

## Ultrasound imaging of tissue overlying the ischial tuberosity: Does patient position matter?



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### ABSTRACT

**Background:** Deep Tissue Pressure Injury (DTI) occurs in the tissues underlying the skin that may not have visible signs of skin breakdown and may be detected by ultrasound. The optimal position for ischial region ultrasound image acquisition to facilitate assessment of the tissue proximal to the ischias not been determined.

**Objective:** To evaluate the mean difference in geometric and grey scale measures of tissues overlying the ischial tuberosity (IT) acquired from ultrasound images in supine and lateral recumbent simulated sitting positions from adults with spinal cord impairment (SCI).

**Methods:** Nine individuals (3 acute and 6 chronic) with SCI or disease with neurological level of injury C4-T12 and AIS A-D and who used a wheelchair for mobility were recruited and underwent ultrasound acquisition in the supine and lateral recumbent positions. One participant was imaged twice on a separate day. Three images from the left (n = 8) and right (n = 2) IT were scanned using a 6 – 18 MHz linear ultrasound probe (Acuson S2000) with participants' hips and knees flexed to 90° in both the supine and lateral recumbent positions using a single rater protocol. MATLAB Image Processing Toolbox with a customized script was used to obtain mean and maximal thickness, echogenicity and contrast of skin, subcutaneous tissue and muscle. Wilcoxon Signed Rank Test and Bland Altman analysis was used to determine if there were differences between the two image acquisition positions and to construct limits of agreement.

**Results:** Thickness and contrast measures were similar in the supine and lateral recumbent positions ( $p > 0.05$ ). Muscle echogenicity was lower in the supine position ( $p = 0.04$ ).

**Conclusion:** There is agreement in geometric and grey scale measures of tissues over the IT between the supine and lateral recumbent positions with the exception of muscle echogenicity, which was lower in the supine position. Since DTI is thought to originate in the muscle and echogenicity plays a role in abnormal tissue imaging diagnosis, further studies are recommended to determine the impact of body position on muscle echogenicity prior to being used in prospective studies.

### 1. Introduction

Pressure injuries occur over bony prominences as a result of prolonged unrelieved pressure or a combination of pressure and shear forces [1]. Deep tissue injury (DTI) is a type of pressure injury that is defined as the appearance of a purple or maroon coloured area or a

blood filled blister over intact skin, as a result of damage to underlying soft tissues [1]. Typically DTI occurs when sustained internal shear stress exceed the tissue's tolerance [2]; tissue and cell deformation [3,4], along with impaired lymphatic drainage [5] occur resulting in irreversible damage occurs secondary to ischemia and anaerobic waste product accumulation [2]. Furthermore, the skin overlying the

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damaged tissue becomes ischemic secondary to reduced blood flow, leading to extravasation of red blood cells into the dermal and interstitial spaces, resulting in the visible purple or maroon appearance of the skin [2]. DTI progresses from the deep subdermal layer to the superficial dermal and epidermal layers [6]. The onset of a visible DTI in the skin can occur within 3–5 days from the initial insult [7]. DTI may develop into full-thickness skin loss approximately 7–10 days following the initial insult [2] or may resolve spontaneously without tissue loss [8].

It is vital that clinicians screen patients for signs of DTI prior to the development of skin manifestations at the surface, in order to prevent the morbidity and mortality associated with DTI including but not limited to: sepsis, osteomyelitis, myocardial infarction, renal failure or multiple organ failure [9]. Since DTI is especially difficult to diagnose in individuals with darkly pigmented skin [1] and other dermatological conditions can be mistaken for pressure injuries, including skin tears, bruising, venous engorgement, arterial insufficiency, and dermatitis, the need to identify reliable methods for screening is of paramount importance.

Several methods are currently used to identify the signs of DTI overlying the ischial tuberosity (IT) including thermography [10], diagnostic ultrasonography [6,11,12], computerized tomography [13,14] and magnetic resonance imaging [15]. Ultrasound imaging offers the advantages of being portable, non-invasive, with few contraindications and rapid result interpretation [6,16–18]. However, several factors can influence the geometric and grey scale measures including transducer rotation, medial/lateral and cranial/caudal tilting and pressure exerted over the tissues [19–22], training and experience [23]. Diagnostic ultrasound imaging can be used to screen individuals at risk for developing a DTI prior to the appearance of tissue breakdown at the skin surface. Accumulation of fluid in the region below the epidermal and dermal tissue, which would appear on ultrasound images as areas of less dense tissue and lower signal intensity (i.e., hypoechogenic) [6,24], has been suggested as a sign of tissue injury [25]. Additionally, the identification of unclear, layered structures, hypoechoic lesions, discontinuous fascia and heterogeneous hypoechoic areas is pathognomonic for DTI [26]. Swaine and colleagues [27] detected abnormal ultrasound signs in 42% of their sample (n = 19) through the appearance of a cystic, solid or combination of a cystic and solid region. The majority of abnormal signs were directly overlying the lowest point of the IT where most of the sustained force is applied during sitting. More detailed quantification of ultrasound imaging using grey scale values (i.e. echogenicity) and measures of homogeneity of tissue layers (i.e. contrast) may provide greater insight into changes in tissue properties when compared with visual appearance prior to the appearance of tissue breakdown.

Pressure injuries of the ischia region are common among individuals with spinal cord impairment (SCI) [26] due to reduced lean tissue mass [28] and prolonged sitting due to wheelchair mobility [29]. Increasingly, clinicians and researchers have become interested in the interactions between ischial tuberosity (IT) geometry, preservation of sacral motor and sensory function as it influences continence and regional sensation, and the sitting surface as risk factors for ischia region DTI among patients with SCI.

While several investigators have described changes in tissues prior to impending DTI, no studies have determined the optimal patient position for acquiring ultrasound images of tissues overlying the IT and the potential influence of positioning on tissue measures. Furthermore, patient position during image capture over the IT is not consistently reported in the literature [6]. Additionally, it has been reported that during sitting, the gluteus muscle moved in a posterior and lateral direction relative to the IT [30,31] potentially contributing to contributing to changes in muscle thickness. Akins et al. [32] acquired ultrasound images over the IT in the lateral recumbent position and found thickness measures of tissue to be reliable and correlated with measures obtained by MRI. Swaine et al. [27] reported poor between

operator reliability of skin thickness for the unloaded IT, however good between operator reliability of fat and muscle thickness during assessment of the loaded and unloaded IT. Although not over the IT, Burke and colleagues used prone and two lateral recumbent positions and reported that position during scan acquisition can affect dermal thickness and density measures of tissue overlying the sacral region [23].

There are two feasible options for simulating the sitting position during image capture: the supine and lateral recumbent positions. However, a prior comparison of ultrasound measures acquired in the supine and lateral recumbent positions over the IT has not been undertaken. The supine position may be uncomfortable to maintain for some individuals with SCI, requires a greater amount of time to set up and requires a height adjustable table and thigh straps, and is difficult for the technologist to maintain the probe in the optimal position during image capture. This study compares the geometric, grey scale, and contrast ultrasound measures of the tissue layers overlying the IT obtained by the same operator from individuals with SCI in two positions: supine and lateral recumbent in order to provide recommendations for patient positioning when acquiring DTI screening images.

## 2. Methods

This study was a local substudy within a larger multi-centre longitudinal observational cohort study among individuals with SCI living in Canada and Australia examining the predictive validity of eight risk factors for sitting acquired pressure injuries (The AusCAN Risk for Sitting-Acquired Pressure Ulcers, NHMRC Grant 634388). A description of the study methods can be found on the Australian New Zealand Clinical Trials Registry (#ACTRN12618000231246). The study was approved by the local Research Ethics Board and adhered to Good Clinical Practice Guidelines and local privacy and regulatory requirements.

### 2.1. Participants

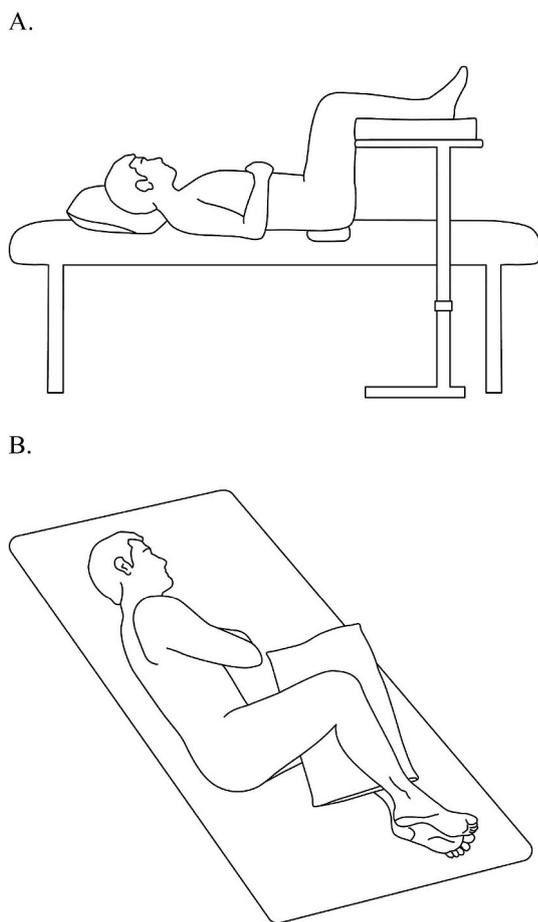
Nine adult individuals previously consented to the AusCAN study were recruited via convenience sampling at a subacute tertiary rehabilitation facility. Inclusion criteria included age of at least 18 years, English comprehension, able to provide informed consent, and the use of a manual or power wheelchair for mobility. Individuals with subacute SCI were recruited into the study if they presented within six months of SCI onset; whereas, individuals with chronic SCI were recruited if they were at least ten years post injury. Exclusion criteria included malignancy, myelomeningocele, a current sitting acquired pressure injury or suspected DTI at the time of study enrollment, history of a surgically closed pressure injury over the buttock, and any surgical interventions of the pelvis or trochanteric bony prominences.

Demographic data collected included age, sex, height (cm), and weight (kg). Injury details including date of injury, neurological level of injury, and ASIA Impairment Scale category as per the International Standards for Neurological Classification of Spinal Cord Impairment [33] were also collected.

### 2.2. Ultrasound imaging protocol

#### 2.2.1. Ultrasound device

Ultrasound images were acquired using a Linear Array B-Mode 6 – 18 MHz Siemens ACUSON S2000 Transducer (Siemens Canada Ltd., Mississauga, Ontario). In order to ensure optimal image quality, gain, depth and time/gain compensation of the ultrasound system settings were standardized for each individual to identify the boundaries of the skin-subcutaneous tissue, subcutaneous tissue-muscle and muscle-ischial tuberosity. The ultrasound system settings remained consistent for both image acquisition positions.



**Fig. 1.** Participant positioning during ultrasound image acquisition in supine (A) and lateral recumbent position (B). In both positions (A) and (B) ultrasound images were acquired parallel and perpendicular to the long axes of the ischial tuberosity.

### 2.2.2. Ultrasonographer

Ultrasound images were acquired by a physical therapist (SG) who received 31.5 h of training in ultrasonography including video instruction, e-learning PowerPoint with self-evaluation, written protocol and face to face training with a certified sonographer. The physical therapist received training in applying the transducer and image optimization to enable the identification of the local anatomic structures including boundaries between the skin, subcutaneous fat, muscle, hamstring tendon and bone. Validation of the correct boundary identification was also completed by the certified sonographer.

### 2.2.3. Image acquisition

Participants were transferred from their wheelchair to a hospital bed with a foam pressure relief mattress via a mechanical lift, transfer board or using a pivot transfer. If a mechanical lift was used, the sling was removed following the transfer. Participants had scans acquired in a supine and lateral recumbent position as described below.

### 2.2.4. Supine position

The participant was placed in the supine position, with their knees and hips flexed at 90° and calves supported on a bedside table (Fig. 1A). The left ( $n = 2$ ) or right ( $n = 8$ ) IT was then exposed with appropriate draping. A pillow and rolled towel were used to ensure that any areas of the body that were in contact with a support surface were offloaded during the image acquisition. In order to ensure that hips were kept in a neutral position of internal/external rotation and abduction/adduction, a neoprene strap (Bodypoint Inc., Seattle, WA, USA) was secured

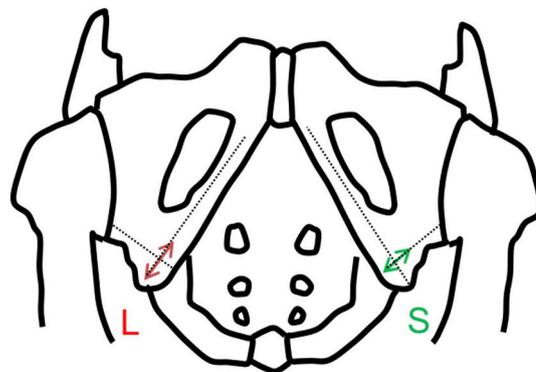
around the participant's thighs. A foam cushion was placed under the buttock to elevate the pelvis in order to enable access to the IT by the ultrasound probe and ensure optimal probe placement. The IT was scanned by the ultrasonographer while the legs were stabilized on a bedside table by a research assistant.

### 2.2.5. Lateral recumbent position

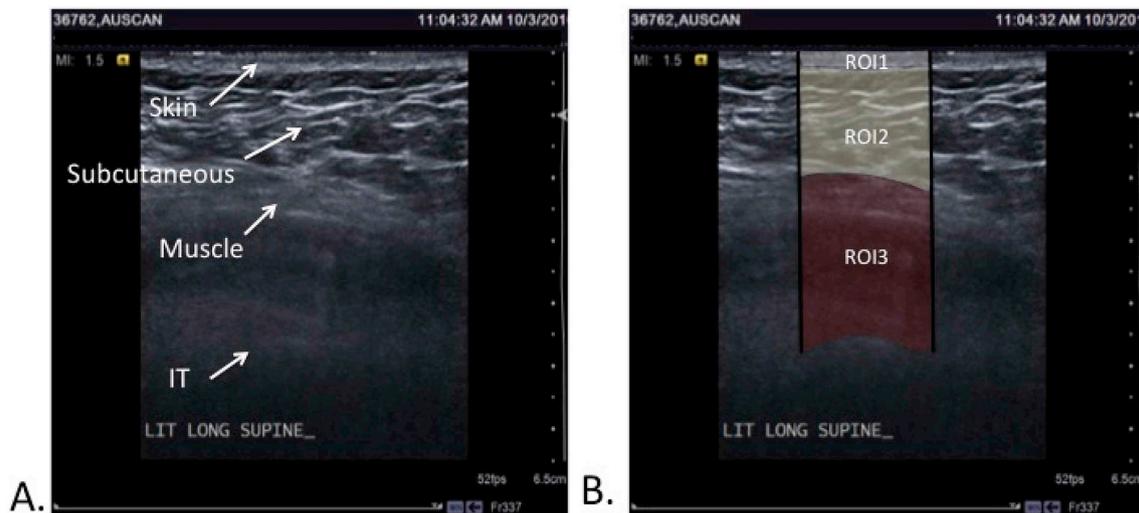
Participants were placed in the lateral recumbent position with the scanning leg uppermost, with the hips and knees flexed to 90° and in neutral hip abduction/adduction and neutral internal/external rotation with a pillow placed between the knees (Fig. 1B).

Once the participant assumed the position for scan acquisition, the IT was palpated and the transducer was placed over the region of interest. Care was taken to ensure that no soft tissue deformation of the buttock tissue occurred during image acquisition by unloading the transducer head. To ensure optimal image acquisition and repeatability, the transducer was positioned perpendicular to the IT to ensure that the bone boundary of the lowest point of the IT as indicated by the most inferior aspect of the hamstring tendon insertion and the boundaries between the skin, subcutaneous fat and gluteus muscle could be visualized in the region of interest.

Three images were acquired parallel and perpendicular to the long and short axis of the IT (Fig. 2) in both the supine and lateral recumbent positions for a total of 12 images acquired from each IT for each participant. The long axis of the IT corresponds to the region of the IT which is continuous with the inferior public ramus. The short axis of the IT corresponds to the region of the IT which is oriented perpendicular to the inferior public ramus. During image acquisition in both the long and short axis, the probe was positioned parallel to the lowest point of the IT. The probe was removed between each image capture and reapplied. The ultrasound settings remained the same for image acquisition in both the supine and lateral recumbent positions for each participant. Ultrasound system settings were optimized for each participant in order to ensure optimal image acquisition with the focal region adjusted in the subcutaneous tissue region. Ultrasound images were captured directly on the ultrasound system, and subsequently transferred in DICOM format onto a computer with an encrypted hard drive for image analysis.



**Fig. 2.** Schematic illustration of the pelvic region from the inferior view. The ultrasound images are acquired along the long (denoted by the red arrow L) and short (denoted by green arrow S) axes of the ischial tuberosity. The long axis of the ischial tuberosity corresponds to the region of the ischial tuberosity which is continuous with a line drawn along the inferior public ramus (noted by the continuous dotted line). The short axis of the ischial tuberosity corresponds to the region of the ischial tuberosity which is oriented in a direction perpendicular to the long axis of the inferior public ramus. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)



**Fig. 3.** Sample ultrasound image of the tissues overlying the long axis of the ischial tuberosity acquired in supine position. The skin, subcutaneous tissue, muscle and ischial tuberosity (IT) are labeled (A). The skin, subcutaneous tissue and muscle are partitioned into three regions of interest (B) in which mean thickness, echogenicity and contrast are calculated (ROI1 = Region of Interest of skin, ROI2 = Region of Interest of subcutaneous fat, ROI3 = Region of Interest of muscle). The image was acquired at a frequency of 8 MHz, a scanning depth of 6.5 cm with a focus at 1 cm.

### 2.3. Image processing

The ultrasound images acquired parallel to the long axis of the IT in DICOM format were imported into MATLAB Image Processing Toolbox (MATLAB 2013, Mathworks, Natick, MA, USA) for 2D viewing and analysis as described in Gabison et al. [34]. The IT, identified as a curved hyperechoic structure with an anechoic bone shadow beneath [35] was defined. A Region of Interest (ROI) overlying the IT was outlined, and the skin-subcutaneous tissue, subcutaneous tissue-muscle and the muscle-ischial tuberosity boundaries were outlined (see Fig. 3b). The skin was identified as a smooth hyperechoic layer with a clearly defined boundary. Subcutaneous tissue was identified as an area of low echo 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 [35] (Fig. 3A). Once all the boundaries were identified by the investigator, the program partitioned the image into three main sub ROIs: skin, subcutaneous tissue and muscle (Fig. 3B). The investigator (SG) confirmed correct partitioning of the boundaries. Any errors in identifying boundaries were corrected. The MATLAB program was used to extract geometric measures (mean and maximal skin, subcutaneous and muscle thickness, and depth of the IT), measurements related to grey scale values (echogenicity and kurtosis) and co-occurrence matrix (contrast) of the skin, subcutaneous tissue and muscle.

### 2.4. Statistical analysis

SPSS Statistics (SPSS Statistics 23, IBM, Armonk, New York, USA) was used to calculate the mean and standard deviation of participants' mean and maximal tissue thickness, echogenicity and contrast of skin, subcutaneous tissue and muscle for the two image acquisition positions where position A was denoted as the supine position for image acquisition and position B was denoted as the lateral recumbent position for image acquisition. Data were tested for normality using the Shapiro-Wilk test.

Wilcoxon Signed Rank Test was undertaken to determine if the two positions used for image acquisition provided similar measures with respect to thickness, echogenicity and contrast of the skin, subcutaneous tissue and muscle.

A Bland Altman Analysis was used to determine if there were differences between the two image acquisition positions and to construct limits of agreement between the measures obtained between supine and

lateral recumbent positions [36]. Bland Altman plots were created where the difference between the two paired measurements were plotted against the mean of the two measurements obtained in supine and the lateral recumbent positions in order to investigate any possible relationship between the two image acquisition positions.

*A priori* limits of acceptability were defined as data falling within 95% confidence intervals. Significance level was set at  $p = 0.05$ .

### 3. Results

Nine individuals (7 males), aged 51–73 years ( $55.9 \pm 11.7$ ), body mass index of  $30.9 \pm 3.2 \text{ kg/m}^2$ , with acute ( $n = 3$ ) or chronic ( $n = 6$ ), tetraplegia ( $n = 5$ ) or paraplegia ( $n = 4$ ) and a mean duration of SCI of  $25.0 \pm 19.6$  years consented for participation. One participant returned for re-assessment, resulting in 10 paired supine versus lateral recumbent position data sets for analysis.

The Shapiro Wilk test revealed that the mean thickness, echogenicity and contrast measures of the skin, subcutaneous tissue and muscle were normally distributed. Wilcoxon Signed Rank Test revealed that there were no significant differences between the supine and lateral recumbent positions for thickness (range: 0.81 - 0.85 mm,  $p > 0.05$ ), echogenicity, (range: 2.69 - 1.05,  $p > 0.05$ ) and contrast measures (range: 2.34 - 0.22,  $p > 0.05$ ) of the skin, subcutaneous tissue and muscle (Table 1) with one exception: echogenicity of the muscle obtained in the supine position was lower when compared with echogenicity of muscle obtained in the lateral recumbent position (14.68,  $p = 0.04$ ) (Fig. 4).

Bland-Altman plot of muscle echogenicity demonstrated a systemic bias of muscle echogenicity measures being lower by 14.67 units in the supine position when compared with the lateral recumbent position with a 95% limit of agreement ranging from -46.72 - 17.36 units.

### 4. Discussion

This study evaluated the supine versus lateral recumbent position for ultrasound image acquisition of the tissues overlying the IT and the influence of position on thickness, grey scale and contrast measures of the skin, subcutaneous tissues and muscle. Mean differences in echogenicity measurements were systematically lower in the muscle layer with images acquired in the supine position as compared with those obtained in the lateral recumbent position. The observed difference in echogenicity may reflect pooling of interstitial fluid in the buttock

**Table 1**

Mean Thickness, Echogenicity and Contrast Measures Obtained in the Supine (n = 10) and Lateral Recumbent Positions (n = 10) with Mean Paired Differences between Supine and Lateral Recumbent Positions and Corresponding p - values.

Mean Thickness	Mean (mm) ± SD Supine	Mean (mm) ± SD Lateral Recumbent	Mean Paired Difference (mm) ± SD	p - value
Skin	2.80 ± 0.13	2.62 ± 0.11	0.18 ± 0.37	0.163
Subcutaneous Tissue	11.15 ± 4.24	10.29 ± 4.37	0.86 ± 3.60	0.472
Muscle	19.27 ± 7.14	20.10 ± 7.17	-0.81 ± 4.71	0.598
Echogenicity	Mean ± SD Supine	Mean ± SD Lateral Recumbent	Mean Paired Difference Mean ± SD	p - value
Skin	52.20 ± 23.58	51.15 ± 20.23	1.05 ± 7.20	0.656
Subcutaneous Tissue	67.77 ± 10.89	70.46 ± 12.37	-2.69 ± 14.06	0.560
Muscle	74.81 ± 21.99	89.49 ± 24.95	-14.68 ± 16.35	*0.019
Contrast	Mean ± SD Supine	Mean ± SD Lateral Recumbent	Mean Paired Difference Mean ± SD	p - value
Skin	7.26 ± 5.45	7.88 ± 5.81	-0.62 ± 2.80	0.498
Subcutaneous Tissue	18.76 ± 9.47	21.10 ± 10.66	-2.34 ± 9.31	0.447
Muscle	3.98 ± 3.23	3.76 ± 1.79	0.22 ± 2.52	0.791

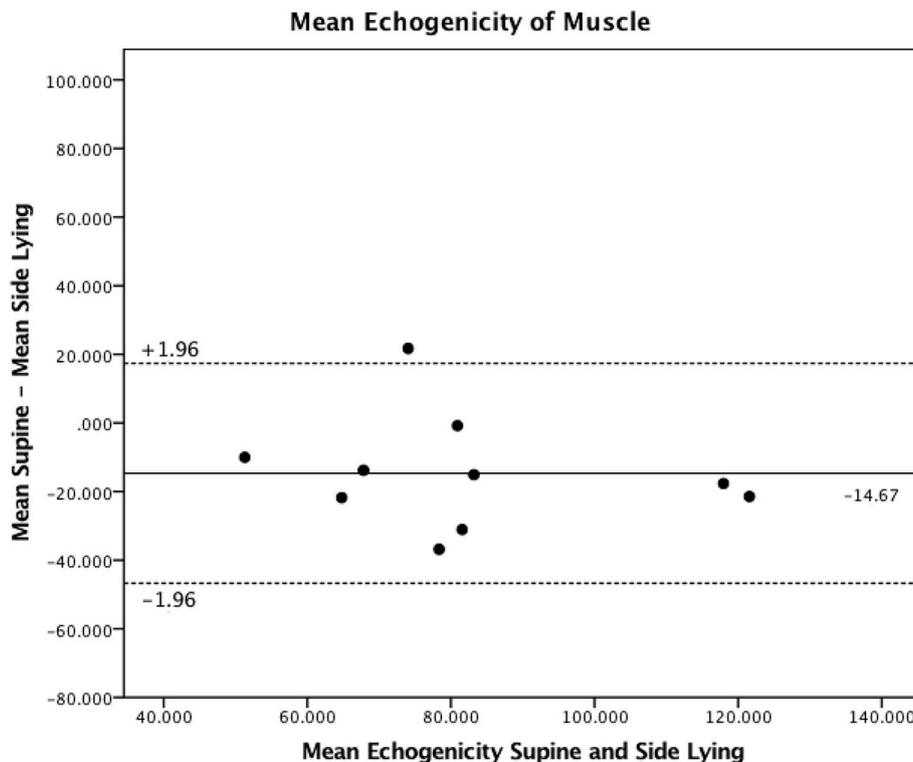
\*denotes significance

region when the legs are elevated in the supine position during scan acquisition. This suggests that the position for scan acquisition may result in incorrect interpretation of gluteus muscle echogenicity; changes in muscle echogenicity not attributable to DTI detection may be incorrectly identified when images are acquired in the supine position. Future studies should explore the potential confounding variable of regional circulation on ultrasound image acquisition and measurement precision.

In contrast, measures of skin, subcutaneous tissue and muscle thickness were not significantly different between images acquired in the supine and lateral recumbent positions. While potential deformation of tissue distal to the foam cushion may have occurred during supine image acquisition in addition to potential sagging of tissue during the different image acquisition protocols, this had no effect on

tissue thickness measures. Additionally, any potential effect of gravity on tissue geometry using the two image acquisition positions was not reflected in any of the geometric measures. Therefore, when evaluating geometric measures of skin, subcutaneous tissue and muscle, we suggest that the supine and lateral recumbent positions produce similar results.

It has been identified that there is considerable variation in tissue thickness over the IT [30,31]. Our measure of gluteus maximus absolute thickness and proportional thickness relative to total tissue were greater than found by other investigators who examined tissues overlying the IT in individuals with SCI [30,31]. It has been recognized that during sitting, the gluteus muscle may move away from the IT [37] in a lateral and posterior direction [30,31]. Additionally, changes in thickness of the muscle may be attributed to the deformation of the gluteus



**Fig. 4.** Bland Altman Plot comparing mean echogenicity values of muscle obtain in the supine versus lateral recumbent positions. The solid horizontal line represents the mean difference with 95% upper and lower limits of agreement.

maximus muscle when the hip is flexed to 90° [30,31,37]. It is possible that difference in muscle thickness between our work and previously published studies may be attributed to the changes in orientation of the gluteus maximus muscle. However, as we did not evaluate the position of the gluteus maximus muscle relative to the IT during image acquisition, we are unable to determine the source of differences between our measures and other investigators. Future studies examining thickness measures of the gluteus maximus muscle should consider its orientation relative to the IT and determine if changes in muscle thickness measures are attributed to atrophy, fat infiltration or change in orientation of the muscle itself. It has been suggested that less padding over bony prominences may reduce the tolerance of the tissues to resist concentrated pressure [38]. Therefore, in the event where the gluteus maximus muscle cannot be found overlying the IT, we recommend that future studies note its presence or absence when thickness measures are recorded as it may lead to misinterpretation of true values.

While grey scale analyses have been used to assess tissue pathology [39–42], studies using ultrasound imaging to examine changes in tissue health as it relates to DTI over the IT have primarily focused on geometric measures [11,17,43–45] with few studies using grey scale analysis [46,47]. Given that DTI originates in the muscle adjacent to the bone [15], efforts to establish grey scale measures to predict DTI are warranted. Grey scale analyses enables one to quantify the depth and the degree of tissue injury through evaluation of mean echogenicity values in the skin, subcutaneous and muscle layers [47]. The image analysis methods used in this study enabled quantitative ultrasound measures of echogenicity and contrast overlying the IT. Examining grey scale measures at a specific depth (e.g. distal 1/3 or at 66% of tissue depth measured from the skin surface) would further enrich the potential for evaluating longitudinal changes in tissue integrity (i.e. progression or resolution of DTI).

Images acquired in the supine position were awkward for participants with involuntary movements due to spasticity, regional pain or lower extremity contracture. The supine position required one additional staff member to position the participant during scan acquisition and to stabilize the lower extremities on the positioning table. The lateral recumbent position was feasible to position the participant for scan acquisition using one staff member and did not require a second staff member to stabilize the participant's lower extremities. As acquiring images in the lateral recumbent position yielded similar findings with the exception of muscle echogenicity, in the interest of reducing participant and technologist burden the choice of position for scan acquisition should be based on participant preference, with lateral recumbent position as the default.

Current methods to evaluate tissue health and detect DTI including photography, thermography [10], computerized tomography [13,14] and magnetic resonance imaging [15] present unique diagnostic and feasibility challenges. With photography, only the superficial tissue is characterized, failing to evaluate the deeper structures [16]. Thermography while feasible provides limited information due to its lack of specificity regarding tissue injury localization. While computerized tomography and magnetic resonance imaging can provide more precise localization of tissue injury, it exposes the individual to ionizing radiation and is costly to use, with limited urgent access. The advantage of using diagnostic ultrasound is its ability to scan the tissues quickly, and allows for rapid bedside interpretation. In individuals with established DTI, diagnostic ultrasound may also evaluate the severity and depth of the DTI, the presence of necrotic and granulating tissue, degree of undermining [47] and the presence of edema in the subcutaneous layer and to monitor treatment response [17]. While our study employed methods to obtain quantitative ultrasound measures overlying the IT, future studies should evaluate the specificity and sensitivity of this method in the assessment of DTI.

It is important to consider that there are several factors that can contribute to measurement error from our ultrasound imaging acquisition protocol. Although we attempted to customized image

acquisition settings including setting the focal zone at the subcutaneous tissue region, differences in the amount of pressure exerted on the tissue with the transducer [48], and changes in the angle and orientation of the transducer relative to the tissue [20,21] may have resulted in incorrect geometric and grey scale measures. Additionally, changes in tissue geometry (e.g. sagging of the skin) during the different image acquisition protocols could have contributed to measurement error. Ishida et al. [21] measured thickness and echogenicity of the rectus femoris while adjusting the tilt angle of the transducer during scan acquisition and found differences in echogenicity which were greater than the minimal detectable change when the transducer was tilted at an angle of 3°. Whittaker et al. [20] found no statistical significance between thickness measures of the transverse abdominis muscle using different transducer orientations. In a separate study, Ishida et al. [22] found significant differences in transversus abdominis thickness measurements under different loads of applied pressure. Although we found significance differences between echogenicity measures acquired in the supine and lateral recumbent positions, we are unsure if our measures of echogenicity between supine and lateral recumbent positions were attributed to participant position, potential changes in the angle or rotation of the transducer relative to the tissue scanned, the amount of pressure exerted by the transducer and the selection of the focal zone in the subcutaneous region. While we attempted to capture images perpendicular to the short and long axis of the IT, we were not able to measure or control the tilt or rotation of the ultrasound head during the scanning protocol or the amount of pressure exerted by the transducer. Additionally, we are unsure if changes in tissue geometry over the IT (i.e. potential sagging of the skin) attributed to the observed differences. Further studies using an instrumented transducer with a force sensor or digital angle gauge and adjustments in the focal zone depth can explore the influence of factors affecting thickness and grey scale measurements when acquiring images over the IT. Changes in tissue geometry as measured by optical coherence tomography [49] during the different scanning positions could also be considered.

This study evaluated two positions for acquiring ultrasound images to determine tissue health overlying the IT. Prior to generalization of the study results, there are several important limitations we wish to acknowledge; the first being our small sample size, second the lack of clarity as to whether grey scale measures are an accurate predictor of DTI and the need for a “gold-standard” comparator such as magnetic resonance imaging to determine the specificity and sensitivity of ultrasound to predict DTI development. While Akins et al. [32] have identified high correlation (ICC = 0.894 – 0.989,  $p < 0.001$ ) between thickness measures of tissues overlying the IT obtained using MRI and ultrasound, the need to determine correlation of grey scale measures acquired between MRI and ultrasound is warranted.

## 5. Conclusions

At present, despite multiple methodologies used to assess tissue health using ultrasound imaging [11,50,51], there are no established consensus derived guidelines for acquisition of ultrasound images over the IT in individuals at risk for developing sitting acquired pressure injuries. The evaluation of soft tissue using diagnostic ultrasound has the potential for earlier detection of DTI, thereby potentially preventing progression of further tissue damage and potentially improving the individual's quality of life. There is good agreement between the supine and lateral recumbent positions for acquisition of tissue thickness and contrast measures of the skin, subcutaneous tissue and muscle overlying the IT. While echogenicity did not vary in skin and subcutaneous tissue between the two positions, lower values for echogenicity were found in the gluteus muscle. The study findings highlight how differences in ultrasound image acquisition in the supine and the lateral recumbent positions in individuals with SCI can systematically influence ultrasound imaging measurements.

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