

Quantitative ultrasound imaging over the ischial tuberosity: An exploratory study to inform tissue health



Sharon Gabison^{a,b,*}, Sunita Mathur^a, Mary C. Verrier^{a,b}, Ethne Nussbaum^c, Milos R. Popovic^{d,e}, Dany H. Gagnon^f

^a Department of Physical Therapy, University of Toronto, 500 University Avenue, Toronto, Ontario, M5G 1V7, Canada

^b SCI Mobility Lab, Lyndhurst Centre, Toronto Rehab-University Health Network, 520 Sutherland Drive, Room 236, Toronto, Ontario, M4G 3V9, Canada

^c MCISc Program in Field of Wound Healing, Western University, School of Physical Therapy, Elborn College, London, Ontario, M8G 1H1, Canada

^d Rehabilitation Engineering Laboratory, Lyndhurst Centre, Toronto Rehabilitation Institute - University Health Network, 520 Sutherland Drive, Room 236, Toronto, Ontario, M4G 3V9, Canada

^e Institute of Biomaterials and Biomedical Engineering, University of Toronto, Rosebrugh Bldg, 164 Room 407, Toronto, Ontario, M5S 3G9, Canada

^f School of Rehabilitation, Université de Montréal, C.P. 6128, Succursale Centre-ville, Montreal, Quebec, H3C 3J7, Canada

A B S T R A C T

Background: Characterization of ischial tissue health using a standardized diagnostic ultrasound protocol capturing thickness and gray scale analysis has not been established.

Objectives: This study evaluates inter-participant and inter-trial reliability of thickness and gray scale analysis of ultrasound images of tissues overlying the ischial tuberosity. It provides recommendations for the number of images required to minimize the standard error of measurement (SEM) and determines the number of images required for thickness, gray scale and contrast values that exceed an a-priori minimal detectable change (MDC) for repeated tissue assessment.

Methods: Brightness mode ultrasound images using a 12 MHz linear probe were collected on the dominant limb in the side lying position for ten healthy participants and partitioned into three regions of interest: skin, subcutaneous tissue and muscle. Thickness and gray scale measures of skin, muscle and subcutaneous tissue were calculated using a customized MATLAB program. Contrast of each region of interest was calculated using the Gray Scale Level Co-Occurrence Matrix. Generalizability theory was used to quantify indices of dependability and corresponding SEMs and MDCs with 90% Confidence Intervals.

Results: Participants accounted for most of the total variance (75.56% to 94.78%). Coefficient of dependability (ϕ) for thickness, grey scale and contrast measures was greater than 0.80 when more than two images were averaged. In order to detect a MDC of 21% in thickness and echogenicity measures, at least three images are required, while at least 5 images are required for a MDC of 25% for contrast measures.

Conclusions: Obtaining reliable thickness, echogenicity and contrast measures of tissue overlying the ischial tuberosity can be achieved from two ultrasound images by a single therapist on an individual participant however three and five images are required to use a MDC of 21% for thickness measures and MDC of 25% for contrast measures respectively.

1. Introduction

Diagnostic ultrasound is a safe, non-invasive, economical, widely available and efficient tool providing information regarding soft tissue integrity of the epidermis, dermis, subcutaneous tissue, muscle, tendons and joints [1–4]. Brightness mode (B-mode) diagnostic ultrasound produces grey scale images [5] which enable visualization of tissue thickness, muscle size and quality, orientation of blood vessels and progression of inflammation. B-mode ultrasound has been used to

document soft tissue injury, assess wound healing, evaluate changes in scar tissue and examine the effect of atrophy secondary to steroid use [6,7] and diseased states including musculoskeletal and neuromuscular disorders [5,8,9]. These findings suggest that diagnostic ultrasound may be a valuable tool to characterize “tissue health” in other disease states.

When using grey scale analysis of ultrasound images, each pixel from an ultrasound image is assigned a gray scale value ranging from 0 to 255, where 0 corresponds to black and 255 corresponds to white. The

* Corresponding author. SCI Mobility Laboratory, Lyndhurst Centre, 520 Sutherland Drive, Room 236, Toronto, Ontario, M4G 3V9, Canada.
E-mail address: shar.gabison@utoronto.ca (S. Gabison).

mean gray scale of the image can be quantified using the average grey scale of the pixels encompassed in a specific region of an image and defined as “echogenicity”. The frequency distribution of the gray scale values from all pixels can be interpreted using a histogram enabling the calculation of variance, symmetry, kurtosis and uniformity. Furthermore, the interrelation of the gray scale of each pixel or subgroup of pixels and its neighbour can be estimated in different orthogonal directions over a specific region of an image using a Gray Level Co-occurrence Matrix (GLCM) by examining patterns of pixel pairs. With GLCM, each pixel is assigned a gray scale value and is compared to a neighbouring pixel in a defined direction and space. The number of occurrences of the pixel pairs are summed and the probability of occurrence of that pixel pair in each of the orthogonal directions is calculated [10] and expressed as the “contrast”, where 0 corresponds to a uniform image of equal gray scale throughout the image [11] yielding information about tissue texture.

Tissue composition and changes in tissue integrity can be captured using ultrasound imaging by evaluating mean echogenicity values. Echogenicity has been measured and its value associated with the presence of intramuscular adipose tissue [12] and muscle density [13]. Echogenicity has been used to distinguish individuals with muscle abnormalities [14–16] enabling the characterization of tissue integrity [17]. Diseased muscles lose their striated patterns, appear hyperechogenic and more brightly spotted on ultrasonography [18]. Dermal edema evaluated by changes in echogenicity has been detected using ultrasound in individuals at high risk for the development of pressure injuries [3,19]. Ultrasound imaging can be used to document tissue healing through the appearance of changes in tissue homogeneity and regularity [6], the appearance of ill-defined layered structures and the presence of hypochoic areas [20]. GLCM has been conducted on various tissues including the rectus femoris [21], vastus lateralis [22], quadriceps and biceps muscles [16, 23], supraspinatus [22,23] and the Achilles tendon [11]. Nadeau et al. [11] demonstrated that higher test-retest reliability was achieved using GLCM than values extracted from the mean gray scale values.

While ultrasound imaging does not replace histopathological examination of tissue, areas of tissue damage may demonstrate reduced ultrasound reflectance (hypoechoic) secondary to edema and changes to tissue architecture [3]. Although high frequency ultrasound can be used to characterize soft tissue health in individuals to study the effect of injury, disease related changes and immobility, image quality, which is dependent on the skill of the operator [24], probe placement, scanning technique and system parameter settings [8] is integral in demonstrating its utility. Furthermore, there is a growing body of literature demonstrating different applications for its use in tissue assessment.

The reliability and validity of B-mode ultrasound has been established in estimating skeletal muscle size and has been compared with both computerized tomography [9] and magnetic resonance imaging (MRI) [25,26], which are considered the “gold standards” [9,26]. Cross-sectional area, volume and thickness of upper and lower limbs muscles have been evaluated in healthy individuals and individuals with neuromusculoskeletal disorders [5]. Echogenicity of the biceps tendon and supraspinatus tendon thickness in wheelchair users has also been studied [27,28]. Skin thickness in individuals with spinal cord injury (SCI) [20], adipose tissue thickness of young adults [29] and median nerve excursion in healthy individuals and in individuals with carpal tunnel syndrome [30] have also been measured. The presence of edema and/or damage in the dermis, subepidermal and subcutaneous tissue identified from high frequency (20 MHz) ultrasound images has been used as an adjunct assessment approach along with clinical skin assessment overlying the heels and coccygeal areas in vascular surgery patients [31].

Using ultrasound imaging to assess tissue integrity over the ischial tuberosity in healthy individuals could serve as a comparator for other populations or as a baseline in diseased states or in cases where tissue damage is suspected. For example, reduced thickness of skin over the

ischium has been found following SCI [20,32] and secondary to adaptation [33]. Additionally, morphological changes in muscles following SCI including atrophy [34], fat infiltration [35] and greater proportion of low-density muscle tissue [36] may be identified using ultrasound imaging.

Reliability estimates of echogenicity and contrast of tissues overlying the ischial tuberosity has not been established. Akins et al. [37] evaluated thickness of tissues overlying the ischial tuberosity and compared findings from measures acquired using MRI and B-mode ultrasound. The authors found that thickness measures obtained using ultrasound were reliable and correlated with measures obtained by MRI. Swaine et al. [38] evaluated between-operator reliability of measuring soft tissue thickness overlying the ischial tuberosity in loaded and unloaded conditions using ultrasound imaging and found that between-operator reliability was good in able bodied individuals and individuals with SCI. In both these studies, echogenicity or contrast were not included in the analysis.

The physiological changes identified with impending pressure injuries [3] and the lack of quantification of buttock tissue health using gray scale analysis necessitates a study to examine whether standardized measures can be quantified using ultrasound imaging. The purposes of the present study were to i) evaluate the reliability of thickness, echogenicity and contrast measures from ultrasound images of tissues overlying the ischial tuberosity, ii) provide recommendations for the number of images required to minimize the Standard Error of Measurement (SEM), and iii) obtain the number of images required for thickness, echogenicity and contrast values that exceed an a-priori minimal detectable change for repeated tissue assessment. We anticipated that our protocol would be able to establish reliable ($\phi > 0.75$) and accurate measures (SEM < 15%) for thickness, echogenicity and contrast measures of the tissue overlying the ischial tuberosity.

2. Materials and methods

This observational study was approved by the Institutional Review Board at the University of Toronto. All testing procedures were performed at the Muscle Performance Lab at the University of Toronto. All participants provided informed consent to take part in the study.

2.1. Participants

Ten healthy participants 18 years of age or older were recruited by convenience sampling for the study and evaluated at two time points spaced 60 min apart. Participants were excluded from the study if they reported any history of prior injury or surgery of the hip, knee, or ankle, history of muscle disease (e.g. muscular dystrophy, polymyositis), or any skin conditions (e.g. dermatitis, psoriasis) to eliminate any confounding variables on ultrasound imaging.

2.2. Ultrasound imaging protocol

2.2.1. Ultrasound device

A Linear Array B-Mode 12 MHz Ultrasound Transducer (GE LOGIQ-E Ultrasound system, GE Healthcare, WI, USA) was used to scan the tissue overlying the ischial tuberosity to capture spatial resolution for skin thickness. Frequency, gain, depth, dynamic range and time/gain compensation were standardized for each individual to ensure within-participant consistency in measures and to ensure optimal image quality.

2.2.2. Ultrasonographer

A physical therapist (SG) captured and recorded all ultrasound images. The physical therapist received 2 h of training in ultrasonography by a physical therapy researcher (SM) with experience in performing ultrasound image acquisition and well published in the field of musculoskeletal ultrasound imaging. The physical therapist received

training in identifying the placement of the ultrasound transducer to capture the region of interest and image optimization to distinguish the superficial fat and muscle layers in addition to the hamstring tendon and the bone.

2.2.3. Image acquisition

In order to quantify tissue health in populations that are at high risk for seated acquired pressure injuries, images were acquired in the position that would best simulate the sitting position. Participants assumed the side lying position for a period of 30 min with the dominant leg (as determined by the kicking leg) uppermost, with the hip and knee flexed to 90° with a pillow between the knees to maintain neutral hip abduction/adduction and neutral internal/external rotation. A goniometer was used to ensure hip and knee flexion of 90°. Once the participant was placed in the scanning position, the lowest point of the ischial tuberosity was palpated and its perpendicular distance at mid-point from a line between the coccyx and greater trochanter was recorded to ensure consistent placement of the ultrasound head. Three longitudinal ultrasound images were captured for each participant on two distinct occasions separated by 60 min. Prior to each image being captured, the probe was removed and then reapplied over the same landmark. Care was taken during image capture to minimize tissue deformation under the probe. System settings on the ultrasound unit remained the same for the two ultrasound scanning sessions for each participant. Three focal points, the subcutaneous-muscle boundary, the mid-depth of the muscle, and the ischial tuberosity were used during image acquisition. Ultrasound images were captured by the GE system, and subsequently transferred in a DICOM format to an encrypted computer for analysis.

2.2.4. Image processing

The ultrasound images in the long axis view of the ischial tuberosity in DICOM format were imported into MATLAB (MATLAB, 2013, Mathworks, USA). The selection of the measurement in the longitudinal view enabled greater visualization of the fascial planes and interfaces between the tissues. The image processing toolbox, which uses reference standard algorithms for image processing (segmentation) and analysis and can be customized using C/C++ code and is available with the MATLAB program, was used for 2D viewing and analysis (Fig. 1a). The ischial tuberosity was traced by the investigator using an image marker. The ischial tuberosity was identified as a curved hyperechoic structure with an anechoic bone shadow beneath [16]. The investigator then outlined a well-defined area over the ischial tuberosity. Within each area overlying the ischial tuberosity, the external fascial boundaries between the skin-subcutaneous tissue and subcutaneous tissue-muscle and the muscle-ischial tuberosity were selected manually using image markers. The skin was identified as a smooth hyperechoic layer with a clearly defined boundary. Subcutaneous tissue was identified as an area of low echoic intensity with echogenic boundaries of connective tissue and muscle was identified as an area of low echoic intensity with fascicular architecture, surrounded by clearly

defined boundaries as per Pillen and van Alfen [16]. Once all boundaries were identified, the image was partitioned into three main regions of interest (ROIs): skin, subcutaneous tissue and muscle. The investigator confirmed correct partitioning of boundaries and any errors in identifying boundaries were corrected (Fig. 1b). Geometric measures (mean and maximal skin, subcutaneous and muscle thickness, and depth of the ischial tuberosity), measurements related to gray scale values (echogenicity of the skin, subcutaneous tissue and muscle) and measurements related to the interrelation of the gray scale of each pixel and its neighbouring pixels in four orthogonal directions (i.e. contrast) in each ROI (skin, subcutaneous tissue and muscle) were extracted.

Image acquisition and image processing was conducted by the same investigator (SG).

2.3. Statistical analysis

For each participant, three images were used to calculate the participant's mean and maximal thickness, echogenicity and contrast in each ROI. SPSS Statistics (SPSS Statistics 23, IBM, USA) was used to obtain group means and standard deviations for mean and maximal thickness, echogenicity and contrast of skin, subcutaneous tissue and muscle in each ROI. A Multivariate Analysis of Variance was used to determine if significant differences existed between Time 1 and Time 2 in mean and maximal thickness, echogenicity and contrast of skin, subcutaneous tissue and muscle using a p-value of 0.05.

Intra-class correlation coefficients (ICC [1,2]) with 95% Confidence Intervals were calculated from repeated measurements on the same image by the same rater in order to determine if the investigator identified boundaries consistently within images.

For reliability analyses, Generalizability Theory (G-Theory), a generalization of classical reliability theory was used. With G-Theory, the results of psychometric tests and relative contribution of sources of error within a measurement are explored. Sources of variance in a measurement are calculated [39], followed by the implementation of a Decision Study (D-Study) in which dependability coefficients (ϕ) ranging from 0 (no reliability) to 1 (perfect reliability), and the standard error of measurement (SEM) are used to make decisions about repeated measures. A Generalizability Study (G-Study) using the GENOVA software (Springer-Verlag, USA) was first conducted to compute the multiple sources of error in the measurements including the participant (P), the ultrasound image (I) and the random errors associated with the interaction effects between the participant and image (PI). Multiple sources of errors in the measurements were expressed as a percentage of the total variance. Then, coefficients of dependability (D-Study) were computed [39] for each measurement to determine the reliability when one or multiple images (i.e. mean of 2, 3, 4 or 5 images) were used. While only 3 images per participant were used for this study in the calculation of the generalizability coefficient, the information gathered from the G-Study enables one to calculate the variance and interaction components of more than one image by dividing the variance by the number of images used in the calculation of the mean [40]. The

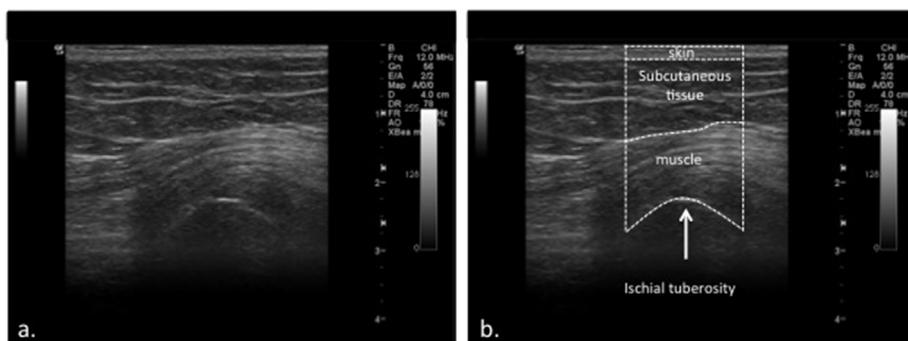


Fig. 1. a and b. Ultrasound image of the tissue overlying the ischial tuberosity for one representative healthy participant (a). The same image was then processed (b) to partition the tissue into three different layers including skin, subcutaneous tissue and muscle to acquire the regions of interest (ROIs). The ischial tuberosity was identified as a hyperechoic structure. The image revealed a homogenous pattern of reflection from the epidermis down to the ischial tuberosity. A clear muscle layer was identified, corresponding to the gluteus maximus muscle. Selective ROIs were analyzed using MATLAB software to calculate mean and maximal thickness of skin, subcutaneous tissue, and muscle, depth of is-

chial tuberosity, echogenicity, and contrast.

following interpretation for reliability was used: values < 0.50 representing poor reliability, values between 0.50-0.75 representing moderate reliability and values > 0.75 representing good reliability [41].

Absolute Standard Error of Measurement (SEM) (expressed as the square root of the absolute variance), Relative SEM (expressed as (SEM/overall mean) x 100) Absolute Mean Detectable Change (Absolute MDC) using 90% Confidence Interval (expressed as $1.65 \times SEM \times \sqrt{2}$) and Relative Mean Detectable Change (Relative MDC) using 90% Confidence Interval (expressed as (Absolute MDC/overall mean) x 100) were calculated for all measures to determine the dispersion of measurement error and spread of the data respectively. The SEM was used to quantify the accuracy of the measurements and to calculate confidence intervals around the measured variables. A higher SEM indicates low accuracy of the measurement, whereas a low SEM indicates a high level of accuracy. MDC enables one to determine the value that is required to establish that a change in measure is not due to chance [41]. Therefore, any value exceeding the MDC was considered to indicate a true change in score.

3. Results

Ten healthy participants (4 males), aged 23–69 (mean age 42.8 ± 15.7) years, mean height 173.4 ± 6.0 cm and mean weight 71.3 ± 12.5 kg were recruited for the study.

Intra-class correlation coefficients (ICC [1,2]) from repeated measurements on the same image by the same rater identified intraclass correlation coefficients between 0.902-0.999.

Table 1 summarizes the descriptive statistics of mean and maximal thickness, echogenicity and contrast of skin, subcutaneous tissue and muscle at the two time points. There were no significant differences between Time 1 and Time 2 for the mean and maximal thickness, echogenicity and contrast values of the skin, subcutaneous tissue and muscle layers and therefore data was pooled for Time 1 and Time 2 for the Generalizability and Dependability studies.

The thickest layer of tissue observed over the ischial tuberosity was the area occupied by muscle (mean 18.82 ± 6.61 mm) followed by subcutaneous tissue (mean 9.56 ± 3.22 mm). Higher echogenicity was observed in the area occupied by skin (89.51 ± 6.51) when compared with subcutaneous tissue (73.33 ± 13.32) and muscle (74.49 ± 12.82).

Table 1

Group mean and standard deviations of thickness, echogenicity and contrast in skin, subcutaneous tissue and muscle over the regions of interest for Time 1 (n = 10), Time 2 (n = 10) and overall (n = 20).

	Time 1 (mean ± 1 SD)	Time 2 (mean ± 1 SD)	Overall (mean ± 1 SD)
Maximum Thickness (mm)			
Skin	2.63 ± 0.46	2.64 ± 0.46	2.64 ± 0.45
Subcutaneous	9.24 ± 3.07	9.89 ± 3.50	9.56 ± 3.22
Muscle	18.21 ± 7.16	19.42 ± 6.33	18.82 ± 6.61
Mean Thickness (mm)			
Skin	2.33 ± 0.40	2.38 ± 0.41	2.36 ± 0.40
Subcutaneous	8.20 ± 3.25	8.77 ± 3.68	8.48 ± 3.94
Muscle	14.49 ± 6.58	15.58 ± 6.07	15.04 ± 6.18
Echogenicity^a			
Skin	91.79 ± 6.18	87.23 ± 6.31	89.51 ± 6.51
Subcutaneous	75.68 ± 13.55	70.99 ± 13.38	73.33 ± 13.32
Muscle	76.59 ± 10.98	72.39 ± 14.72	74.49 ± 12.82
Contrast^b			
Skin	16.83 ± 5.58	15.33 ± 4.79	16.08 ± 5.12
Subcutaneous	19.02 ± 8.27	18.16 ± 6.90	18.59 ± 7.42
Muscle	11.16 ± 4.55	11.56 ± 4.23	11.36 ± 4.28

^a Echogenicity is quantified using a gray scale range from 0-255, where 0 = black, 255 = white.

^b Contrast is quantified at 0 = no contrast (image is completely homogeneous, all pixels have the same gray scale).

Table 2

Magnitude of variance components expressed as a percentage of the total variance for each source of variance (P = participant, I = image) and interaction (PI = interaction of participant and image) for all measures. Data obtained from the G-Study.

	P	I	PI
Maximum Thickness			
Skin	75.56	0.00	24.43
Subcutaneous	85.64	0.00	14.36
Muscle	89.77	0.00	20.23
Mean Thickness			
Skin	82.01	0.00	17.99
Subcutaneous	90.04	0.00	9.96
Muscle	91.48	0.00	8.52
Echogenicity			
Skin	82.07	0.00	17.93
Subcutaneous	94.78	0.00	5.22
Muscle	87.15	0.00	12.85
Contrast			
Skin	66.97	0.00	33.03
Subcutaneous	87.91	0.20	11.89
Muscle	71.88	0.00	28.12

3.1. G-Study: Magnitude of component variances

The largest proportion of the total variance (75.56%–94.78%) was associated with the participants (P), whereas the lowest proportion of the variance ($\leq 0.001\%$) was attributed to the image (I). Participant-image interaction demonstrated that there were substantial interaction effects across all outcome measures, ranging from 5.22% to 33.03% (Table 2).

3.2. D-Study: Coefficients of dependability, inter-trial and inter participant reliability

The dependability coefficients (ϕ) for each region of tissue overlying the ischial tuberosity are represented in Table 3. Mean thickness measures reached good reliability across the three layers investigated, with the highest value reached for the muscle. Coefficients of dependability for mean thickness were lowest for the skin (0.82–0.93) and highest for the muscle (0.92–0.97). Measures for echogenicity and contrast were lowest for the skin (0.82–0.93 and 0.67–0.86, respectively) and highest for the subcutaneous tissue (0.95–0.98, and 0.88–0.96, respectively).

Of the three layers examined, relative SEM for thickness and echogenicity measures were lower in the skin when compared with

Table 3
Inter-trial reliability (measured by coefficient of dependability ϕ) obtained for a mixed D-Study design with 1 evaluator using 1 image, the average of 2 images, 3 images, 4 images and 5 images for thickness, echogenicity and contrast.

	1 Image	2 Images	3 Images	4 Images	5 Images
Maximum Thickness					
Skin	0.76	0.86	0.90	0.92	0.94
Subcutaneous	0.86	0.92	0.95	0.96	0.97
Muscle	0.90	0.95	0.96	0.97	0.98
Mean Thickness					
Skin	0.82	0.90	0.93	0.95	0.96
Subcutaneous	0.90	0.95	0.96	0.97	0.98
Muscle	0.92	0.96	0.97	0.98	0.98
Echogenicity					
Skin	0.82	0.90	0.93	0.95	0.96
Subcutaneous	0.95	0.97	0.98	0.99	0.99
Muscle	0.87	0.93	0.95	0.96	0.97
Contrast					
Skin	0.67	0.80	0.86	0.89	0.91
Subcutaneous	0.88	0.94	0.96	0.97	0.97
Muscle	0.71	0.84	0.88	0.91	0.93

Table 4
Relative standard error of measurement (SEM-rel) in thickness, echogenicity and contrast of repeated measures of skin, subcutaneous tissue and muscle thickness.

	Image 1	Image 2	Image 3	Image 4	Image 5
Maximum Thickness (mm)					
Skin	9.65	6.82	5.57	4.82	4.32
Subcutaneous	13.10	9.27	7.57	6.56	5.86
Muscle	11.09	7.84	6.40	5.54	4.96
Mean Thickness (mm)					
Skin	8.12	5.74	4.69	4.06	3.63
Subcutaneous	12.87	9.10	7.43	6.44	5.76
Muscle	11.94	8.44	6.90	5.97	5.34
Echogenicity					
Skin	3.27	2.31	1.89	1.64	1.46
Subcutaneous	4.83	3.41	2.79	2.41	2.16
Muscle	5.95	4.20	3.43	2.97	2.66
Contrast					
Skin	23.68	16.74	13.67	11.84	10.59
Subcutaneous	14.48	10.24	8.36	7.24	6.47
Muscle	22.18	15.68	12.80	11.09	9.92

subcutaneous tissue and muscle (Table 4). The skin yielded the lowest relative SEM for maximal and mean thickness and echogenicity measures (4.32–9.65, 3.63–8.12, 1.46–3.27) when compared with subcutaneous tissue (5.86–13.11, 5.75–12.87, 2.16–4.83) and muscle (4.96–11.09, 5.34–11.94, 2.66–5.95). With increasing number of images averaged, a decrease in the relative SEM was found for all measures.

Relative MDC using a 90% confidence interval for each variable is presented in Fig. 2a-d. The figures depict the relative MDC required to establish that a change in measure is not attributed to chance variation based on the number of images that would be averaged to obtain that measure. The skin yielded the lowest MDC for maximal thickness (10.07–22.51), mean thickness (8.48–18.96) and echogenicity measures (3.42–7.64). With increasing number of images used to obtain the average measure, a decrease in the relative MDC was noted. Relative MDC was less than 21% for maximal and mean thickness and echogenicity measures when averaging three or more images. Relative MDC was less than 25% for contrast measures when averaging five images.

4. Discussion

The purpose of this study was to determine the reliability of repeated ultrasound imaging over the ischial tuberosity, to provide recommendations for the number of images required to minimize the

standard error of measurement (SEM) and to determine the number of images required to obtain tissue thickness, gray scale and contrast values that exceed an a-priori minimal detectable change for repeated tissue assessment overlying the ischial tuberosity.

When comparing our findings to other studies investigating tissues overlying the ischial tuberosity, we found thinner subcutaneous tissue [32,42–44] and thicker muscle when compared with Sonenblum et al. [42] and Swaine et al. [38] but thinner muscle when compared with Makhous et al. [32] and Shabshin et al. [44]. It is possible that not only the offloading device but also differences in scanning positions and image acquisition systems (MRI vs. ultrasound) could explain the differences. Previous investigators [32,38,42] had participants sit on a device designed to offload the ischial tuberosity. Since our participants were in a side lying position already offloading the ischial tuberosity, there was no need to provide any offloading device. Deformation of the tissue with the seated device used in other studies may account for these differences.

Our study showed that the highest component of measurement variance was attributed to the participants themselves (66.97%–94.78%), with limited amount of variance attributed to the images and a minimal amount of variance attributed to the interaction effects of the participants and image (5.22%–33.03%). Several factors linked to participants have been reported to account for variance in skin, subcutaneous and muscle tissue including Body Mass Index (BMI) [45], sex [46], and age [47,48]. Addition of participant BMI, sex and age could provide more granularity to the contributors for variance that would have utility for comparing tissue metrics with other populations.

The participant-image interaction effect suggests that the variance across images is affected by the participants. We attribute the participant-image interaction effect to be a function of both the device used and system settings used when acquiring ultrasound images. Obtaining quality images for each participant required optimization of ultrasound system parameters (frequency, gain, depth, dynamic range and time/gain compensation) [49]. Pillen and van Alfen [16] recommend using standardized muscle ultrasound protocols with the same system settings for each individual. However, a standardized protocol with the same system settings for each participant may reduce the variance attributed to the interaction effects of participants and ultrasound settings at the expense of a potential optimal image quality. Obtaining optimal image quality in our study was essential for identification of the delineation of the skin, subcutaneous and muscle tissue used in our analysis.

It is well established that ultrasound imaging is operator dependent [50] and obtaining high quality images requires experience [51]. During image acquisition, the pressure applied while scanning will influence the amount of tissue deformation, thereby affecting quantification of tissue measures and image quality. We attempted to minimize measurement error by ensuring consistent placement and pressure of the ultrasound head, standardizing participant position and obtaining measurements from a well-defined ROI over the ischial tuberosity. We were unable to measure the pressure exerted on the ultrasound transducer during scanning as our transducer was not instrumented for such. The use of an external reference marker system such as a scanning template and the application of standardized pressure using a dense foam cube during scanning may have further reduced this measurement error by minimizing tissue deformation. However, it is important to note that the use of a scanning template on the lumbar multifidus muscles [51] and a dense foam cushion on the transverse abdominis muscles [52] to standardize transducer orientation have been shown to have limited effect on improving reliability when the measurement protocol is precisely defined.

The present study has established that measures of skin, subcutaneous and muscle thickness demonstrate good inter-trial reliability ($\phi = 0.903$ – 0.982) in individuals between the ages of 23–69 when averaging three images. When more than four images were averaged for all measures of echogenicity and contrast, the coefficient of dependability demonstrated good reliability ($\phi > 0.8$) and with increasing

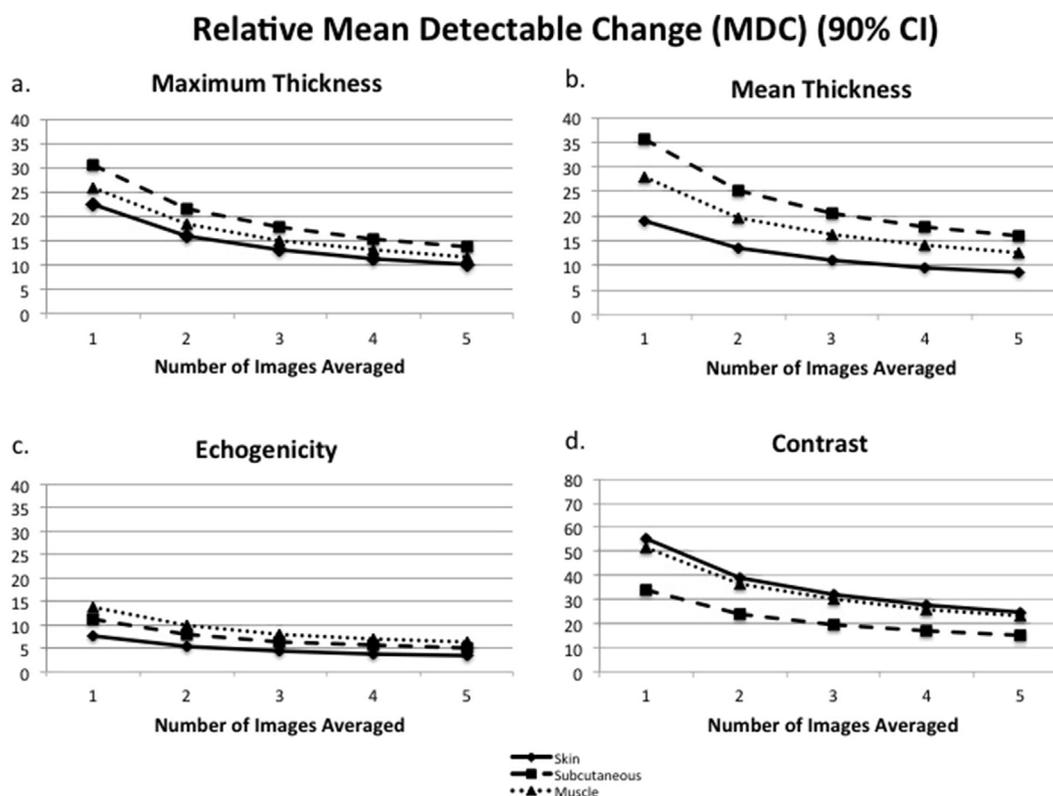


Fig. 2. a–d. Summary of the Relative Mean Detectable Change with 90% Confidence Interval (MDC90%-rel) noted on the vertical axis for maximal and mean thickness, echogenicity and contrast of the skin, subcutaneous tissue and muscle.

number of images averaged to obtain thickness, gray scale and contrast measurements of skin, subcutaneous tissue and muscle, the coefficient of dependability increased. It is not surprising that the coefficient of dependability is lower for contrast measurements compared with thickness and gray scale measurements. Given that contrast measures incorporate variance of echogenicity in the calculations, the ability to discriminate changes in pixel properties is more challenging. For example, changes in tissue secondary to edema will result in a change of the mean gray scale of the pixel. The contrast measures will provide a numeric calculation that reflects the interrelation of the gray scale of each pixel or subgroup of pixels and its neighbour in different orthogonal directions from that tissue. The ability to discriminate these changes in tissue contrast may be more challenging when compared with thickness and gray scale measures.

When measuring muscle thickness using ultrasonography, our study demonstrated a higher reliability for mean muscle thickness measures ($\phi = 0.82\text{--}0.92$) when compared with maximum thickness measures ($\phi = 0.76\text{--}0.90$). Our customized program enabled us to obtain mean thickness measures by averaging the values across the entire length of the ROI. In order to obtain more reliable outcome measures in research and clinical practice, one would have to have a fundamental understanding of the pathology to determine if mean or maximum thickness is the better measure to use. Until this is addressed in each population, we suggest that both should be used.

Based on the findings of our study, we recommend that at least three images should be averaged when the primary outcome measures are linked to geometric measures (e.g., thickness and echogenicity) and that at least four images should be averaged whenever there is an interest to characterize texture within a ROI (e.g. contrast measures). At least three images are required in order to detect a minimal change of 21% for thickness and echogenicity, whereas at least five images are required to detect a minimal change of 25% for contrast measures. Determining the number of images that are required for each parameter to detect minimal change in the desired range needs to be established.

Therefore, it may be prudent for the researcher to determine which measures are required depending on the research question prior to image acquisition so that the minimal number of images can be collected.

Due to the small sample size, caution is advised when generalizing these results to a population of healthy adults. Based on the results of our study, three images are required to detect a 21% difference in thickness measures in skin. Assuming a two-sided significance of 0.05, a power of 0.80, mean skin thickness of 2.64 mm and standard deviation of 0.45 mm, a total of 11 participants would be required to detect a significant difference beyond the minimal detectable change whenever thickness is selected as the main outcome measure.

Our paradigm was constructed as a best-case scenario to be able to identify the structures of interest as participants were asked to maintain the side lying position for 30 min during data collection. We scanned the buttock region of able-bodied individuals, in which interfaces between skin, subcutaneous tissue and muscle could be easily identified and used a customized image analysis program that provided a user-interface to enable manual detection of the boundaries of skin, subcutaneous tissue and muscle. While recognizing that some thickness measures may require that a single rater be used due to poor reliability, this approach may not be as applicable for clinical situations where, for example multiple assessors are used and studies examining inter-assessor variance are required. Furthermore individuals with neuromuscular disorders may be unable to maintain a prolonged side lying position to obtain quality images and interfaces may be more difficult to visualize due to atrophy or fat infiltration of skeletal muscle. However, this exploratory approach demonstrates proof of principle.

5. Conclusion

Participants accounted for most of the total variance (75.56%–94.78%). Coefficient of dependability (ϕ) for thickness, gray scale and contrast measures was greater than 0.80 when more than two

images were averaged. In order to detect a MDC of 21% in thickness and echogenicity measures, at least three images are required, while at least five images are required for a MDC of 25% for contrast measures.

This study is the first to report ultrasound gray scale measures characterizing the integrity of the tissue overlying the ischial tuberosity. Skin, subcutaneous and muscle thickness, echogenicity and contrast can be measured reliably in able bodied individuals by averaging two or more images when using the same evaluator at a single time point. In order to detect a minimal detectable change of 21% for thickness and echogenicity measures and 25% for contrast measures on repeated assessment, three and five images are required respectively. Using this method to assess changes over time may have some value in the clinical setting. Ultrasound may be used to determine changes in deeper tissues that may not be visible at the surface in individuals who are at risk of developing pressure injuries. BMI, age and sex will be important additional variables to consider in future studies using this methodological approach, particularly when attempting to assess other populations with multiple comorbidities in which pressure injuries develop.

Conflicts of interest

None.

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