A novel evaluation of density differences in subcutaneous abdominal adipose tissue layers in pregnancy using elastography

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Abstract

Introduction: Chronic inflammation leads to adipose tissue (AT) fibrosis through excessive accumulation of extracellular matrix proteins. An increasing degree of fibrosis in AT is associated with increasing body mass index (BMI) and insulin resistance. Anecdotally AT has been observed to vary with ease of ultrasound penetration on medical examinations. Ultrasound strain elastography (SE) is a useful tool in assessing fibrosis in liver disease but has not previously been used to assess AT fibrosis. This study assesses the variance in density of the two anatomical layers of subcutaneous AT, superficial subcutaneous adipose tissue (SSAT) and deep subcutaneous adipose tissue (DSAT) in pregnancy using SE.

Method: Women (n = 210) recruited in early pregnancy. Density of SSAT and DSAT were assessed using SE at five-time points throughout pregnancy and post-partum. Semi-quantitative density measures were achieved using two methods, strain values (SV) of the two layers and ImageJ software to calculate the percentage colour pixels in the elastography image and correlated with the SSAT/DSAT thickness and BMI.

Results: Adipose tissue demonstrated a difference in density with the SSAT layer being denser than DSAT. Correlation of tissue density measures with BMI was poor. There was slight change of AT density during pregnancy with a tendency towards harder SSAT and softer DSAT in the third trimester. Post-partum SSAT became softer associated with an increase in SSAT thickness.

Conclusion: Elastography demonstrated density differences in adipose tissue. SE is a new method of assessing the AT demonstrating density differences in adipose tissue. Information on AT density may determine AT fibrosis and be valuable for metabolic disease risk.

Keywords: abdominal, adipose tissue, density, elastography, pregnancy, subcutaneous fat, ultrasound, ultrasound.

Introduction

Obesity, an excess of energy influx, may lead to adipose tissue (AT) fibrosis, characteristic of metabolically dysfunctional AT. Rapid AT expansion can lead to fibrosis affecting metabolic health and insulin sensitivity.¹⁻³ An increase in adipocyte size and number and resulting rapid tissue expansion causes the AT tissue to outgrow the vascular matrix leading to cellular hypoxia, particularly in larger adipocytes.¹ An inflammatory response occurs, and extracellular matrix (ECM), a normal mechanical support system of AT, responds to signalling to maintain
flexibility and expansion of the AT, it is also a fundamental response for repair and regeneration after damage or death of adipocytes; however, persistent inflammation causes excessive ECM production and is the primary cause of AT fibrosis. The excessive ECM limits adipocyte size and reduces flexibility, inhibiting the expansion of adipocytes and decreasing the ability of additional storage or weight loss. Fibrosis causes stress on expanding adipocytes leading to adipocyte necrosis, resulting in chronic AT inflammation, a key mediator in insulin resistance. Pregnancy is a natural state of chronic inflammation and produces increased concentrations of inflammatory markers. Adipose tissue fibrosis may augment inflammation and cocontribute to metabolic disease in pregnancy.

Fibrosis associated with AT expansion is more prevalent in obesity and may be heterogeneous in the AT. An association of fibrosis with BMI has not been supported in all studies in either the subcutaneous AT or visceral AT. Adipose tissue fibrosis has been demonstrated in different AT compartments such as the subcutaneous AT and omental AT. Density of subcutaneous AT using elastography has been investigated, however, differentiating the density of the two layers of subcutaneous AT has not been investigated.

Elastography is a relatively new imaging technique performed by magnetic resonance imaging (MRI) and ultrasound to determine the plasticity of tissue to investigate cancerous lesions. Ultrasound elastography uses one of two methods to obtain tissue density; strain elastography (SE) or shear wave imaging used by acoustic radiation forces impulse imaging (ARFI) or transient elastography (TE). Fibrosis of the liver fibrosis has also been successfully assessed using elastography. FibroScan® (Echosens) was developed specifically for liver fibrosis assessment using TE to measure shear wave speed to assess tissue density and has more recently been used to demonstrate subcutaneous AT fibrosis.

Strain elastography (SE) measures the change in tissue compression to detect strain and determine tissue density and has also been successful in defining liver fibrosis measuring the tissue elasticity in a region of interest, symbolising strain values by placing a colour box over a B-mode image. The advantage over TE is the ability to visualise an image of the region of interest with two-dimensional ultrasound whilst determining elasticity. Strain elastography has not previously been used to assess the density of AT.

Obesity in pregnancy is associated with pregnancy complications, and increased BMI and central obesity are known to increase the risk of complications. Ultrasound has demonstrated a difference in AT mobilisation in BMI categories during pregnancy and the potential for abdominal subcutaneous AT to predict adverse pregnancy outcomes highlighting AT as an important endocrine organ that functions differently with expansion effecting metabolic parameters.

The purpose of this study was to assess the density difference in subcutaneous AT layers and if the density changes in pregnancy and post-partum. A secondary purpose to determine a correlation of BMI and SSAT/DSAT thickness with AT density. We hypothesised there would be a difference in AT density layers in pregnancy which may vary post-partum.

Method
Two hundred and 14 women were recruited to the study at (M1) 12–14 weeks pregnant as part of a longitudinal study on subcutaneous tissue from April 2015–April 2016. Measurements were made at the initial ultrasound (M1) examination and at three other time-points during pregnancy, 18–20 weeks (M2), 26–29 weeks (M3), 33–36 weeks (M4) gestation and 6 weeks post-partum (M5). Elastography was performed on 210 women. Demographic information such as height, weight, age and ethnicity were collected. Informed and written consent was obtained from each participant. Ethical approval was obtained by the Nepean Blue Mountains Hospital Local Health District (Study number 15/4 – LNR/15/NEPEAN4/).

Ultrasound imaging
Abdominal subcutaneous AT was measured using ultrasound. The participants were positioned supine and the was transducer placed medial to the anterior superior iliac spine in the longitudinal plane. At this level, the two layers of the subcutaneous AT SSAT and DSAT can be differentiated. This technique has previously been described. Measurements of the thickness of the AT layers were taken using a linear transducer ML6-15 with elastography capabilities. Measurements were made from the inferior surface of the skin to the superficial fascia (SAT), and from the superficial fascia to the rectus abdominal muscle (DSAT). The measurements and the changes in AT thickness described by BMI, have previously been reported in another publication.

Elastography
Strain elastography uses strain to determine the density of the imaged AT, utilising mechanical methods of compression and decompression. The displacement of ultrasound speckles is measured, which in turn calculates the percentage of deformation which is called strain. Qualitative data is produced by placing the colour box over the region of interest. The strain values (SV) are depicted by colours ranging from red (soft tissue) to green (moderate tissue) and blue (hard tissue). The strain displays comparative information of the tissue in the region of interest. Small strain value indicates small compression and decompression. Normal breathing was often enough to achieve tissue distortion however, when necessary, distortion was induced by external force generated by gentle bouncing of a linear ML6-15 transducer (GE Healthcare Austria GmbH & Co, Tiefenbach, Austria) on the superficial tissue. A “quality” bar governs the quality of the elastography results ensuring that there is adequate strain achieved for the assessment. This could only be achieved using light pressure from the ultrasound probe on the targeted area. Semiquantitative data was obtained from...
the machine produced strain values using a GE Voluson E8/E10 ultrasound machine (GE Healthcare Austria GmbH & Co) and from the elastography images using an image processing and analysis software, ImageJ software (version 1.51) (Wayne Rasband National Institutes of Health, Bethesda, MD, USA) to count the percentage of colour pixels. In this study, the ROI was placed to include the AT (SSAT and DSAT) and the rectus abdominal muscle as a comparative tissue (Figure 1). One operator (NK) performed all the elastography measures using the strain and ImageJ software.

**Reproducibility**

Reproducibility, inter- and intra-operator consistency were assessed on a smaller number (n = 20) of participants performed by two operators both operators had over 20 years’ experience in ultrasound. The first operator obtained an image from the participant and measured the strain values of the AT layers. The second operator also obtained an image and measured the strain values of the AT layers. Both operators then measured strain on the other’s image. Both operators were blinded to the other’s measurements. The second image and measurements were performed at the same examination.

**Statistical analysis**

Power calculation was not performed as this is the first exploratory research in this area. Variation of SSAT and DSAT density was graphically displayed with a box plot. Correlation of strain values and percentage pixel colour with BMI and AT thickness were made using Spearman’s or Pearson’s correlation, as appropriate.

Descriptive statistics are presented as number (per cent) or mean values (± standard deviations) for numeric data. Student’s two-sample t tests with confidence intervals were used to compare hard versus soft SSAT and DSAT at the M1 (11–14 weeks) time-point. Generalised estimating equations (GEE) were used to model changes in the elastography over time accounting for repeated measurements within individuals. This also enabled all available observations to be included in the analysis regardless of missing data at specific time-points. These analyses were performed using SAS® version 9.4 (Copyright © 2013 SAS Institute Inc. SAS and all other SAS Institute Inc. product or service names are registered trademarks or trademarks of SAS Institute Inc., Cary, NC, USA) and STATA version 14 (StataCorp. 2015. Stata Statistical Software: Release 14. College Station, TX: StataCorp LP).

**Results**

**Demographics**

There were 214 women recruited of which 210 had elastography measures at 12–14 weeks gestation. The mean age of the women was 27.13 (6.4) years, mean weight 73.4 (±18.5) kg and BMI 27.1 (6.4) kg/m². There were 192 (91%) women returning for assessment at 18–20 weeks, 177 (84%) at 26–29 weeks and 161 (77%) women at 33–36 weeks gestation. Post-partum, 118 (56%) women returned for elastography assessment. Dividing the women into BMI categories at M1, 90/210 (43%) were normal weight, 54/120 (26%) were overweight and 66/120 (31%) were obese. At M5 48/118 (41%) were normal weight, 30/118 (25%) overweight and 40/118 (34%) were obese.

**Density results**

Box plots demonstrating the range of density at M1 within the two layers of subcutaneous AT are demonstrated in Figure 2. Using a two-sample t-test to assess the density difference of the SSAT, the mean difference of red pixels (soft SSAT) to blue pixels (hard SSAT) was −16.9 (−20.8 to −13.0) P < 0.001 and the mean difference for DSAT soft to hard DSAT was 6 (1–10.6) P < 0.001 (Figure 2a). Testing the strain values at M1 the mean difference of SSAT to DSAT was −0.05 (−0.06 to −0.39) P = 0.02 (Figure 2b).

![Figure 1: Ultrasound Image Showing the Two Layers of SF: SSAT and DSAT. Strain Elastography Colour Overlays the Two Layers of SF and the Rectus Abdominis Muscle as Comparative Tissue. The Colour Demonstrates the Changes in Density. SF, Subcutaneous Adipose Tissue; SSAT, Superficial Subcutaneous Adipose Tissue; DSAT, Deep Subcutaneous Adipose Tissue.](image-url)
Subcutaneous AT layer changes in pregnancy
The mean SSAT and DSAT layers decreased over pregnancy and then increased significantly post-partum (Table 1).

Correlation with BMI and AT thickness
There was poor correlation of tissue density measures with BMI: SSAT: r = 0.00–0.19 and DSAT: r = 0.05–0.21. There was also poor correlation of SSAT and DSAT density with respective thickness SSAT: r = 0.05–0.18 and DSAT: r = 0.00–0.12.

Longitudinal density changes in pregnancy
Although there were statistically significant differences in the elastography variables over time, these were small changes in the strain values of density of AT at the different time-points during pregnancy (Figure 3). The percentage of SSAT hard tissue (blue pixels) increased in the early third trimester suggesting the tissue became denser. The percentage of DSAT soft tissue (red pixels) increased in the third trimester suggesting softer tissue. This was also reflected in the strain

Table 1: Mean density of superficial subcutaneous adipose tissue (SSAT) and deep subcutaneous adipose tissue (DSAT) throughout pregnancy and post-partum. AT, adipose tissue (*significantly different (P < 0.00) to M1).

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<tr>
<td><strong>SSAT thickness</strong></td>
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<tr>
<td>Number (%)</td>
<td>210</td>
<td>192 (91%)</td>
<td>177 (84%)</td>
<td>161 (77%)</td>
<td>118 (56%)</td>
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<tr>
<td>Mean cm (95% CI)</td>
<td>1.14 (1.05–1.24)</td>
<td>1.07 (0.98–1.17)</td>
<td>1.0 (0.91–1.09)</td>
<td>0.99 (0.83–0.99)*</td>
<td>1.34 (1.2–1.44)*</td>
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<td><strong>SSAT density</strong></td>
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<td>Soft AT (Red)</td>
<td>0.12 (0.10–0.14)</td>
<td>0.11 (0.09–0.13)</td>
<td>0.1 (0.08–0.12)</td>
<td>0.1 (0.08–0.12)</td>
<td>0.24 (0.22–0.27)*</td>
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<td><strong>Mean (95% CI)</strong></td>
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<td>Hard AT (Blue)</td>
<td>0.29 (0.26–0.32)</td>
<td>0.31 (0.28–0.34)</td>
<td>0.36 (0.32–0.39)*</td>
<td>0.34 (0.31–0.38)*</td>
<td>0.24 (0.21–0.27)</td>
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<tr>
<td><strong>SSAT Mean (95% CI)</strong></td>
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<td>Strain (kPa)</td>
<td>0.2 (0.19–0.21)</td>
<td>0.19 (0.18–0.21)</td>
<td>0.19 (0.18–0.20)</td>
<td>0.19 (0.18–0.20)</td>
<td>0.28 (0.26–0.3)</td>
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<td><strong>DSAT thickness</strong></td>
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<td>Mean cm (95% CI)</td>
<td>0.73 (0.64–0.81)</td>
<td>0.70 (0.63–0.77)*</td>
<td>0.65 (0.58–0.71)</td>
<td>0.63 (0.56–0.71)</td>
<td>0.74 (0.64–0.91)*</td>
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<td><strong>DSAT density</strong></td>
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<tr>
<td>Soft AT (Red)</td>
<td>0.27 (0.24–0.3)</td>
<td>0.29 (0.26–0.32)</td>
<td>0.34 (0.31–0.38)*</td>
<td>0.39 (0.36–0.42)*</td>
<td>0.27 (0.24–0.29)</td>
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<td><strong>Mean (95% CI)</strong></td>
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<tr>
<td>Hard AT (Blue)</td>
<td>0.21 (0.18–0.24)</td>
<td>0.19 (0.16–0.21)</td>
<td>0.17 (0.15–0.2)*</td>
<td>0.13 (0.11–0.15)*</td>
<td>0.25 (0.22–0.27)*</td>
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<td><strong>DSAT Mean (95% CI)</strong></td>
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<tr>
<td>Strain (kPa)</td>
<td>0.25 (0.24–0.27)</td>
<td>0.26 (0.25–0.27)</td>
<td>0.27 (0.25–0.29)</td>
<td>0.29 (0.27–0.31)*</td>
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values (Table 1). Post-partum there was a significant difference in the strain values compared to M1 with the SSAT strain at 0.28 (2.3) and DSAT 0.31 (0.12) suggesting softer post-partum AT in both layers. In the second method of assessment for SSAT, soft fat (red) increased to 0.24 (0.15) post-partum and the amount of hard fat (blue) 0.24 (0.15) decreased resulting in softer AT. In the DSAT, soft fat (red) remained the same at 0.27 (0.14) and hard fat (blue) increased to 0.25 (0.15) however, becoming marginally harder post-partum. These changes of density throughout pregnancy and post-partum are plotted in Figure 3.

Reproducibility
There was excellent interoperator and intra-operator agreement when measuring strain on the same image with an intraclass coefficient (ICC) ICC: 0.91 (P < 0.001) for SSAT and ICC: 0.99 (P < 0.001) for DSAT. However, there was only fair interoperator agreement between images ICC (CI): 0.45 (−0.57–0.81) for SSAT and 0.31 (−3.9–0.65) ICC for DSAT.

Discussion
This is the first study to explore and describe the density differences in the two layers of subcutaneous AT. Adipose tissue fibrosis is a relatively recent finding in obesity research and has largely been studied using the histological approach and metabolic markers.4,5,7,10 To our knowledge SE has not been used to specifically assess the density of AT. We have described a method to image AT with SE and demonstrated a variation in the density of subcutaneous AT and its layers. Fibrosis occurring in subcutaneous AT is caused by excessive accumulation of the extracellular tissue (ECM), this occurs when there are adipocyte necrosis and low-grade inflammation. As part of the regenerative process the influx of macrophages and immune cells respond and if damage persists, the result is extra collagen and fibronectin production replacing normal AT.4 The excessive ECM then increases density and reduces the flexibility of the AT. This process, similar to liver fibrosis is not homogeneous throughout the tissue.5 The variation of density of the two subcutaneous AT layers we have demonstrated on SE may be reflective of this process. Others have used an adaptation of the FibroScan® to assess subcutaneous AT density prior to bariatric surgery2,11 finding a correlation with adipose fibrosis, hypertension, adiponectin and liver fibrosis. Their method used TE which does not allow direct visualisation of the tissue imaged and therefore was unable to determine differences between AT layers. A limitation of their research was participants needed >2 cm of AT to avoid artefacts from diffraction and pressure and shear wave coupling, a problem we did not encounter.

Our data demonstrated poor correlation between BMI and SSAT/DSAT thickness with the density of SSAT and DSAT. Previous research has also demonstrated a poor correlation with AT fibrosis (which is associated with AT density) and BMI.5 The process of the formation of AT fibrosis leads to an assumption that with the expansion of the AT layer as in increased BMI there would be an increase in fibrosis,5 such that rapid AT expansion, may be the key for fibrosis formation. Heterogeneity of AT distribution and metabolic markers is also found in those with an increased BMI who are healthy, that is the “metabolically healthy obese”.26 This heterogeneity may be explained using the AT expansion theory, where an individual has a limited AT storage within the superficial subcutaneous AT layer.27 Once this storage capacity is reached due to the limits of expansion of adipocytes and pre-adipocyte numbers, then AT storage must go to secondary storage areas such as DSAT, visceral AT and the organs decreasing the metabolic health.28, 29 The increase in adipocyte size in a AT compartment at capacity results in inflammation and increased ECM production5 causing fibrosis. The capacity limitation of AT storage varies between individual. This combined with rapid AT expansion, fibrotic restriction of adipocyte expansion and the inflammatory response of adipocytes, may explain the heterogeneity of AT fibrosis and lack of correlation across BMI categories and with AT thickness.

![Figure 3: Plots of Changes of Adipose Tissue Density Through Pregnancy. (Superficial, Superficial Subcutaneous Adipose Tissue; Deep, Deep Subcutaneous Adipose Tissue).](image-url)
There was a difference in the density of the two layers of subcutaneous AT with the SSAT being denser than the DSAT. The SSAT is the primary layer of subcutaneous AT for triglyceride deposit and has been found to be protective of disease. SSAT has been described as the primary layer of AT.\textsuperscript{28} When storage capacity in this compartment is reached the adipocytes becomes enlarged and pre-adipocyte differentiation is limited. This leads to adipocyte dysfunction and low-grade inflammation which in turn causes excessive ECM and fibrosis.\textsuperscript{28} This theory potentially demonstrates SSAT is at greater risk of fibrosis and may explain increased density of SSAT in our results. In comparison, the DSAT compartment has more pre-adipocyte availability and may be less likely to become fibrotic. Fibrosis in the DSAT compartment has not been reported as prior research has not differentiated the two layers of subcutaneous AT. However, fibrosis in omental AT has been discovered, but in contrast to subcutaneous AT fibrosis has been found to be beneficial, being associated with lower circulating triglycerides.\textsuperscript{3} Conceivably, other AT compartments may contain varying AT fibrosis and cause varying effects on metabolic health, particularly in pregnancy when chronic inflammation is a natural response and affecting risk of metabolic dysfunction in pregnancy.\textsuperscript{30}

Density changes within the two layers of subcutaneous AT were minimal throughout pregnancy. There was a marked difference in AT density post-partum with the SSAT layer becoming softer; this was associated with a rapid expansion of AT thickness post-partum in this study. This is an interesting finding and is perhaps a normal physiological adaption for breast feeding but requires further investigation. In an over-feeding study where rapid AT accumulation occurs, it was demonstrated that ECM remodelling peaks closer to day 56 and therefore fibrotic changes caused by excessive ECM in the new AT may not have begun at the time of our post-partum assessment.\textsuperscript{31} Alternatively, there may be an unknown threshold for AT to become fibrotic.

Measurement reproducibility was excellent; however, image reproducibility assessing interoperator variability was only fair. This may be due to the heterogeneity of AT fibrosis, with a slight difference in area assessed. The AdipoScan\textsuperscript{TM} (Echosens) demonstrated only fair reproducibility \textit{in vivo} and this poor performance was explained by the heterogeneity of AT fibrosis.\textsuperscript{11} This inaccuracy may be minimised by producing multiple images on the participant and averaging the results when performing this examination. Further research should investigate the best method to ensure reproducibility. Other studies have shown good to moderate reproducibility in liver elastography and other sites of the body.\textsuperscript{16}

There are several limitations to this study. As a preliminary study, there were small numbers and attrition of study numbers latter in the pregnancy and post-partum may have affected the strength of the findings. No metabolic or pathological parameters available to make comparison or to classify AT fibrosis. Further studies should use such parameters to assess the association with AT fibrosis and metabolic status.

**Conclusion**

This preliminary research has demonstrated that SE can determine the different densities in AT and has the potential to assess AT fibrosis an important indicator of metabolic health. There is a difference in AT compartments, with the SSAT being denser than DSAT until postpartum when increase in AT thickness decreases the density of SSAT. Future research should compare SE data on AT with biopsy or metabolic markers in pregnancy, perhaps at the time of caesarean section. Assessment of SE to predict adverse pregnancy outcomes would be interesting. Further research on the deposition and density of AT in post-partum breastfeeding women would be of value. This measure of density may also prove to be useful in non-pregnant people and people with metabolic disorders.

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**Ethics approval**

Ethics approval was granted by the Nepean Blue Mountains Hospital Local Health District (Study number – 15/4 – LNR/15/NEPEAN/4).

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**Disclosure of interests**

There are no conflicts of interest for any of the authors.

**Authorship contribution**

RN and NK developed the idea. MP NK AEQ RB contributed to planning and equipment for the study. NK and CB conducted data analysis. NK drafted the paper and AEQ contributed to the writing of the manuscript. All authors interpreted the data and gave valuable input at all stages. All authors have approved the final version of the article for publication.

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