject control design) (Dudley-Javoroski and Shields 2008, Dudley-Javoroski and Shields 2008, Shields and Dudley-Javoroski 2006, Shields and Dudley-Javoroski 2006), between-limb muscle and tendon size differences could potentially be attributable to image acquisition variability.

The purposes of this study were to 1) determine within-operator and between-operator reliability (including the operator's image analysis technique) for images of the Achilles tendon, soleus, patellar tendon, and vastus lateralis in healthy subjects; and 2) determine the responsiveness (ability to detect change) of ultrasound imaging to training adaptations in individuals with SCI who participated in unilateral electrical muscle stimulation.

METHODS

Subjects and Ultrasound Operators

The University of Iowa Human Subjects Institutional Review Board approved the protocol. All subjects provided written informed consent before participating. 10 healthy, recreationally active adults (5 females, 5 males) (Table 1) participated in the first arm of the study, designed to explore within- and between-operator variability during sonographic measurements of muscle and tendon dimensions. Exclusion criteria were pain or swelling (pitting edema) in any of the tested locations at rest or during activity or any conditions known to affect tendon morphology (ankylosing spondylitis (Genc et al. 2005), rheumatoid arthritis (Genc et al. 2005), type 2 diabetes mellitus (Akturk et al. 2007), familial hypercholesterolemia (Tsouli et al. 2008)). Two novice operators underwent 6.5 hours of training in acquisition of images. To learn the rudiments of the ultrasound system, the novice operators reviewed the US operator's manual. The novice operators then received the written study protocol that detailed scan procedures (Appendix 1). Using an anatomy text for reference, the novice operators practiced obtaining images according to the criteria set in the study protocol. At the end of training, their

proficiency was determined during scans of pilot subjects, without cuing or assistance. An ultrasound operator with more extensive imaging experience as compared to the novice operators analyzed all images captured by the novice operators. The experienced operator had logged in excess of 500 hours scanning muscles and tendons in preparation for this protocol.

The more experienced operator obtained muscle and tendon images in a separate cohort of 6 healthy adults (3 females, 3 males)(Table 1). As with the novice operators, within-rater variation was conceptualized as the operator's ability to capture dimensionally uniform images on repeated trials in a single subject. The more experienced operator traced muscle and tendon dimensions for all subjects in the study. As such, the experienced operator's repeatability in tracing an image over several blinded trials was separately determined ("tracing variation").

The experienced operator also conducted scans of seven men with clinically complete (ASIA-A) (American Spinal Injury Association 2002) chronic SCI (Table 1). The men with SCI all were participants in a long-term unilateral electrical muscle stimulation program (Shields and Dudley-Javoroski 2006) designed to induce hypertrophy of one limb (within-subject control design). At the time of the study, these subjects had completed up to 6 years of unilateral, isometric, supramaximal soleus or quadriceps stimulation, performed on 5 days per week. Each subject demonstrated visible hypertrophy of the trained limb as well as force-production (Shields and Dudley-Javoroski 2006), force-potential (Shields et al. 2006), and bone mineral density adaptations (Dudley-Javoroski and Shields 2008, Dudley-Javoroski and Shields 2008, Shields and Dudley-Javoroski 2006, Shields et al. 2006). We included this SCI cohort to examine the responsiveness of ultrasound because a sound scientific basis exists to expect a difference in muscle tissue dimensions between trained and untrained limbs. The experienced operator used the same procedures employed for the non-SCI subjects to examine between-limb differences in muscle thickness and tendon CSA. Three subjects trained one soleus muscle and four subjects trained one quadriceps muscle group, allowing between-limb comparisons to be made for the soleus, vastus lateralis, Achilles tendon, and patellar tendon dimensions (Table 1).

We elected not to employ training-based exclusion criteria for the non-SCI cohort to avoid undue restriction of the range of variation in measured dimensions of muscle and tendon structures. A key unanswered question in SCI research, as mentioned in the introduction, is whether tendon dimensions change post-injury, becoming smaller or perhaps even by becoming larger. By placing no restrictions on the activity level of non-SCI subjects, we attempted to capture scores with a relatively large degree of variation. SCI group scores would be more likely to fall within the range of non-SCI values using this approach, setting up rigorous criteria to establish between-group differences. This conservative tactic favors the null hypothesis of no tendon change post-SCI. Though we do not compare groups statistically due to the small sample size of this study, future investigations should consider selecting non-SCI subjects with a range of training histories.

Image Acquisition

B-mode ultrasound images were obtained with a LOGIQi sonographic imaging system (GE Healthcare, Milwaukee, WI) using a 5-12 MHz transducer (model 12L-RS). A strip of thin (1 mm), flexible plastic was affixed on the transducer surface approximately 0.5 cm from the edge of the scanning field. This strip cast an echo in the image that was used as a landmark during image tracing. Muscle and tendon regions were traced using the integrated software of the LOGIQi unit. Patellar tendon and vastus lateralis images were obtained at the sites depicted in Figure 1. Achilles tendon and soleus images were obtained at the sites depicted in Figure 2. A detailed description of the imaging technique appears in Appendix 1. At each site, each operator captured five axial-plane images, removing and repositioning the probe between each image ("independent scans"). Images were analyzed using the integrated software of the LOGIQi

system. Using the system's caliper and tracing tools, the experienced ultrasound operator measured vastus lateralis and soleus thickness and patellar tendon and Achilles tendon CSA. The operator was blinded to the dimensions obtained during tracing. Likewise, the operator was blinded to the results of Novice Operator 1 while processing images obtained by Novice Operator 2. Detailed tracing procedures appear in Appendix 2.

The variation attributable to the experienced operator's image tracing technique was separately determined ("tracing variation"). An assistant used a random number generator to select one subject from the pool of 16 non-SCI participants and then randomly selected an image from each anatomic location for that subject. These four images were presented twice to the experienced operator in randomized, blinded fashion. The operator traced the images without receiving knowledge of results. These re-traced results were then combined with the results of the operator's original measurement of the images. The operator and assistant repeated this process for three additional, randomly-selected non-SCI subjects. At each anatomic location, the operator's intra-class correlation coefficient (ICC (3,1)) (Portney and Watkins 2009) was computed as "tracing variation." The tracing variation is considered to be one contributing source of the experienced operator's "total variation."

Data Reduction—Mean values for the non-SCI subjects were computed from ten scans at each anatomic site (5 independent images captured by each of the 2 novice operators). For the non-SCI subjects scanned by the experienced operator, mean values were obtained from five independent scans. Data from these two non-SCI cohorts were pooled to obtain overall mean non-SCI muscle and tendon values.

Mean values for each subject with SCI were obtained from five independent scans performed by the experienced operator.

Statistical Analysis—For the non-SCI subjects, mean, standard deviation, and 95% confidence intervals were calculated for each anatomic site. A coefficient of variation (CV) was calculated for the five images collected by each operator for each subject. Within-operator variation (reliability) was calculated as the mean coefficient of variation (CV) for all subjects scanned by that operator (n=10 for the novice operators, n=6 for the experienced operator). Within-operator variation was also expressed as an intraclass correlation among repeated images (ICC(3,1)) (Portney and Watkins 2009). The experienced operator's "Tracing variation" was similarly estimated by calculating CV and ICC (3,1).

The mean tendon and muscle values for each subject were compared in pairwise fashion between the two novice operators. A one-way ANOVA was performed to determine whether systematic differences existed between the two operators. The level of agreement between the operators was determined by an intraclass correlation coefficient (ICC(2,1)) for each anatomic site.

To examine the overall responsiveness we determined if ultrasound imaging could detect a significant change in tissues exposed to long-term training. We pooled all trained and untrained muscle values, performed a Kolmogorov-Smirnoff test for normality, and followed with parametric statistics (a one-way ANOVA (repeated measures)). We repeated this procedure for pooled tendon values. Significance was set at $p < 0.05$.

To more fully describe the sample, a one way ANOVA was used to determine whether tendon or muscle parameters differed according to gender. Pearson correlation coefficients were obtained for subject height and weight versus muscle or tendon dimensions.

RESULTS

Within-Operator Reliability

Mean and standard deviation (SD) tendon and muscle dimensions and 95% confidence intervals for all non-SCI subjects appear in Table 2. Table 3 illustrates the within-operator variation estimates for the novice and experienced ultrasound operators. Mean CV ranged from 2.93 to 12.08% for the novice operators. ICCs (3,1) ranged between 0.58 to 0.95, depending on the anatomic location. For the experienced operator, mean CV ranged from 1.85 to 6.22% and ICCs (3,1) ranged from 0.86 to 0.99.

For four randomly-selected subjects, tracing variation (CV) for the experienced operator ranged between 0.84% (vastus lateralis) and 3.83% (Achilles tendon) (Table 3). ICCs(3,1) ranged from 0.93 to 1.00.

Between-Operator Reliability

No systematic differences existed for images captured by Novice Operator 1 and Novice Operator 2 (all sites, $p > 0.05$) (Table 4). Percent difference and absolute (signless) percent difference appear in Table 4. Pearson correlation coefficients were moderate between the two raters (range 0.4068 to 0.6408, Table 4). Concordance between raters (ICC(2,1)) ranged between 0.623 (soleus) and 0.742 (vastus lateralis).

Responsiveness of US: SCI cohort

Figure 3 illustrates the muscle and tendon values obtained for the SCI cohort (untrained limbs only) compared to non-SCI mean values. Achilles tendon CSA for all SCI subjects' untrained limbs exceeded the mean CSA for the non-SCI group by > 1 SD. Patellar tendon CSA for all subjects with SCI exceeded the non-SCI group mean. In contrast, soleus and vastus lateralis thickness for all SCI subjects typically fell below the non-SCI mean thickness values. (The exception was the soleus of subject S1, the largest subject in the study).

Figure 4 depicts representative images from the trained and untrained limbs of subjects with SCI. The untrained muscles appeared hyperechoic and lacked the striations visible in trained muscle. Figure 5 illustrates differences in trained and untrained limb muscle and tendon dimensions, expressed as a percent of the untrained limb value. The between-limb difference for soleus and vastus lateralis (21.0% (14.1) and 13.2% (13.1), $n=3, 4$ respectively) both exceeded the experienced operator's coefficient of variation for these sites (5.15%, 1.85%, respectively). To examine the general effect of training on muscle, regardless of anatomic location, we pooled soleus and vastus lateralis thickness values and compared trained and untrained limbs. As the data satisfied the assumption of normal distribution, we performed a follow-up paired t-test, which revealed a significant effect of training ($p = 0.026$). Statistical power for this test was 0.635.

For the soleus training subjects, Achilles tendon CSA was descriptively 14.4% larger in the untrained limb (% CV for this site = 5.8%). However, for the quadriceps training group, the between-limb difference observed in the patellar tendon (3.0% in favor of the trained limbs) did not exceed the experienced operator's coefficient of variation for that site (6.22%). To examine the general effect of training on tendon CSA, regardless of anatomic location, we pooled Achilles and patellar tendon CSA values and compared trained and untrained limbs. As the data satisfied the assumption of normal distribution, we performed a follow-up paired t-test, which revealed no significant effect of training ($p = 0.933$).

Demographic Factors

For the non-SCI subjects, a significant effect of gender was present for patellar tendon CSA (M>F, $p < 0.01$) but not for the other sampled sites. When both genders were considered, Pearson correlations were low between height and tendon/muscle dimensions ($r^2 = < 0.279$) (Table 5). Pearson correlations were weak to moderate between subject weight and tendon/muscle dimensions when both genders were considered ($r^2 = 0.213$ to 0.378) (Table 5). For females, a low-to-moderate correlation existed between weight and soleus thickness ($r^2 = 0.429$), but correlations were very low for other parameters ($r^2 < 0.06$). Females demonstrated moderate correlations between height and patellar tendon and vastus lateralis dimensions ($r^2 = 0.436$, 0.662). Males demonstrated a moderate correlation between height and Achilles tendon CSA ($r^2 = 0.403$). Males also demonstrated a moderate correlation between weight and soleus thickness ($r^2 = 0.566$) and a very strong correlation between weight and Achilles tendon CSA ($r^2 = 0.928$).

DISCUSSION

The first purpose of this study was to establish the reliability of ultrasound imaging in distinguishing muscle and tendon variation in individuals without SCI. Specifically we determined within-operator and between-operator reliability for images of the Achilles tendon, soleus, patellar tendon, and vastus lateralis and determined the reliability associated with the operator's image analysis technique (image tracing). Using these estimates of variation, we examined the responsiveness of ultrasound imaging to detect change in muscle and tendon parameters in subjects with SCI who participated in unilateral electrical muscle stimulation.

Within-Operator Reliability

Because ultrasonography is not a voxel-based imaging modality, the resolution of measurement depends on an assortment of factors such as insonation frequency, beam aperture, depth of the targeted tissue, and the resolution of the display screen (Erickson 1997, Jacobson 2007). In the present study, maximum axial resolution of the ultrasound system was 0.13 mm (Pillen et al. 2008), considerably better than the typical resolution of MRI (0.3 mm) (Erickson 1997)). The challenge of ultrasound imaging, however, lies in its dependence upon operator technique to generate high-quality images. Variations in operator hand position or pressure can markedly affect the appearance of imaged structures (Erickson 1997). When determination of muscle or tendon dimensions is an objective, as may be the case in numerous clinical situations, operator-generated sources of variation must be estimated.

In the present study, the experienced operator traced muscle and tendon structures in all images, including those collected by the novice operators. In this way we attempted to determine the level of operator variability due solely to the scanning technique of the novice operators. However, poor tracing technique by the experienced operator could inflate the variability estimates for the novice operators. We therefore separately determined the level of variation contributed by the experienced operator's tracing technique. Portney and Watkins suggest that ICC values above 0.75 indicate acceptable concordance (Portney and Watkins 2009). During blinded retracing of randomly-selected images, ICCs ranged from 0.92 to 0.99. Low coefficients of variation ($< 3.83\%$) likewise support that variation due to tracing was low. Previous investigators have noted similarly low tracing variation estimates for other anatomic sites (ICCs > 0.96 (Kopenhagen et al. 2009, O'Sullivan et al. 2007)).

The two novice ultrasound operators underwent 6.5 hours of self-directed training in image acquisition, after which they demonstrated proficiency in the scan criteria listed in Appendix 1. Within-operator coefficients of variation for five repeated scans ranged between 2.93% to 12.08% and ICCs ranged from 0.58 to 0.95. The novice raters achieved acceptable concordance

for all measurements except two (Table 4: patellar tendon, Rater 2 and vastus lateralis, Rater 1). Pressler and colleagues report that a novice rater with a similar level of training (> 3 hours) and educational background (entry-level physical therapy degree) obtained comparable within-operator ICC values (< 0.80) during repeated scans of the lumbar multifidus muscles (Pressler et al. 2006). A previous study with presumably more experienced operators reported ICC values in a similar range (0.81) (Ying et al. 2003). All ICC values for the more experienced operator in the present report exceeded 0.86.

No previous studies offer within-operator variation estimates for soleus or vastus lateralis thickness. For the novice and experienced operators in the present study, ICCs for muscle thickness generally exceeded concordance levels for tendon CSA (Table 3). Previous studies with experienced operators have likewise demonstrated low within-operator variability for muscle thickness measures (ICCs > 0.96) for transverse abdominis (Koppenhaver et al. 2009), lumbar multifidus (Koppenhaver et al. 2009), and lower trapezius (O'Sullivan et al. 2007).

Between-Operator Variation

No systematic differences in tendon or muscle values emerged between images obtained by the two novice raters ($p > 0.05$ for all sites). However, no between-operator concordance values met the 0.75 criterion suggested by Portney and Watkins (Portney and Watkins 2009). Several previous studies report that ICC values exceeding 0.75 are achievable for other muscle groups (Cameron et al. 2008, Koppenhaver et al. 2009, O'Sullivan et al. 2007). In the present study and in others (Brushoj et al. 2006, O'Connor et al. 2004), between-operator variation has generally been found to exceed within-operator variation. We concur with other authors who suggest that utilizing a single scan operator is desirable during ultrasound studies of muscle or tendon (Brushoj et al. 2006, O'Connor et al. 2004).

Adaptations to Training

Achilles and patellar tendon CSA values in subjects with SCI exceeded mean values obtained from the non-SCI cohort. Anthropometric and demographic factors may help explain this finding. In the non-SCI group, a significant effect of gender ($M > F$) was present for patellar tendon CSA. Mean patellar tendon CSA was not statistically different for male subjects with or without SCI (0.86 versus 0.98 cm², $p = 0.231$). Thus the gender of the SCI subjects likely contributed to their large patellar tendon CSA values.

Anthropometric factors likely contributed to the SCI subjects' Achilles tendon CSA values (all exceeded the mean non-SCI value). Male non-SCI subjects demonstrated a moderate correlation ($r^2 = 0.403$) between height and Achilles tendon CSA. The SCI group unilateral soleus training subjects were among the tallest individuals in the study, which may have predisposed them to high Achilles tendon CSA. Body weight for the soleus training group (87.7 kg) was also higher than body weight for male non-SCI subjects (82.0 kg). The very high correlation between male subject weight and Achilles tendon CSA ($r^2 = 0.93$) was thus another probable contributing factor to the high Achilles CSA in the soleus training group. These observations suggest that investigations of tendon adaptations to SCI may not be straightforward. The confounding influences of gender and anthropometric factors may mask adaptations when unmatched subject cohorts are compared. Matching subjects according to gender and height may be required for clear examination of potential post-SCI tendon adaptations.

Muscle adaptations to SCI (atrophy) have been extensively studied (Castro et al. 1999, Shah et al. 2008, Shah et al. 2006), but tendon structural adaptations remain largely unknown. Until recently, modes of disuse such as bed rest and limb suspension in the non-SCI model were the

primary sources of insight (de Boer et al. 2007, Kubo et al. 2000, Reeves et al. 2005). No such reduced-use models have reported alterations of tendon CSA. Similarly, subjects with > 1 year history of stroke demonstrated no difference in Achilles tendon CSA between the hemiparetic and more-functional limbs (Zhao et al. 2009). Uniquely, subjects with chronic SCI were reported to have 17% smaller patellar tendon CSA than age and gender-matched control subjects (Maganaris et al. 2006). We have reported extensive adaptations of muscle and bone to electrically-stimulated loading (Dudley-Javoroski and Shields 2008, Dudley-Javoroski and Shields 2008, Shields and Dudley-Javoroski 2006, Shields et al. 2006) and reasoned that tendon, the link between muscle and bone, would also be exposed to a strong anabolic stimulus. We predicted that the routine loads experienced by tendons in trained limbs would partially offset the loss of tendon CSA reported by Maganaris and coauthors (Maganaris et al. 2006). This was not the case for the overall assessment of tendon CSA between the trained and untrained limbs in this study. Subjects who unilaterally trained the quadriceps muscle revealed an observed 3.0% between-limb patellar tendon CSA difference, which did not exceed the operator's measurement variability (6.22% CV). Moreover, the subjects who trained one soleus muscle showed that the untrained Achilles tendon was, on average, 14.4% larger. This difference value exceeded the experienced operator's coefficient of variation (5.80%), but given the limited sample size, precludes us from concluding that training reduces the CSA of paralyzed muscle tendons. This unexpected result is reminiscent of previous studies that reported larger Achilles tendon CSA for elderly subjects, who generated lower plantar flexor forces and presumably experienced lower habitual tendon loading than younger subjects (Magnusson et al. 2003).

Interpretation of vastus lateralis and soleus training effects were more straightforward: trained muscles were significantly thicker than untrained muscles as determined by ultrasound imaging (13.2% and 21.0% thicker for vastus lateralis and soleus, respectively). These difference values exceeded the experienced operator's coefficient of variation (1.85%, 5.15%), supporting that the difference exceeded imaging variability.

While sonographic assessment of muscle thickness may therefore be useful for post-SCI muscle training studies, further work is needed to explore the relationship between ultrasound and MRI-based measures of hypertrophy. Magnetic resonance images of subject S2's trained and untrained limbs were presented in a previous report (Shields and Dudley-Javoroski 2009) and demonstrated a 73% difference in soleus cross sectional area between limbs. Calf circumference for this subject (Table 1) differed by only 4.7% from the MRI-derived circumference, suggesting that calf anatomic structures were reasonably consistent between the ultrasound and MRI scanning days. Thus while correlations between MRI-derived CSA and ultrasound-derived muscle thickness are likely to be limited, the training effect visible via MRI offers support for the validity of the training effect observed via ultrasound in the present study.

Demographic Factors

In this cohort of 16 healthy adults, patellar tendon CSA (mean 0.78 cm²) was commensurate with previously-published MRI reports (Couppe et al. 2008) (~0.80 cm²). Patellar tendon CSA for males in the present study significantly exceeded CSA for females ($p < 0.05$). Westh and coauthors reported a similar gender dimorphism among female and male runners (.67 vs. .99 cm², respectively) (Westh et al. 2008).

Achilles tendon CSA in the present study (0.40 cm²) was generally smaller than observed in previous studies (.57 to .95 cm²) (Brushoj et al. 2006, Magnusson and Kjaer 2003, Pang and Ying 2006, Ying et al. 2003). However, few previous studies reported clear criteria for scan site selection; most stated that images were acquired "at the level of the medial malleolus" (Brushoj et al. 2006, Pang and Ying 2006, Ying et al. 2003). Because the dimensions

of the tendon vary along its length (Magnusson and Kjaer 2003), differences in image site placement may have contributed to the lower CSA values observed in the present study.

Correlations between subject anthropometric factors (height, weight) and tendon dimensions were generally low to moderate ($r^2 < 0.66$), as has been observed in previous studies (Magnusson et al. 2003, Magnusson and Kjaer 2003, Pang and Ying 2006). An unusual finding is the very high correlation between male subject weight and Achilles tendon CSA ($r^2 = 0.93$). A correlation of this magnitude has not previously been reported and may be partially influenced by the correlation between height and weight exhibited by this cohort ($r^2 = 0.589$).

Imaging Considerations

Though the non-SCI subjects in the present study reported no tendon symptoms, hypoechoic regions suggestive of sub-clinical tendonopathy were occasionally observed. This is consistent with Schmidt and coauthors, who found hypoechoic areas in 13% of Achilles tendons (Schmidt et al. 2004). Enlargement of the retrocalcaneal bursa was also apparent on several scans, another incidental finding consistent with Schmidt's sample (fluid detected in 24% of retrocalcaneal bursae).

Paralyzed, untrained muscle displayed an atypical sonographic appearance, with enhanced echogenicity and obscure fascia planes. Particularly in the vastus lateralis, high-signal striations corresponding to perimysial divisions between muscle fibers (Erickson 1997) were absent. A similar sonographic appearance has been described for other neuromuscular disorders (Pillen et al. 2008) and has been correlated with adipose deposition (Reimers et al. 1993) and fibrosis (Pillen et al. 2009). Histologic findings of adipose deposition and fibrosis in paralyzed muscle (Kern et al. 2008, Shah et al. 2008) support that these processes likely underlie the sonographic appearance of paralyzed muscle. The trained vastus lateralis muscle, on the other hand, appeared qualitatively similar to non-paralyzed muscle. This hints that electrical stimulation training may prevent adipose deposition and/or fibrosis that would otherwise occur after SCI. With further advances in quantitative echotexture analysis (Zaidman et al. 2008), sonographic imaging may one day offer noninvasive insight into this possible adaptation to electrically stimulated muscle training.

Important Clinical/Research Considerations

The ultrasonography procedures used in the present study were designed to provide an estimate of operator error due strictly to the operator's sonography technique. For this reason, the experienced operator palpated and marked the scan sites and performed all image tracing. The within-operator concordances obtained by the novice operators would likely differ in a more "clinical" context in which the novice operator conducted the entire procedure. Secondly, the discovery of abnormal echotexture in paralyzed muscle raises the question of whether a novice operator could obtain interpretable images of tissues from persons with SCI. Although novice operators generally achieved acceptable concordance when scanning healthy tissues, this may not be the case for tissues with underlying pathology. Moreover, error estimates obtained from healthy subjects may or may not be universally applicable to subjects with pathological tissues. Ultrasound imaging variability is routinely estimated using a variety of materials such as non-living tissue ("phantoms") (Farrell et al. 2001, Ferrari et al. 2006, Martins et al. 2007, Roberts et al. 1999), and normal healthy tissues (Brushoj et al. 2006, O'Connor et al. 2004). The applicability of any of these error estimates to tissues with any particular diagnosis is open for further investigation. Finally, because all scans were conducted on a single day for each subject, this study does not provide an estimate of within-subject, between-session variability. Particularly for longitudinal studies, the normal fluctuation in tissue dimension across days should be estimated.

Although the spatial resolution of ultrasound is superior to MRI (Erickson 1997, Pillen et al. 2008), it has not supplanted MRI as the “gold standard” for musculoskeletal imaging, particularly of large muscles. Muscle CSA, obtainable via MRI, is clearly a superior indicator of muscle hypertrophy or atrophy than ultrasound-derived muscle thickness. One previous study described a method to sonographically determine CSA of the entire vastus lateralis, with good correspondence to MRI (Reeves et al. 2004). However, this method would seem to require the skill of a seasoned sonographer, likely beyond the training of novice operators or general clinicians. Though the vastus lateralis thickness assessment described in the present study does not lend itself to direct MRI comparison, novice operators achieved reasonable levels of within-operator concordance with minimal training. Given the convenience and cost-effectiveness of ultrasound, the muscle thickness measurement method in this report may be more amenable to clinical assessment situations than other, more complex methods (Reeves et al. 2004).

The tendon CSA scan locations selected for this study were intended to mimic the common locations of tendon pathology, including rupture (Theobald et al. 2005). However, Achilles (Arampatzis et al. 2007, Magnusson and Kjaer 2003) and patellar tendon (Couppe et al. 2008) CSA vary along tendon length, and adaptations to increased use may not be uniform along the tendon length (Arampatzis et al. 2007, Couppe et al. 2008, Magnusson and Kjaer 2003). This may cause greater between-group CSA differences in some tendon regions than in others. Future studies may benefit from sampling several tendon locations to gain a more complete insight into potential between-group differences.

If tendon atrophy does indeed occur after SCI, muscle-bone loading interventions may offer an ideal stimulus to preserve tendon structure. Like muscle and bone, tendon is a metabolically-active tissue that adapts to the mechanical loads it encounters. Equine (Firth et al. 2004) and human athletes (Magnusson and Kjaer 2003, Rosager et al. 2002, Ying et al. 2003) have higher tendon CSA than untrained controls, suggesting that chronic exposure to exercise may enhance tendon architecture. Short-term exposure to strengthening exercise (12–14 weeks) has yielded tendon CSA enhancements in some studies (Arampatzis et al. 2007, Kongsgaard et al. 2007). However, vigorous tendon loading can also lead to overuse symptoms, many of which have been correlated with sonographically-detected tendon hypertrophy (Jacobson 2007). In these cases, tendon calcification, fibrillar derangement, and noncollagenous matrix enhancements may underlie hypertrophy, rather than anabolism of collagen fibrils (Hae Yoon et al. 2003). Any future detection of CSA alterations after SCI, particularly in studies of muscle-tendon loading, should trigger investigation of the histologic nature of tendon adaptations.

In this study we did not attempt to standardize ankle position among subjects, in particular between subjects with and without SCI. The loss of ankle dorsiflexion after SCI (McDonald et al. 2005) and loss of plantar flexor sarcomeres in series (Williams and Goldspink 1984) may cause passive tension to be higher at any given dorsiflexion angle for the subjects with SCI than for the non-SCI subjects. Pilot work indicated that adding passive tension to the Achilles-plantar flexor unit adversely affected image quality (collagen fibrils were more echogenic and less discrete from the surrounding tissues). This finding diminished the appeal of passively dorsiflexing the ankles of SCI subjects to match typical non-SCI positioning (Table 1). Moreover, factors such as time of day, noxious input below the level of injury, and timing and dosage of anti-spasmodic medications can cause wide fluctuations in muscle hypertonicity in a single day. The clasp-knife phenomenon causes resistance to wane during passive dorsiflexion, further complicating the process of normalizing plantar flexor tension between groups. We elected to scan the Achilles tendon of all subjects with the foot in the naturally-occurring plantarflexed position. However, it is important to bear in mind the potential confounding influence of passive tension and hypertonicity in future investigations of post-SCI tendon adaptations.

Summary

The present study quantified between- and within-operator reliability during ultrasound imaging of four muscle and tendon sites. In 6 of 8 cases, acceptable within-operator variability was achieved by novice operators with limited training. Relatively high between-operator variation supports the use of a single ultrasound operator. Variability during image processing (tracing, experienced operator) was considerably lower than variation contributed by operator technique during scan acquisition. We explored the responsiveness of ultrasound imaging to changes in muscle and tendon parameters in subjects with SCI who performed unilateral electrical stimulation training. Ultrasound was responsive to training effects in muscle (hypertrophy) but not in tendon, suggesting that tendon adaptations to training may be subtle. The reliability and responsiveness estimates provided by the present study should alert the musculoskeletal research community that US merits further investigation for post-SCI studies.

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Appendix 1. Image Acquisition Procedures

Due to the internal parallel orientation of collagen fibrils, tendons demonstrate anisotropy; that is, the reflected echo is strongest when the beam strikes perpendicular to the long axis of the tendon fibrils. For Achilles and patellar tendon images, the operators sought to maximize the tendon echo as a technique to standardize beam orientation across scans. To avoid deforming the dimensions of muscle or tendon tissues, the operators took care to minimize transducer pressure and ensured that a layer of coupling gel was visible above the skin during all scans. Proficiency of the novice operators was determined according to eight criteria during a scan of a pilot subject, without cuing or assistance: Appropriate image depth; appropriate beam frequency; beam foci at appropriate depth; correct probe orientation; probe pressure causes no deformation; tendo echo is maximized; muscle deep border is clear; images appropriately labeled.

Patellar tendon imaging

Subjects lay supine with the knee in 30° of flexion. An investigator (SDJ) palpated the distal pole of the patella and placed an ink mark over the patellar tendon 3 cm distal to this bony landmark (Figure 1). An ultrasound operator obtained five axial-plane images, removing and repositioning the probe between each image. Image depth was 3 cm and the beam frequency was 12 Hz. The second ultrasound operator obtained five additional images using the same procedure.

Vastus lateralis imaging

With the subject remaining in 30° of knee flexion, the experienced operator measured femur length by palpating the proximal limit of the greater trochanter and the distal limit of the lateral femoral condyle. The experienced operator marked the subject's skin as a single point at 50% of this distance (Figure 1). Leaving the tape measure in place over the greater trochanter, the operator moved the distal end of the tape to the superior pole of the patella. The operator made an ink mark on the skin at the intersection of this new line and the line previously drawn at 50% of femur length (Figure 1). (In contrast, previous studies have positioned the transducer “in the midsagittal plane”, which does not appear optimal for imaging the vastus lateralis.) Pilot work indicated that the fascia between the vastus lateralis and vastus intermedius is sonographically distinct at this location, facilitating delineation of these muscles during image processing. A novice ultrasound operator positioned the probe along the longitudinal axis of the limb, placing the probe's plastic strip atop the intersection of the skin markings. This caused a shadow to fall in the image at the location of the externally-derived landmark. The novice operator obtained five images of the vastus lateralis, removing and repositioning the probe

between each image. Image depth was 5 cm and the beam frequency was 10 Hz. The second novice operator obtained five additional images using the same procedure.

Soleus Imaging

Subjects moved to a prone position with the knee extended and the foot hanging at rest off the end of the exam table. The experienced operator determined the subject's resting plantar flexion angle with a goniometer. The operator measured tibia length between the proximal limit of the medial tibial crest and the distal limit of the medial malleolus then marked 30% and 66% of this distance (Figure 2). The operator then obtained calf circumference at 66% of tibia length. Pilot data indicated that at 66% of limb length, the soleus is thick but the transverse intermuscular septum (separating the soleus and the deep compartment of the leg) is not consistently visible. To ensure correct differentiation of the soleus and the deep flexors, sonographic images were obtained at 30% of tibia length, where the transverse intermuscular septum is sonographically distinct. A novice ultrasound operator positioned the probe in the mid-sagittal plane, placing the probe's plastic strip atop the skin mark at 30% (Figure 2). This caused a shadow to fall in the image at the location of the externally-derived landmark. The novice operator obtained five sagittal plane images of the soleus, removing and repositioning the probe between each image. Image depth was 6 cm and the beam frequency was 8 Hz. The second novice operator obtained five additional images using the same procedure. The more experienced operator then sonographically determined the most distal limit of the medial gastrocnemius and denoted this location as a percent of tibia length (Table 1)

Achilles tendon imaging

The subject remained prone for imaging of the Achilles tendon. The experienced operator placed an ink mark on the skin 3 cm proximal to the palpated insertion of the Achilles tendon on the calcaneus (Figure 2). During axial-plane imaging of the Achilles tendon, the width of the probe (3.5 cm) was larger than the width of the tendon. To ensure that adequate gel remained under the leftmost and rightmost sides of the probe, the operator taped lightweight cardboard strips along the sides of the tendon. This created a "trough" into which a copious amount of gel was placed, ensuring image quality at the margins of the probe field. A novice ultrasound operator positioned the probe over the ink mark, tilted the probe until the tendon was maximally echogenic and obtained five axial-plane images, removing and repositioning the probe between each image. Image depth was 3 cm and the beam frequency was 12 Hz. The second novice operator obtained five additional images using the same procedure.

Appendix 2. Image Tracing Procedures

Tracing techniques for the patellar tendon and the vastus lateralis appear in Figure 1. The operator traced the contour of the patellar tendon, excluding the peritendinous sheath (visible as a distinct, highly echogenic region superficial and deep to the tendon). To obtain vastus lateralis thickness, the operator first noted the location of the shadow cast by the probe's plastic strip. The operator placed a line along the superficial surface of the vastus lateralis (the iliotibial band), ending at the shadow cast by the plastic strip. Using the software angle-generation tool, the operator then drew a line at ninety degrees from the first line, extending through the vastus lateralis and into the vastus intermedius. Using the software caliper tool, the operator measured vastus lateralis thickness between the vertex of the ninety degree angle and the fascia between the vastus lateralis and the vastus intermedius. In this way, muscle thickness was obtained orthogonal to the fascia planes of the vastus lateralis, rather than in relationship to the on-screen *x* and *y* axes.

Tracing procedures for the Achilles tendon and the soleus were similar to the patellar tendon and vastus lateralis methods. In axial-plane images, the Achilles tendon appears as an ellipse that tapers medially. The operator traced the outline of the Achilles tendon using the software tracing tool, excluding the peritenon (Figure 2). The operator obtained soleus thickness in the same manner as for the vastus lateralis, using the shadow of the plastic strip as a landmark and measuring thickness orthogonal to the soleus' superficial fascia.

Figure 1.

(A) For patellar tendon imaging, the probe was positioned in the transverse plane 3cm distal to the inferior pole of the patella. (B) Patellar tendon axial images. The dotted line in B denotes the tendon borders. (C) For longitudinal images of the vastus lateralis, the probe was positioned at 50% of femur length as determined by palpation of bony landmarks (depicted in A). Muscle thickness was measured orthogonal to the superficial fascia plane, in the interval denoted by arrows. The shadow of a transducer-mounted plastic strip (dotted line in C) denoted the location of the 50% landmark.

Figure 2.

(A) For soleus images, the probe was positioned at 30% of tibia length in the mid-sagittal plane. (B) Sagittal image of soleus. Muscle thickness was measured orthogonal to the superficial fascia plane, in the interval denoted by arrows. The shadow of a transducer-mounted plastic strip (dotted line in B) denoted the location of the 30% landmark. (C) For Achilles tendon axial images, the probe was positioned in the axial plane 3cm proximal to the insertion of the tendon on the calcaneus (depicted in A). The dotted line in C denotes the tendon borders.

Figure 3. Mean (SD) non-SCI muscle and tendon dimensions (bars), compared to values obtained from the untrained limbs of SCI subjects (horizontal lines).

Figure 4. Representative images of trained and untrained vastus lateralis muscle for SCI subject S4 (top) trained and untrained Achilles tendon for subject S3 (bottom). AD = adipose tissue, VL = vastus lateralis, VI = vastus intermedius.

Figure 5.

Percent difference between trained and untrained limbs for subjects who trained one soleus (n=3, left panel) and for subjects who trained one quadriceps (n=4, right panel). Dotted lines represent the experienced operator's percent CV for sonographic measurement of that structure. Overall, muscle thickness was significantly increased in the trained limbs ($p = 0.05$) while the overall tendon thickness was not significantly changed with training ($p = 0.43$).

Table 1

Subject demographics.

Non-SCI Subject		Sex	Age	Height (cm)	Weight (kg)	Limb Dom.	Side Tested	Femur Length (mm)	Tibia Length (mm)	Calf Circ. (mm)	Medial Gastroc Insert. %	Resting PF angle
N1	M	23	174	63.6	R	R	R	410	408	342	39.7	37
N2	M	24	169	70.5	R	R	R	432	370	352	44.3	19
N3	F	24	166	56.4	R	L	L	454	378	402	39.2	32
N4	F	23	175	57.3	R	L	L	438	398	364	41.2	32
N5	F	23	168	58.6	R	R	R	462	368	384	35.9	32
N6	M	25	188	85.9	R	L	L	488	432	396	49.1	29
N7	F	24	168	69.1	No pref.	R	R	434	361	376	48.2	34
N8	M	24	184	90.0	R	R	R	544	418	412	35.4	21
N9	M	28	180	95.0	L	L	L	484	406	422	40.4	29
N10	F	26	169	81.0	R	L	L	468	366	398	49.2	31
N11	M	29	184	96.8	R	R	R	482	428	418	35.4	23
N12	M	31	173	72.6	R	R	L	478	404	341	46.1	24
N13	M	32	175	81.4	L	L	L	454	378	318	43.4	20
N14	F	19	165	63.6	R	L	L	456	362	394	40.3	30
N15	F	37	170	71.8	R	R	R	454	356	407	36.0	28
N16	F	31	168	77.3	R	R	L	430	354	402	45.2	29
Mean		26	174	74.4				461	387	383	41.8	28
s.d.		4.5	7.1	13				31	26	31	4.9	5.3

SCI Subject	Sex	Age	SCI Level	Years post-SCI	Height (cm)	Weight (kg)	Muscle Tested	Trained Limb(s)	Femur Length (mm)	Tibia Length (mm)	Calf Circ. (mm)	Medial Gastroc Insert. %	Resting PF angle
S1	M	38	T9	8.9	175	113	Sol	R		398	406 (414)	41.7 (47.2)	46 (48)
S2	M	29	T4	7.5	188	65.9	Sol	R		420	302 (338)	45.7 (45.7)	51 (53)
S3	M	27	T4	6.2	188	84.1	Sol	R		432	299 (339)	45.3 (45.1)	30 (25)
S4	M	30	T6	2.3	193	71	Quad	L	498				
S5	M	28	T6	2.5	185	77	Quad	R	480				
S6	M	45	T4	0.9	175	85	Quad	L	480				

SCI Subject	Sex	Age	SCI Level	Years post-SCI	Height (cm)	Weight (kg)	Muscle Tested	Trained Limb(s)	Femur Length (mm)	Tibia Length (mm)	Calf Circ. (mm)	Medial Gastroc Insert. %	Resting PF angle
S7	M	34	T6	2.1	187	95	Quad	R	509				
Mean		32.8		3.6	183	87.3			492	415	336 (364)	44.2 (46.0)	35 (34)
s.d.		6.4		3.1	6.7	14.9			14	17	61 (44)	2.2 (1.1)	11 (15)

Non-SCI subjects 1–10 were scanned by the two novice operators. Non-SCI subjects 11–16 and the SCI subjects were scanned by the experienced operator. The limb to be tested was randomly selected. SCI subjects are grouped into unilateral soleus trainers and unilateral quadriceps trainers. Untrained limb data are presented. The experienced operator collected all anthropometric measurements. “Limb Dom.” = limb dominance (self-reported). “Calf Circ.” = calf circumference. “Medial Gastroc Insert.” = the location of the medial gastrocnemius insertion as a percentage of tibia length. “Resting PF angle” = Resting plantar flexion angle during prone positioning. For SCI subjects S1-3 (unilateral soleus trainers), trained limb values appear in parentheses.

Table 2

Tendon and muscle dimensions for non-SCI subjects (n=16).

	Mean (SD)	95% CI
Patellar Tendon (cm ²)	0.78 (0.13)	0.71 – 0.84
Vastus Lateralis (cm)	2.32 (0.43)	2.11 – 2.53
Achilles Tendon (cm ²)	0.40 (0.07)	0.36 – 0.43
Soleus (cm)	1.16 (0.31)	1.01 – 1.31

Table 3

Within-operator variation.

	Patellar Tendon		Vastus Lateralis		Achilles Tendon		Soleus	
	CV	ICC (3,1)	CV	ICC (3,1)	CV	ICC (3,1)	CV	ICC (3,1)
Novice Operator 1	9.12	0.82	6.35	0.66	9.46	0.76	8.39	0.93
Novice Operator 2	12.08	0.58	2.93	0.95	9.10	0.77	10.02	0.94
Experienced Operator (total)	6.22	0.86	1.85	0.99	5.80	0.92	5.15	0.92
Experienced Operator (tracing)	3.77	0.94	0.84	1.00	3.83	0.93	1.21	1.00

Within-operator variation, estimated by coefficient of variation (CV) and intraclass correlation (ICC(3,1)). The experienced operator's variation during repeated, blinded retracing of selected images is shown in the bottom row.

Table 4

Between-operator variation for the two novice operators.

	P-value	R-squared	ICC (2,1)	% diff (SD)	Abs % diff (SD)
Patellar Tendon (cm ²)	0.176	0.5063	.686	-5.75 (14.58)	11.19 (11.03)
Vastus Lateralis (cm)	0.057	0.6408	.742	-8.25 (12.76)	10.65 (10.18)
Achilles Tendon (cm ²)	0.606	0.4068	.654	1.82 (16.34)	12.39 (1.00)
Soleus (cm)	0.265	0.4088	.623	-11.82 (34.45)	21.75 (27.51)

Percent difference (% diff) and absolute (signless) percent difference (Abs % diff) are expressed as mean (SD).

Table 5

Anthropometric correlations for non-SCI subjects.

Height	Patellar Tendon	Vastus Lateralis	Achilles Tendon	Soleus
Both genders	0.2791	0.0352	0.0772	0.0023
Females only	0.4359	0.6621	0.0621	0.0560
Males only	0.0744	0.0027	0.4027	0.1189
Weight	Patellar Tendon	Vastus Lateralis	Achilles Tendon	Soleus
Both genders	0.3788	0.2263	0.2132	0.2618
Females only	0.0207	0.0602	0.0333	0.4293
Males only	0.2342	0.1340	0.9279	0.5656

Pearson correlations (r^2) between tendon/muscle dimensions and height (top panel) and weight (bottom panel).