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Adaptation of a MR imaging protocol into a real-time clinical biometric ultrasound protocol for persons with spinal cord injury at risk for deep tissue injury: A reliability study

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ABSTRACT

Background: High strain in soft tissues that overly bony prominences are considered a risk factor for pressure ulcers (PUs) following spinal cord impairment (SCI) and have been computed using Finite Element methods (FEM). The aim of this study was to translate a MRI protocol into ultrasound (US) and determine between-operator reliability of expert sonographers measuring diameter of the inferior curvature of the ischial tuberosity (IT) and the thickness of the overlying soft tissue layers on able-bodied (AB) and SCI using real-time ultrasound.

Material and methods: Part 1: Fourteen AB participants with a mean age of 36.7 ± 12.09 years with 7 males and 7 females had their 3 soft tissue layers in *loaded* and *unloaded* sitting measured independently by 2 sonographers: tendon/muscle, skin/fat and total soft tissue and the diameter of the IT in its short and long axis. Part 2: Nineteen participants with SCI were screened, three were excluded due to abnormal skin signs, and eight participants (42%) were excluded for abnormal US signs with normal skin. Eight SCI participants with a mean age of 31.6 ± 13.6 years and all male with 4 paraplegics and 4 tetraplegics were measured by the same sonographers for skin, fat, tendon, muscle and total. Skin/fat and tendon/muscle were computed.

Results: AB between-operator reliability was good (ICC = 0.81–0.90) for 3 soft tissues layers in *unloaded* and *loaded* sitting and poor for both IT short and long axis (ICC = −0.028 and −0.01). SCI between-operator reliability was good in *unloaded* and *loaded* for total, muscle, fat, skin/fat, tendon/muscle (ICC = 0.75–0.97) and poor for tendon (ICC = 0.26 *unloaded* and ICC = −0.71 *loaded*) and skin (ICC = 0.37 *unloaded* and ICC = 0.10).

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Conclusion: A MRI protocol was successfully adapted for a reliable 3 soft tissue layer model and could be used in a 2-D FEM model designed to estimate soft tissue strain as a novel risk factor for the development of a PU.

1. Introduction

Pressure ulcers (PU) are localised areas of tissue breakdown in skin and/or underlying tissues and represent one of the most significant problems in individuals with spinal cord impairment (SCI) with an incidence ranging from 23% to 33% per year and a lifetime risk of 85% [1,2]. PUs generate significant indirect and direct health care costs. Sitting-acquired pressure ulcers (SAPUs) are a subset of PUs that can occur in individuals with SCI who use wheelchairs [4]. SAPUs occur over bony prominences of the pelvis that support upper body weight in sitting, typically at the ischial tuberosity, posterior greater trochanter and the coccyx or the sacrum when the pelvis is tilted posteriorly. SAPUs may be categorised as either superficial PUs or deep tissues injuries (DTIs), the latter of which are initiated in the subcutaneous tissues and can progress to the epithelium before clinical signs are noted and thus often present with variable prognosis [3].

Risk factors associated with the development of pressure ulcers in persons with SCI are numerous and multifactorial in nature and include immobility and lack of sensation [4–6]. Risk prediction, as modeled using Finite Element methods (FEM), has determined that the development of deep SAPUs in SCI is associated with increased stresses and strains within the soft tissues adjacent to the ischial tuberosities [7–9]. This modeling requires three types of data, namely, the geometry of the individual tissue layers, constituent properties of the layers, and the representative mechanical boundary conditions [3,10].

The current gold standard system for measuring the magnitude of tissue deformation is Magnetic Resonance Imaging (MRI), although its use is not feasible on a large scale due to its cost and inaccessibility. Ultrasound provides an alternative non-invasive, safe and portable solution and its performance, in terms of feasibility and reliability has recently been reported for individuals with SCI [11–14]. Ultrasound biometrics of soft tissue thickness layers (total, muscle and adipose) and the radius of curvature of the lowest point of the ischial tuberosity in simulated sitting was conducted on six participants, four of which presented with SCI [11]. The results indicate good between-operator reliability (ICC = 0.95) for combined tissue thickness measurements that highly correlated with measurements using MR imaging. It was interesting to note that between-operator reliability for IT radius of curvature was good for ultrasound (ICC = 0.71), but poor for MRI (ICC = 0.21) [11]. However, the study did not involve measurements in real-time, there were issues associated with landmark registration on the IT; and there were no reliability estimates or measurements of the different tissue layers.

To date there has been no published studies of reliability involving sonographers measuring soft tissues overlying the buttocks and the diameter of the inferior aspect of the IT in sitting, using a standardised ultrasound protocol in real time.

This provides the motivation of the present study, namely to adapt a MRI protocol into an ultrasonic protocol and to determine the intra- and between-operator reliability of sonographers measuring the thickness of both the soft tissues overlying the ischium and its bony dimensions on able-bodied and persons with spinal cord impairment in *loaded* and *unloaded* sitting.

2. Materials and methods

2.1. Design and sample size determination

This study involved a cross-sectional design divided into two parts: Part 1 involved a sample with able-bodied (AB) participants serving as a feasibility and safety study for Part 2, which involved individuals with SCI. For Part 1, a power analysis was carried out using pilot data from 3 AB participants and G Power software (version 3.1.7; Heinrich Heine University, Dusseldorf, Germany) with an acceptable level of between-operator reliability of $\rho H0 = 0.75$ and the target reliability estimate of $\rho H1 = 0.95$, a significance level of 0.05, and a statistical power of 0.90. This resulted in a required sample size of 14 participants and measurements were performed 10 times on 3 soft tissue layers and 2 bone axes by two sonographers. For Part 2, this replicate number was reduced to 3 replicate observations based on safety concerns for maintaining skin integrity by reducing sitting duration and the fact that no differences were evident after the first 3 repetitions using repeated measures ANOVA ($p > 0.05$) in Part 1. In addition, the resulting reliability estimates from Part 1 supported this reduction [15]. The sample size for Part 2 was increased from 14 to 19 to account for an anticipated higher dropout rate for participants with SCI.

2.2. Participants

A convenience sample of fourteen adult AB participants with no history of pelvic fractures and no evidence of a current pressure ulcer were recruited. Nineteen adults with SCI (traumatic or non-traumatic) resulting in permanent injury and who used a wheelchair for mobility were recruited. Exclusion criteria involved SCI participants with a history of pelvic fracture or a Category 3 or 4 PU or who presented with a current PU or deep tissue injury overlying the ischium of the pelvis. All participants were recruited through verbal contact with people in the community and hospital.

2.3. Ethical issues and approval

Ethical approval was obtained from Human Research Ethics Committees at the hospital and university (SMAHS #9/326 and SMAHS #9/414). All participants signed informed consent and all data was de-identified and confidentiality was assured.

2.4. Instrumentation

2.4.1. Anthropomorphic measurements

The height and weight of the AB participants were measured in standing and the height of SCI participants was measured in a lying position using a standard protocol. Body weight of SCI participants was estimated from the difference in weight when they sat in their wheelchair from that of their wheelchair alone.

2.4.2. Ultrasound biometrics chair

All ultrasound biometrics were obtained whilst the participants sat in a purpose-built ultrasound chair (Fig. 1a). The chair's seat

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depth, width and height and back tilt/recline angle and footrest height were adjusted to match participant's anthropometric measurements in their typical sitting posture as per a written protocol. There were two conditions for both studies, namely *unloaded* sitting and *loaded* sitting. In Part 1, *unloaded* sitting involved the use of a custom padded toilet seat (Fig. 1b) to provide full access to the soft tissues overlying the IT (Fig. 1c), while *loaded* sitting used a water-filled cushion overlying a rigid planar seat with 2 rectangular apertures (Fig. 1d). This support was later deemed unsafe for SCI use due to the solid bar in the midline and difficulties adjusting the amount of water in the cushion specifically for insensate individuals. Accordingly, in Part 2, *unloaded* sitting used the same padded toilet seat (Fig. 1c) and loaded sitting used a 0.75 mm thick polyvinyl sheet that was cut and secured across a rigid seat with a cut-out for the buttocks (Fig. 1e). US gel was liberally applied to the top and bottom of both seats in *loaded* sitting. Participants were transferred into the US chair using a cut-out sling and powered floor hoist (Invacare IPL 205, U.S.A.).

2.4.3. Ultrasound instrumentation and data acquisition

B-mode ultrasound images were obtained with a Philips CX50 (Philips, Eindhoven, NL) ultrasound imaging system using a 3–12 MHz linear transducer and a 1–5 MHz curved transducer. Musculoskeletal pre-sets were optimized and saved for the 3–12 MHz linear transducer with both sonographers prior to the data collection. The biometrics were performed using the onboard Philips calipers and algorithms in the software to measure thicknesses and bony diameters.

A standardised musculoskeletal ultrasound protocol was developed and was first pilot tested on a sample of 5 AB participants and adjustments were made. The Part 1 US protocol was documented (Appendix 1) and reviewed and revised for Part 2 to address feasibility and safety concerns for participants with SCI (Appendix 1). This protocol was pilot tested with one paraplegic and one tetraplegic participant.

For Part 1, two soft tissue layers (total thickness and skin/fat thickness) between the lowest point of the IT and overlying skin surface were each measured ten times by both sonographers in both *unloaded* and *loaded* sitting. Using the same image, the thickness of the subcutaneous fat and skin was estimated from the distance of the echogenic interface of the gluteus maximus muscle fascia to the skin surface (Fig. 2).

Repeated measurements were made for the right side only. The thickness of the combined hamstring tendon and gluteus maximus muscle layer were estimated by subtracting the skin and fat thickness from the total thickness.

In addition, in *loaded* sitting alone, the diameter of the inferior curvature of the IT in its short and long axis was measured 10 times by both sonographers (Fig. 2 b,c).

For Part 2, five soft tissue layers (total, skin, fat, tendon and muscle) were measured between the lowest point of the IT and overlying skin surface three times each by each sonographer in both *unloaded* and *loaded* sitting on both the left and right ischium. Two additional thickness parameters with the combination of tendon/muscle and skin/fat were estimated during post processing.

For Parts 1 and 2, the sonographers and the test order were randomised and sonographers were blinded to participants' medical histories, their colleague's measurements and their own measurements by occluding the on-screen measurements. All images and measurements were obtained independently and all annotations and measurements were completed in real-time.

For Part 2, any abnormal ultrasounds images were confirmed, measured and described by both sonographers and reviewed by the senior medical consultant of the SCI unit.

2.4.4. Statistical analysis

All of the data were analysed with the IBM SPSS Statistics for Windows (Version 22.0). For AB and SCI participants, mean and standard deviation were calculated for each layer and bone biometric. A coefficient of variation (CV) was calculated for the 10 images acquired by each sonographer for each AB and the 3 images acquired for each SCI. The mean CV for all participants for each sonographer in both *unloaded* and *loaded* sitting was estimated [16–18]. The ICC for within-operator reliability among repeated measures was estimated using the model $ICC_{3,1}$ which is a 2-way mixed model with consistency agreement and a single measure [19,20]. A threshold reliability of 0.75 was identified *a priori* as a 'good' rating based on both Portney and Watkins [19] and in systematic reviews of the reliability of US to measure muscle biometrics [21,22]. ICC estimates below this threshold were reported as 'poor'.

The between-operator reliability for between sonographers utilised the model $ICC_{2,1}$ which is a 2-way random model with absolute agreement and single measure [19,20]. The ICC estimate

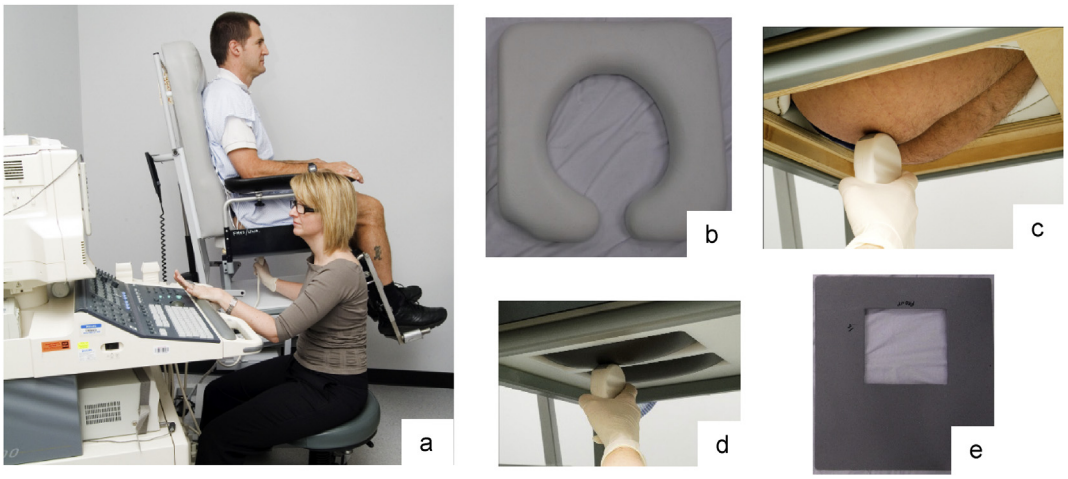

Fig. 1. Purpose built US biometric chair with power elevating seat, tilt and recline (a); padded commode seat for sitting unloaded used for AB and SCI studies (b); padded commode seat for unloaded for AB and SCI with transducer placement for soft tissue scanning (c); water-filled cushion for loaded sitting for AB (d); solid seat with polyvinyl plastic overlay for loaded sitting for SCI.

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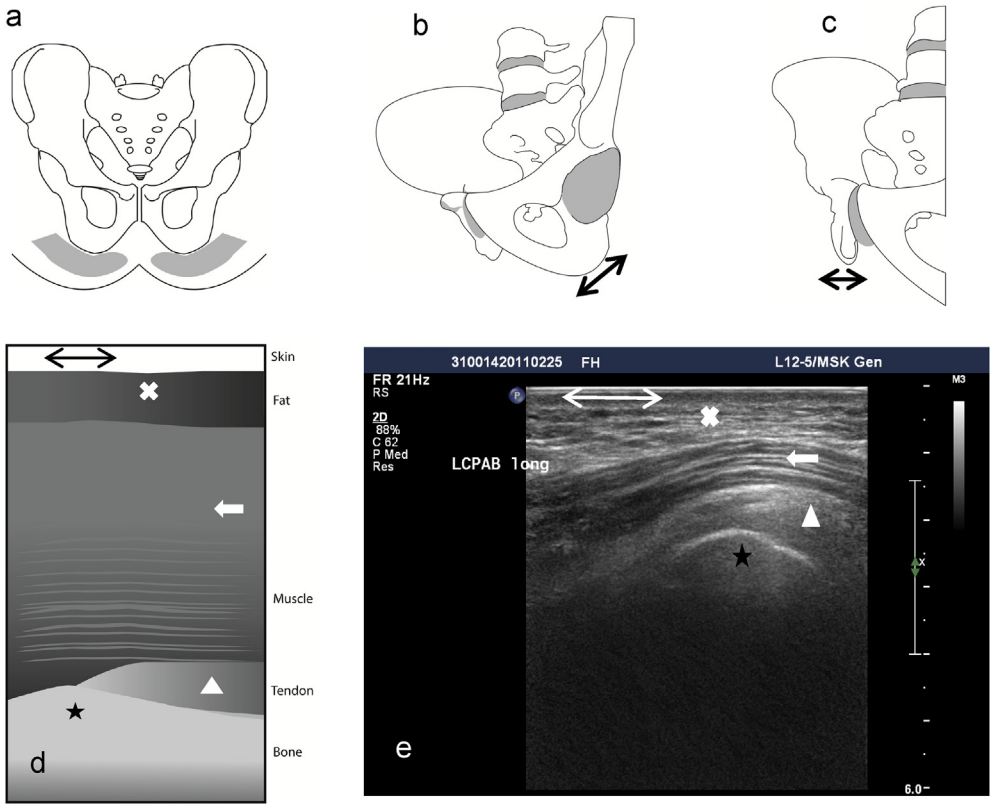

Fig. 2. Illustration of pelvis in the Cartesian plane (coronal view) with soft tissue layers under the ITs (a); illustration of IT with the long axis of the bone (arrow) used in US protocol (b); illustration of IT with the short axis of the bone (c). Illustration of the 5 soft tissue layers (d). US image of long axis view of the IT (star), hamstring tendon which is the conjoint tendon (triangle) with the proximal end of the biceps femoris muscle and the semitendinosis muscle which originates on the IT (star), gluteus muscle layer (arrow), fat layer (x), skin layer (chevron) and skin (double headed arrow) (e).

represents the reliability attainable when using either one of the averages obtained by either sonographer [23,24]. A one-way repeated measures ANOVA was performed to determine whether differences existed between sonographers for each parameter [19]. The Bland–Altman analysis was subsequently used as a measure of the level of agreement [17,25,26]. The mean difference (bias) between the two sonographers was estimated in conjunction with the 95% limits of agreement (LoA) [23,25,26] and the percent mean difference for each parameter was also estimated [25].

Data quality was measured using a source verification audit procedure to determine the accuracy of caliper placement in accordance with the US protocol for all soft tissue measurements [27]. An *a priori* proportion correct threshold of 95% for each sonographer measuring all soft tissue layers in both groups was defined as 'accurate' caliper placement for this study [27].

To determine whether the first 3 observations of the thickness for tendon/muscle and skin/fat were different between the AB and SCI in *unloaded* and *loaded* sitting, a Mann-Whitney *U* test was used with significance set to $p < 0.05$. The effect size was estimated by converting the resulting z statistic to an *r* value [28] and Cohen's [29] conventions applied whereby $r = 0.1$ is considered a small effect size, $r = 0.3$ is medium, and $r = 0.5$ is large.

3. Results

In Part 1, two senior sonographers measured 14 AB participants, with 10 repetitions for each 3 soft tissue layers and bone diameters in 2 axes. In Part 2, the same 2 senior sonographers measured 8 SCI participants, with 3 repetitions for each 5 soft tissue layers.

3.1. Participant characterisation

Fourteen AB participants (7 males and 7 females) were measured in Part 1 with a mean age of 36.7(±12.09) years and a mean BMI of 23.7(±2.4) kg/m^2. Nineteen participants with SCI were screened for US, three were excluded due to abnormal skin signs. An additional eight participants (42%) were excluded as they were adjudged to exhibit abnormal ultrasound signs with no clinical evidence of deep tissue injury or non-blanching erythema [30]. These 8 participants had a total of 9 abnormal US signs with 8 (89%) directly overlying the IT. Eight abnormal signs (89%) could be identified in specific soft tissue layers where three were cystic (33%), two were solid (22%), and four were a combination (45%). The remaining eight SCI participants (all males) were included in Part 2 with a mean age of 31.6 (±13.6) year; and a mean BMI of 23.4 (±4.8) kg/m^2. This cohort included 4 paraplegics and 4 tetraplegics with a mean time post SCI of 99.6(±136.5) months.

3.2. Sonographer characterisation

Two expert sonographers with postgraduate level of education who were instructors in a university sonography program and who worked in tertiary hospitals with more than 10 years musculoskeletal experience developed the protocol.

3.3. Part 1 reliability

The descriptive statistics for the US biometrics in *unloaded* and *loaded* sitting for AB are summarised in Tables 1 and 2 respectively.

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The within-operator reliability estimates for sonographers measuring the 3 soft tissue layers ranged between 0.86 and 0.98 in both *unloaded* and *loaded* sitting and the mean CV for ten repeated scans for each soft tissue layer ranged between 2.8% and 6.9% for *unloaded* and 3.9% to 8.1% for *loaded* sitting (Tables 1 and 2). The within-operator reliability of the sonographers measuring the radius of the lowest point of the IT in both the short and long axes, ranged between 0.50 and 0.92 and the mean CV ranged between 4.2% and 10.3%.

Between-operator reliability values for AB for sonographers measuring all three soft tissues layers in *unloaded* and *loaded* sitting ranged between 0.81 and 0.90 (Table 3). Three biometrics: skin/fat in *unloaded* ($p = 0.03$), total in *loaded* ($p = 0.04$), and tendon/muscle in *loaded* ($p = 0.04$) confirmed systematic differences between sonographers. The Bland-Altman mean differences (bias) for soft tissue layers in *unloaded* and *loaded* sitting ranged between −0.178 and 0.13 cm, resulting in a difference ranging from −0.4 to 9.7%. Fig. 3a demonstrates the Bland-Altman plot for mean tendon/muscle thickness in *unloaded* sitting whereby Sonographer 2 measurements were 0.03 cm (0.4%) higher than Sonographer 1.

The between-operator reliability estimate for the IT bone diameter in its short axis was −0.28 and −0.01 in its long axis. Systematic measurement differences between sonographers was confirmed for the IT diameter short axis ($p = 0.01$). The Bland-Altman mean difference (bias) estimate for the IT short axis was −0.680 cm resulting in a difference of 19.2% and for the IT long axis, it was a mean difference of 0.22 cm (8.1%) (Fig. 3b).

3.4. Part 2 reliability

The descriptive statistics for the 5 soft tissue layers and the corresponding estimates for within-operator reliability in *unloaded* and *loaded* sitting are summarised in Tables 4 and 5, respectively. A total of 8 participants had all required measurements done by both sonographers, except for one participant had a missing skin measurement from one sonographer. Both left and right sides were used for a total sample of 16 and all soft tissue layers were identifiable and measureable on US. The within-operator reliability estimates for sonographers measuring the 5 soft tissue layers ranged between 0.38 and 0.98 in both *unloaded* and *loaded* sitting and the mean CV ranged between 7.0% and 17.4% for *unloaded* and 7.2% to 17.2% for *loaded* sitting (Tables 4 and 5).

Table 6 indicates that between-operator reliability of the sonographers for both *unloaded* and *loaded* sitting ranged between 0.10 and 0.97. Skin biometrics in *unloaded* ($p = 0.04$) and skin in *loaded* confirmed systematic differences between sonographers ($p < 0.001$). The Bland-Altman mean differences (bias) for soft tissue layers in *unloaded* and *loaded* sitting ranged between −0.009 to 0.085 cm (0.5–28.6%).

The source verification audit for Parts 1 and 2 resulted in correct caliper placement ≥95% for all soft tissue layers, excluding Part 2 where tendon and skin accuracy was 15% and 8% respectively.

3.5. Group differences

A Mann-Whitney *U* test indicated that mean tendon/muscle thickness in the SCI participants in *unloaded* sitting (Mean Rank = 4.5, n = 8) and *loaded* sitting (Mean Rank = 4.75, n = 8) were significantly lower than AB participants in *unloaded* sitting (Mean Rank = 15.50, n = 14, U = 0.00, z = −3.822, $p < 0.001$, two-tailed) and *loaded* sitting (Mean Rank = 15.36, n = 14, U = 2.0, z = −3.6, $p < 0.001$, two-tailed). The effect size for *unloaded* sitting tendon/muscle was $r = 0.81$ and $r = 0.77$ for *loaded*. No significant differences were noted for the skin/fat layer (both $p > 0.05$).

4. Discussion

The present study was designed to establish the reliability of US biometric imaging in measuring the diameter of the IT and the thickness of the overlying soft tissues in *unloaded* and *loaded* sitting. The present study indicates reliability coefficients that met the *a priori* threshold of ICC ≥0.75 for good reliability set out by Portney and Watkins [19] for the three-layer model consisting of total thickness, skin/fat and tendon/muscle in both AB and SCI but poor for the five-layer model as a direct result of poor reliability estimates for tendon and skin. To our knowledge, the within-and between-operator reliability of real-time US for these soft tissues measurements by sonographers has not been previously reported.

In the AB group, the ICC values for the diameter of the inferior curvature of the bone in both axes were negative and violate reliability model assumptions [24]. Indeed given the value of the ICC is between 0 and 1, negative values were interpreted as zero or no reliability [19,31]. It is well established that the shape of the inferior curvature of the IT can vary [7] and, in the present study, both sonographers expressed their concern with the lack of operational definition as previously reported [11] and the lack of registration points for this anatomical site when compared to those in other imaging protocols [32]. Specifically, in the present study, the lowest point of this bone during sitting was dependent on each participant's pelvic posture in the anterior-posterior plane. Pelvic tilt may have changed within and between sonographer sessions. This result is in contrast to Akins and colleagues [11] who reported an higher concordance value (ICC = 0.712), whilst translating a similar MRI protocol. However, this earlier study utilised a single scan operator method to acquire images with participants placed in more supportive positions of supine and lateral recumbent and used two operators to post-process the data on the same images. Such a post processing protocol will inevitably uncouple the decisions an operator must make during real-time image acquisition and measurement.

No previous studies offer reliability estimates of the thickness for the hamstring tendon and skin overlying the inferior curvature of the ischium for AB and SCI in *unloaded* and *loaded* sitting using real-time sonography; however, the reliability estimates were poor in the present study. The two major reasons for low ICC values in any study are lack of variation between measurements and

Table 1
Within-operator reliability for Part 1, sonographers 1 and 2 measuring able-bodied participants (n = 14) in *unloaded* sitting.

Unloaded Sitting					
Soft Tissue Thickness (cm)		Mean (cm) (SD)	ICC $_{3,1}$	95%CI	CV%
Total	Sono 1	4.86 (0.76)	0.96	0.92–0.98	2.93
	Sono 2	4.76 (0.83)	0.97	0.94–0.99	2.83
Tendon & muscle	Sono 1	3.64 (0.59)	0.95	0.90–0.98	3.61
	Sono 2	3.67 (0.70)	0.95	0.91–0.98	4.13
Skin & fat	Sono 1	1.22 (0.47)	0.98	0.99–0.99	5.71
	Sono 2	1.09 (0.34)	0.94	0.89–0.98	6.93

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Table 2
Within-operator reliability for Part 1, sonographers 1 and 2 measuring able-bodied participants (n = 14) in *loaded* sitting.

Loaded Sitting					
Soft Tissue Thickness (cm)		Mean (cm) (SD)	$ICC_{3,1}$	95%CI	CV%
Total	Sono 1	2.61 (0.63)	0.95	0.90–0.98	4.88
	Sono 2	2.78 (0.59)	0.96	0.93–0.99	3.94
Tendon & muscle	Sono 1	1.58 (0.51)	0.93	0.85–0.97	8.12
	Sono 2	1.74 (0.47)	0.86	0.71–0.94	6.60
Skin & fat	Sono 1	1.03 (0.30)	0.94	0.89–0.98	6.41
	Sono 2	1.04 (0.28)	0.94	0.88–0.98	6.94
IT Diameter (cm)					
IT (short)	Sono 1	2.42(0.50)	0.58	0.39–0.79	10.32
	Sono 2	3.10(0.64)	0.92	0.86–0.97	4.98
IT (long)	Sono 1	6.00 (0.89)	0.50	0.31–0.74	8.68
	Sono 2	5.78(0.67)	0.86	0.75–0.94	4.22

Table 3
Between-operator reliability for Part 1, sonographers 1 and 2 measuring able-bodied participants (n = 14) in *unloaded* and *loaded* sitting.

Soft Tissue Thickness (cm)	Mean (cm) (SD)	ICC 2,1	95%CI	$F_{1,13}$	*p*	Mean Difference (cm) (SD)	% Difference (SD)
Unloaded Sitting							
Total	4.81 (0.78)	0.87	0.66–0.96	0.92	0.36	0.102 (0.398)	2.4 (8.1)
Tendon & muscle	3.65 (0.64)	0.90	0.72–0.97	0.16	0.70	−0.032 (0.297)	−0.4 (8.1)
Skin & fat	1.16 (0.41)	0.85	0.49–0.95	6.45	0.03	0.134 (0.197)	9.7 (14.5)
Loaded Sitting							
Total	2.69 (0.60)	0.85	0.55–0.95	5.09	0.04	−0.178 (0.295)	−7.0 (11.3)
Tendon & muscle	1.66 (0.24)	0.81	0.46–0.94	5.34	0.04	−0.165 (0.267)	−5.8 (23.7)
Skin & fat	1.03 (0.28)	0.85	0.60–0.95	0.10	0.76	−0.013 (0.156)	2.8 (19.6)
IT Diameter (short)	2.77(0.52)	−0.28	−0.26-(-0.35)	11.40	0.01	−0.680 (0.753)	−19.2 (21.9)
IT Diameter (long)	5.89(0.64)	−0.01	−0.53–0.51	0.83	0.38	0.223 (0.914)	8.1 (18.5)

disagreement within one sonographer or between two sonographers' measurements [19]. The within-operator tendon measurements met the criteria set out by Portney and Watkins [19] for heterogeneous measurements. It is recognised, however, that others have measured the thickness of the long head of the biceps femoris tendon at its origin site on the IT in AB using the more common posture of prone lying [33] and reported a higher reliability estimate [34]. Nonetheless, as the present protocol dictated the measurement of the tendon at the most inferior curvature of the IT in sitting, this approach may have included both the conjoint tendon of the biceps femoris and semitendinosus muscles and, in some cases, the tendon for the semimembranosus muscle [35]. Small postural changes in the anterior-posterior plane could have altered tendon measurements as the tendon thickness varies along its structure [35–37]. This could explain the variability of the tendon-related measurements. In addition, the inaccuracies associated with some caliper placements by the sonographer would inevitably introduce measurement errors. The combined measurement of the tendon and muscle thickness removed the uncertainty of identifying the tendon-muscle interface, and eliminated caliper placement issues that resulted in good reliability estimates in *unloaded* and *loaded* sitting for both AB and SCI groups.

Within-operator reliability estimates for skin thickness were poor for both sonographers in *loaded* sitting and sonographer 1 in *unloaded* sitting, although it was good for sonographer 2. Measurement heterogeneity was confirmed [19], therefore the poor reliability coefficients were due to the disagreement across repeated measurements of the same sonographer which was also reported by Akesson et al. [38] who used a 10 MHz probe. The resulting between-operator reliability estimates were poor for both *unloaded* and *loaded* sitting with ICC confidence intervals that included zero or no reliability [19,31]. However, the combined skin/fat measurement eliminated the variability and resulted in better reliability estimates.

It is recommended that thresholds of acceptable 95% limits of agreement (LoA) for each comparison be identified *a priori*; however, these were not made in the present study due to lack of published acceptable LoA levels for the application of FEM and computing soft tissue strains [25,39,40]. In general, the mean differences between sonographers were low except for skin and tendon. Given the future aim is to use these US biometrics for FEM to compute the strains over time with newly injured patients, a change that exceeds the mean difference should reflect a real change as opposed to that due to measurement reliability [41]. The 95% LoAs for skin and tendon indicated a range of measurement errors that appeared to be too high, given their small dimensions.

An estimate of the compressive strain in the tissues based on thickness changes for AB was estimated by using the simple definition of a nominal strain:

$$\varepsilon = \frac{t - t_0}{t_0}$$

with t_0 the original thickness and t the thickness during loading.

The results for the total strain was −0.44; tendon/muscle layer was −0.54; and fat/skin layer was −0.11. These strains are of very similar magnitude very similar to those reported in MRI studies [42,43]. This strongly suggests that the US protocol provides a realistic indication of the internal strains in this specific loading condition for individual SCI participants.

The present study clearly included a limited cohort of both AB and SCI participants. The SCI participants who were screened, exhibited an unexpected large proportion of abnormal ultrasound images and a separate publication will describe details of these abnormal results. Four of the SCI participants did not have ASIA Impairment Scale assessments [44] and while this would not be predicted to impact on estimates of reliability, it would impact on thickness measurements with muscle atrophy in denervated muscles [7,11]. In the present study, the SCI participants had significantly thinner tendon/gluteus muscle and these effect sizes

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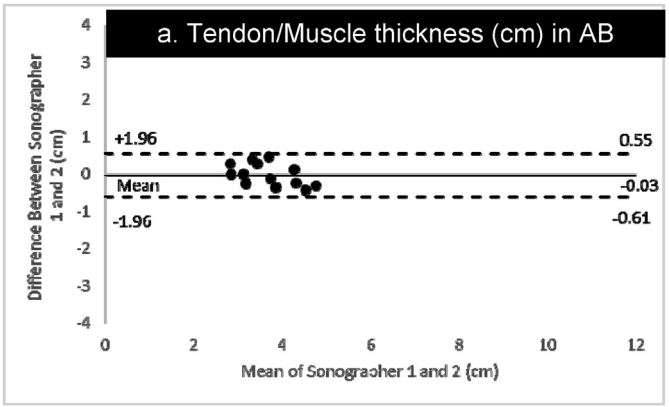

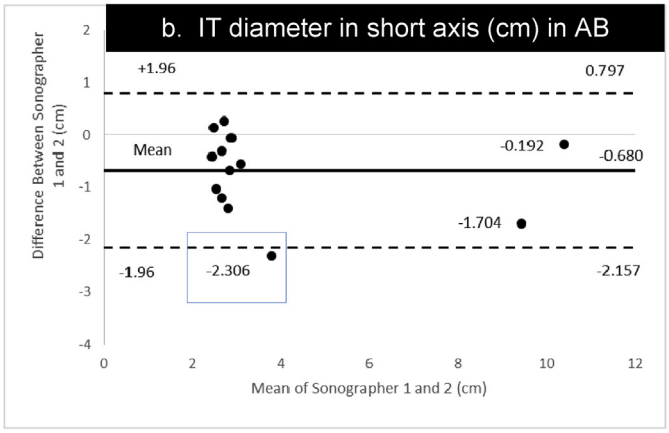

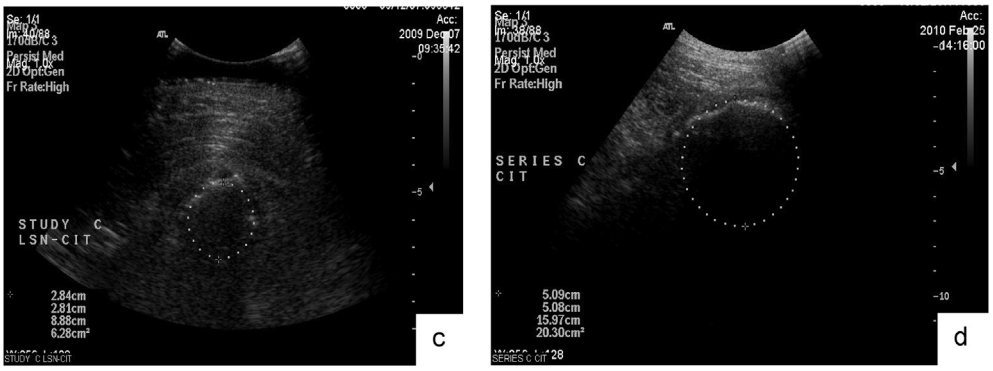

Fig. 3. Bland-Altman plots for (a) tendon/muscle thickness in AB *unloaded*; and (b) diameter of IT in short axis in AB *loaded*. Bland-Altman plots: the horizontal line represents the mean difference and the upper and lower 95% limits of agreement. Ultrasound images: image (c) is one of ten images for Sonographer 1 measuring diameter of IT in short axis in AB *loaded* and (d) is Sonographer 2. These images correspond to participant on the Bland-Altman plot (b) with a difference between sonographers of −2.3 cm (square box).

could be described as large in both *unloaded* and *loaded* sitting using Cohen's conventions [29]. Atrophy of gluteus muscle in this region has been well documented [7,45] and may contribute to the higher shear strains found in individuals with paraplegia [7].

In the present study, expert sonographers were chosen to minimise variability associated with novice sonographers [12,46], but measurement error was still identified. Error attributable to within the raters in both tendon and skin biometrics was greater than other layers and several factors may contribute. Skin and tendon were the smallest thicknesses measured and small differences in measurement would result in larger variations and lower ICCs. The coefficients of variation were within the range of other measurements. Variations in the measured values may be significantly reduced by implementing training with a focus on correct caliper placement. Use of a newer conventional ultrasound machine with a higher frequency probe could reduce measurement error related to the technical limits of the present US machine. Pelvic posture may also alter the site of measurements, therefore minimising changes in pelvic posture of the participants through additional supports in the US chair would serve to reduce changes during imaging.

Computational biomechanical models require simplifications and assumptions of the modelled anatomical structures [47]. Given the small magnitude of the differences in measurements within and between sonographers, even with poor ICCs, the impact on a 5-layer FE model of the buttocks, may not be significant as other factors such as boundary conditions and material properties of the tissue layers have substantial effect [7]. In addition, variation in skin

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Table 4
Within-operator reliability for Part 2, sonographers 1 and 2 measuring participants with SCI (n = 16) in *unloaded* sitting.

Unloaded Sitting					
Soft Tissue Thickness (cm)		Mean (cm) (SD)	$ICC2_{3,1}$	95%CI	CV%
Total	Sono 1	2.77 (1.06)	0.97	0.94–0.99	7.24
	Sono 2	2.73 (0.82)	0.96	0.90–0.98	6.97
Tendon	Sono 1	0.33 (0.09)	0.50	0.19–0.76	17.02
	Sono 2	0.31 (0.09)	0.55	0.25–0.79	17.20
Muscle	Sono 1	1.00 (0.58)	0.92	0.82–0.97	17.38
	Sono 2	1.06 (0.60)	0.92	0.82–0.97	11.32
Fat	Sono 1	1.34 (0.55)	0.85	0.69–0.94	12.18
	Sono 2	1.32 (0.57)	0.92	0.83–0.97	10.89
Skin	Sono 1	0.20 (0.01)	0.75	0.53–0.90	8.51
	Sono 2	0.18 (0.05)	0.65	0.38–0.84	14.48
Tendon & muscle	Sono 1	1.33 (0.59)	0.91	0.81–0.97	13.12
	Sono 2	1.36 (0.64)	0.87	0.75–0.95	8.39
Skin & fat	Sono 1	1.44 (0.65)	0.93	0.84–0.97	12.20
	Sono 2	1.35 (0.52)	0.85	0.69–0.94	12.66

Table 5
Within-operator reliability for Part 2, sonographers 1 and 2 measuring participants with SCI (n = 16) in *loaded* sitting.

Loaded Sitting					
Soft Tissue Thickness (cm)		Mean (cm) (SD)	$ICC_{(3,1)}$	95%CI	CV
Total	Sono 1	1.47 (0.63)	0.96	0.92–0.99	7.59
	Sono 2	1.51 (0.64)	0.98	0.95–0.99	7.19
Tendon	Sono 1	0.26 (0.07)	0.52	0.22–0.78	16.17
	Sono 2	0.24 (0.08)	0.68	0.43–0.86	16.11
Muscle	Sono 1	0.43 (0.22)	0.93	0.85–0.97	13.21
	Sono 2	0.46 (0.24)	0.84	0.68–0.94	10.01
Fat	Sono 1	0.73 (0.44)	0.91	0.81–0.97	16.87
	Sono 2	0.67 (0.44)	0.96	0.90–0.98	17.17
Skin	Sono 1	0.21 (0.03)	0.38	0.07–0.68	9.79
	[a]Sono 2	0.17 (0.04)	0.58	0.28–0.82	12.84
Tendon & muscle	Sono 1	0.69 (0.26)	0.93	0.79–0.98	10.67
	Sono 2	0.70 (0.29)	0.88	0.66–0.97	8.30
Skin & fat	Sono 1	0.78 (0.41)	0.91	0.74–0.98	15.23
	Sono 2	0.80 (0.42)	0.78	0.45–0.95	15.47

[a] Skin measurements for Sonographer 2 based on n = 15 due to missing data.

Table 6
Between-operator reliability for Part 2, sonographers 1 and 2 measuring participants with SCI (n = 16) for *unloaded* and *loaded* sitting.

Soft Tissue Thickness (cm)	Mean thickness (SD) (cm)	$ICC_{2,1}$	95%CI	$F_{1,15}$	*p*	Mean Difference (cm)	%Difference
Unloaded Sitting							
Total	2.75 (0.96)	0.91	0.76–0.97	0.14	0.72	0.039(0.418)	2.0 (21.6)
Tendon	0.32 (0.08)	0.26	−0.25–0.66	0.75	0.40	0.021(0.095)	11.1 (32.7)
[a]Muscle	1.03 (0.61)	0.96	0.90–0.99	1.77	0.21	−0.067 (0.162)	−4.5 (25.2)
Fat	1.33 (0.55)	0.80	0.52–0.93	0.02	0.89	−0.018 (0.404)	4.2 (27.3)
Skin	0.19 (0.04)	0.37	−0.07–0.71	4.98	0.04	0.026 (0.046)	16.5 (22.3)
[c]Tendon & muscle	1.36 (0.61)	0.94	0.85–0.98	0.78	0.39	−0.046 (0.209)	1.2 (23.6)
[c]Skin & fat	1.40 (0.57)	0.75	0.43–0.91	0.71	0.41	0.085 (0.403)	5.0 (31.1)
Loaded Sitting							
Total	1.49 (0.65)	0.97	0.93–0.99	1.08	0.31	−0.039 (0.147)	0.5 (19.1)
Tendon	0.25 (0.06)	0.71	0.35–0.89	3.26	0.09	0.021 (0.047)	14.7 (25.4)
Muscle	0.45 (0.23)	0.85	0.64–0.95	0.94	0.35	−0.030 (0.124)	−10.1 (32.2)
Fat	0.70 (0.44)	0.91	0.76–0.97	1.85	0.19	0.063 (0.185)	9.8 (37.5)
[b]Skin	0.19 (0.03)	0.10	−0.13–0.44	21.54	<0.001	0.051 (0.044)	28.6 (25.0)
[c]Tendon & muscle	0.70 (0.27)	0.88	0.69–0.96	0.68	0.80	−0.009 (0.139)	1.3 (22.4)
[c]Skin & fat	0.79 (0.42)	0.94	0.83–0.98	0.59	0.45	−0.029 (0.153)	−0.8 (26.6)

[a] Sonographer 1 on loaded sitting had missing data, n = 14.
[b] Sonographer 2 in loaded sitting had missing data, n = 15.
[c] Calculated variables from sonographers measuring total, tendon, skin, and fat.

and tendon thickness would be evaluated in a sensitivity analysis during model development [48]. Therefore, the clinical significance of the measurement variability in skin and tendon may be small but the contribution of these layers within the FE model may be significant.

5. Conclusion

Experienced sonographers demonstrated good within- and between-operator reliability in the real-time ultrasound measurement of 3 soft tissue layers overlying the inferior curvature of the IT in both AB and individuals with SCI and poor reliability for the

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diameter of the IT whilst sitting upright. Replicating this protocol in simulated sitting in a supine or a lateral recumbent position would eliminate the need for the US chair and establish the reliability of the 3-layer soft tissue US protocol that uses a preferred position of SCI participants and less ergonomic demands on the sonographer. The current sitting 3-layer model can be used in 2-D FEM to calculate internal strains in these soft tissues as a potentially new risk factor for the development of PUs in the SCI population.

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Conflict of interest

No conflict of interest was noted for any authors.

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Appendix 1

Ultrasound Protocol

To acquire images for soft tissue measurement with the linear transducer, sonographers first located the IT. This was performed by scanning the buttock back and forth in the coronal plane. The transducer was then rotated into a sagittal plane, where the hamstring tendon insertion was visualized and served as an anatomical landmark. By rotating the transducer slightly away from the sagittal plane, alignment with the muscle fibers of the gluteus maximus muscle was achieved. This allowed optimal differentiation between the muscle and overlying subcutaneous fat, which increased the sonographer's confidence in measurement caliper placement. Transducer pressure was an important aspect of technique. The transducer was off loaded until only contact with the skin was achieved, to be able to measure the full thickness of soft tissue underlying the IT free of compression. This often resulted in a contact artefact at the periphery of the image where the edges of the transducer face lost contact with the skin due to the curvature of the buttock.

Measurements of curvature were made in the long and short axes of the IT using the curvilinear transducer. The IT was located as just described, and the lowest point was taken as that closest to the skin. For the long axis image, the transducer was rotated to align with the length of the ischium, approximately 45° to the mid sagittal plane (MSP). The images chosen for capture contained the lowest point of the ischium in its long axis. For the short axis images, the transducer was rotated 90° from the position for the long axis. Again, the lowest point of the IT was sought and a caliper placed on this point using the integrated software of the Philips imaging system. Measurements of curvature were made by fitting a circle to the outer bony surface of the IT, from the lowest point of the ischium. The circles were placed such that they conformed to the surface of the bony cortex without crossing over it. Ten measurements were made for both the long and short axes, with the probe being removed from the skin between each image capture.

Part 2 Protocol Alterations

Part A ultrasound protocol was reviewed for safety and feasibility concerns and it was decided to reduce the overall sitting time on the rigid surface for *loaded* sitting. In Part A, the AB participants provided verbal feedback to the sonographer when they felt the pressure of the probe over their IT whilst sitting on the water cushion. In Part B, since the majority of the SCI participants were insensate, it was decided to have the medical student conducting the skin checks, also palpate the ITs and mark the overlying skin with an "X" which could be viewed through the clear polyvinyl seat (1e). This marking provided an efficient visual orientation of the IT for the sonographer before following the US protocol used in Part A.

References

[1] Garber SL, Rintala DH, Hart KA, Fuhrer MJ. Pressure ulcer risk in spinal cord injury: predictors of ulcer status over 3 years. Arch. Phys Med Rehabil 2000;81:465–71.
[2] Fuhrer MJ, Garber SL, Rintala DH, Clearman R, Hart KA. Pressure ulcers in community-resident persons with spinal cord injury: prevalence and risk factors. Arch Phys Med Rehabil 1993;74:1172–7.
[3] Coleman S, Nixon J, Keen J, Wilson L, McGinnis E, Dealey C, et al. A new pressure ulcer conceptual framework. J Adv Nurs 2014;70(10):2222–34.
[4] Gelis A, Dupeyron A, Legros P, Benaim C, Pelissier J, Fattal C. Pressure ulcer risk factors in persons with SCI: Part I: acute and rehabilitation stages. Spinal Cord 2009;47(2):99–107.
[5] Gelis A, Dupeyron A, Legros PB, Benaim C, Pelissier J, Fattal C. Pressure ulcer risk factors in persons with spinal cord injury: Part 2: the chronic stage. Spinal Cord 2009;47:651–61.
[6] Marin J, Nixon J, Gorecki C. A systematic review of risk factors for the development and recurrence of pressure ulcers in people with spinal cord injuries. Spinal Cord 2013;51(7):522–7.
[7] Linder-Ganz E, Shabshin N, Itzchak Y, Yizhar Z, Siev-Ner I, Gefen A. Strains and stresses in sub-dermal tissues of the buttocks are greater in paraplegics than in healthy during sitting. J Biomech 2008;41(3):567–80.
[8] Linder-Ganz E, Yarnitzky G, Yizhar Z, Siev-Ner I, Gefen A. Real-time finite element monitoring of sub-dermal tissue stresses in individuals with spinal cord injury: toward prevention of pressure ulcers. Ann Biomed Eng 2009;37(2):387–400.
[9] Luboz V, Petrizelli M, Bucki M, Diot B, Vuillerme N, Payan Y. Biomechanical modeling to prevent ischial pressure ulcers. J Biomech 2014;47(10):2231–6.
[10] Lin F, Moran B, Bankard J, Hendrix R, Makhsous M. A subject-specific FEM model for evaluating buttocks tissue response under sitting load. In: Proceedings of the 26th annual international conference of the IEEE EMBS; 2004. p. 5088–91. San Fransisco, CA, USA.
[11] Akins JS, Vallely JJ, Karg PE, Kopplin K, Gefen A, Poojary-Mazzotta P, et al. Feasibility of freehand ultrasound to measure anatomical features associated with deep tissue injury risk. Med Eng Phys 2016;38(9):839–44.
[12] Dudley-Javoroski S, McMullen T, Borgwardt MR, Peranich LM, Shields RK. Reliability and repsonsiveness of musculoskeletal ultrasound in subjects with and without spinal cord injury. Ultrasound Med Biol 2010;36(10):1594–607.
[13] Yalcin E, Akyuz M, Onder B, Unalan H, Degirmenci I. Skin thickness on bony prominences measured by ultrasonography in patients with spinal cord injury. J Spinal Cord Med 2013;36(3):225–30.
[14] Pillen S, van Alfen N. Skeletal muscle ultrasound. Neurol Res 2011;33(10):1016–24.
[15] Walter SD, Eliasziw M, Donner A. Sample size and optimal designs for reliability studies. Stat Med 1998;17:101–10.
[16] Lucas N, Macaskill P, Irwig L, Moran R, Rickards L, Turner R, et al. The reliability of a quality appraisal tool for studies of diagnostic reliability (QAREL). BMC Med Res Methodol 2013;13(1):111.
[17] Mc Auliffe, S., et al., A systematic review of the reliability of diagnostic ultrasound imaging in measuring tendon size: Is the error clinically acceptable? Physical Therapy in Sport.
[18] Atkinson G, Nevill AM. Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. Sports Med 1998;26(4):217–38.
[19] Portney LG, Watkins MP. Foundations of clinical research: applications to practice. third ed. , Philadelphia, PA: F.A. Davis Company; 2015.
[20] Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. Psychol Bull 1979;86(2):420–8.
[21] Hebert JJDC, Koppenhaver SLPPT, Parent ECPPT, Fritz JMPPTATC. A systematic review of the reliability of rehabilitative ultrasound imaging for the quantitative assessment of the abdominal and lumbar trunk muscles. Spine 2009;34(23):E848–56.
[22] Mc Auliffe S, O'Sullivan K, Mc Creesh K. A systematic review of the reliability of diagnostic ultrasound imaging in measuring tendon size: is the error clinically acceptable? Br J Sports Med 2014;48(7):635–6.
[23] Kottner J, Audigé L, Brorson S, Donner A, Gajewski BJ, Hróbjartsson A, et al.

Guidelines for reporting reliability and agreement studies (GRRAS) were proposed. J Clin Epidemiol 2011;64(1):96–106.
[24] Streiner DL, Norman GR. Health measurement scales fourth edition. fourth ed. , Oxford: Oxford University Press; 2008.
[25] Giavarina D. Understanding Bland altman analysis. Biochem Medica 2015;25(2):141–51.
[26] Jones M, Dobson A, O'Brian S. A graphical method for assessing agreement with the mean between multiple observers using continuous measures. Int J Epidemiol 2011;40(5):1308–13.
[27] Houston L, Probst Y, Humphries A. Measuring data quality through a source data verification audit in a clinical research setting. Stud Health Technol Inf 2015;214:107–13.
[28] Clark-Carter D. Quantitative psychological research: a student's handbook. second ed. , New York: Psychology Press; 2004.
[29] Cohen J. Statistical power analysis for the behavioral sciences. second ed. , Hillsdale, New Jersey: Erlbaum; 1988.
[30] Australian Wound Management Association. Pan pacific clinical practice guideline for the prevention and management of pressure injury. Osborne Park, Western Australia: Cambridge Media; 2012.
[31] Giraudeau, B., Negative values of the intraclass correlation coefficient are not theoretically possible. J Clin Epidemiol. 49(10): p. 1205–1206.
[32] Gameraddin M, Alhaj B, Alabdeen MZ. The reliability of biparietal diameter and femoral length in estimation the gestational age using ultrasonography. J Gynecol Obstet 2014;2(6):112–5.
[33] Bianchi S, Martinoli C. Ultrasound of the musculoskeletal system. Berlin Heidelberg Germany: Springer-Verlag; 2007.
[34] Tosovic D, Muirhead JC, Brown JMM, Woodley SJ. Anatomy of the long head of biceps femoris: an ultrasound study. Clin Anat 2016;29(6):738–45.
[35] Koulouris G, Connell D. Hamstring muscle complex: an imaging review. RadioGraphics 2005;25(3):571–86.
[36] Kellis E, Galanis N, Natsis K, Kapetanos G. Validity of architectural properties of the hamstring muscles: correlation of ultrasound findings with cadaveric dissection. J Biomech 2009;42(15):2549–54.
[37] Miller SL, Webb GR. The proximal origin of the hamstrings and surrounding anatomy encountered during repair: surgical technique. J Bone Jt Surg 2008;90(Supplement_2_Part_1):108–16.
[38] Åkesson A, Forsberg L, Hederström E, Wollheim F. Ultrasound examination of skin thickness in patients with progressive systemic sclerosis (scleroderma). Acta Radiol Diagn 1986;27(1):91–4.
[39] Bland JM, Altman DG. Agreement between methods of measurement with multiple observations per individual. J Biopharm Stat 2007;17:571–82.
[40] Bland JMAD. Statistical methods for assessing agreement between 2 methods of clinical measurement. Lancet 1986;8.
[41] Øverås CK, Myhrvold BL, Røsok G, Magnesen E. Musculoskeletal diagnostic ultrasound imaging for thickness measurement of four principal muscles of the cervical spine -a reliability and agreement study. Chiropr Man Ther 2017;25(1):2.
[42] Linder-Ganz E, Shabshin N, Itzchak Y, Gefen A. Assessment of mechanical conditions in sub-dermal tissues during sitting: a combined experimental-MRI and finite element approach. J Biomech 2007;40:1443–54.
[43] Oomens C, Bressers O, Bosboom E, Bouton C, Bader D. Can loaded interface characteristics influence strain distributions in muscle adjacent to bony prominences? Comput Methods Biomech Biomed Eng 2003;6(3):171–80.
[44] DeVivo M, Biering-Sorensen F, Charlifue S, Noonan V, Post M, Stripling T, et al. International spinal cord injury core data set. Spinal Cord 2006;44(9):535–40.
[45] Castro JM, Apple Jr FD, Hillegass AE, Dudley AG. Influence of complete spinal cord injury on skeletal muscle cross-sectional area within the first 6 months of injury. Eur J Appl Physiol Occup Physiol 1999;80(4):373–8.
[46] Hammond K, Mampilly J, Laghi FA, Goyal A, Collins EG, McBurney C, et al. Validity and reliability of rectus femoris ultrasound measurements: comparison of curved-array and linear-array transducers. J Rehabil Res Dev 2014;51(7):1155–64.
[47] Oomens CWJ, Brekelmans M, Baaijens F. Biomechanics concepts and computations. , New York: Cambridge University Press; 2009.
[48] Oomens CWJ, Broek M, Hemmes B, Bader DL. How does lateral tilting affect the internal strains in the sacral region of bed ridden patients? — A contribution to pressure ulcer prevention. Clin Biomech 2016;35:7–13.

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