Diabetes status is associated with plantar soft tissue stiffness measured using ultrasound reverberant shear wave elastography approach

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Abbreviations: (RSWE) Reverberant Shear Wave Elastography, (FBS) Fasting Blood Sugar.

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Abstract:

Introduction: The purpose of this study was to investigate the association between the mechanical properties of plantar soft tissue and diabetes status.

Method: 51 (M/F-21/30) participants with pre-diabetes onset (Fasting Blood Sugar Level-FBS>100 mg/dL), age>18 years, and no lower limb amputation were recruited after ethical approval was granted from Pontificia Universidad Catolica del Peru ethical review board. Ultrasound reverberant shear wave elastography was used to assess the soft tissue stiffness at the 1st MTH, 3rd MTH and the heel at both feet.

Results: Spearman's rank-order correlation (rho) test indicated a significant ($P < .05$) positive correlations between FBS level and the plantar soft tissue shear wave speed at the 1st MTH: *r*ho = 0.402 (@400 Hz), rho = 0.373 (@450 Hz), rho = 0.474 (@500 Hz), rho= 0.395 (@550 Hz), and rho = 0.326 (@600 Hz) in the left foot and rho = 0.364 (@450 Hz) in the right foot. Mann-Whitney U test indicated a significantly ($P < .05$) higher shear wave speed in the plantar soft tissue with the following effect sizes (r) at the 1st MTH of the left foot at all tested frequencies: $r = 0.297$ (@450 Hz), $r = 0.345$ (@500 Hz), $r = 0.322$ (@550 Hz), and $r = 0.275$ (@600 Hz), and at the 1st MTH of right foot $r = 0.286$ (@400 Hz) in diabetes as compared with the age and body mass index matched prediabetes group.

Conclusion: An association between fasting blood sugar level and the stiffness of the plantar soft tissue with higher values of shear wave speed in diabetes vs prediabetes group was observed. This indicated that the proposed approach can improve the assessment of the severity of diabetic foot complications with potential implications in patient stratification.

a. Introduction

The plantar soft tissues of the foot are the first point of contact with the ground during normal gait. The main role of these plantar soft tissues is to act as a shock absorber, to reduce the effect of ground reaction forces during activities such as walking, running and standing.

The plantar soft tissue of the foot consists of highly specialized tissues which present with complex nonlinear, visco-elastic mechanical properties in addition to a complex internal structure comprising of both skin and plantar fat pad [1].

Previous studies show that diabetes is associated with changes in the plantar soft tissue mechanical properties as a result of glycation which is associated with an increased blood sugar level over prolonged periods of time. Specifically, the plantar tissue in people with diabetes compared to the age-matched groups of non-diabetic is thicker [2], stiffer [3,4], harder [5]; and also tends to have less energy return efficiency [6]. In a previous study the changes in the diabetic fat pad was reported to impair the cushioning ability of the plantar soft tissue in distributing pressure [7]. These changes make the plantar soft tissue more vulnerable to tissue damage and ulceration.

While it has been generally agreed that these changes could be due to the histological changes inside the tissues as a result of glycation [8], an in-vivo study using ultrasound indentation technique in people with diabetes revealed a significantly higher stiffness of heel pad in people with higher levels of fasting blood sugar [9]. Fasting blood sugar level is commonly recognised as a risk factor for the diabetes severity.

Prediabetes (a condition in which the patient has intermediate blood sugar level [10]) has been previously implicated to associate with complications like microvascular and cardiovascular disease [11,12] and with microvascular impairment in skin and retina [13]. While plantar pressure distribution pattern during walking in patients with prediabetes were found to be similar to participant with diabetes [14], different gait and balance parameters were found to be affected in patients with pre-diabetes and those with diabetes [15]. Despite these, the role of blood sugar level on changes in the mechanical properties of the plantar soft tissue has not been previously studied, and it Is not known how the mechanical properties of plantar soft tissue different between pre-diabetes vs the diabetes patients.

Glycated haemoglobin was recognised as a risk factor for diabetic foot ulceration [16]. Indeed our previous study show a direct link between plantar soft tissue mechanical properties and the risk of ulceration using ultrasound strain elastography technique [17]. We also showed that this mechanical properties can be used to enhance the prediction of diabetic foot ulceration [18].

Using strain elastography together with an stand-off material as a reference has addressed a number of issues that are traditionally associated with ultrasound strain elastography and allowed a quantitative measure of the plantar soft tissue [17]. However, as the tissue acts as a viscoelastic material , the strain rate dependency indicates that the frequency at which the tissue undergoes deformation affect the measures of strainability hence the stiffness [19,20]. While in strain elastography the tissue is deformed at low frequency (1-2 Hz), to manually maintain the exact frequency is challenging.

Shear wave elastography is another non-invasive, ultrasound-based method to assess soft tissue stiffness through the generation of shear waves inside the tissue and measure their propagation speed as they expand laterally in the field of view. However the use of conventional shear wave elastography is limited in the foot, as the shear waves cannot be formed close to the bone due to excess reflections from the surface of the bone [21,22] .

To address this limitation, recently a new method known as Reverberant Shear-Wave Elastography (RSWE) was introduced that allows vibrating the entire tissue with minimal shadowing as the vibration sources propagates in all directions within the medium.

The technique has been previously used for the in vivo assessment of the elastic properties of human liver [23] and breast tissues [24]it has not previously been applied to assess the plantar soft tissue where the proximity of bony prominences where reflections of waves exist in conventional ultrasound shear wave elastography methods. Furthermore, the ability of the method to assess the plantar soft tissue of people with diabetes in a clinical setting and association of those properties with disease severity has not been investigated.

Therefore, the aim of this study was to provide a clinically viable method for assessing the mechanical properties of the soft tissue of the foot in people with pre-diabetes and diabetes and to investigate the association between these parameters and the measures commonly associate with diabetes severity.

b. Method

Participants

After ethical approval was obtained, 51 (M/F 21/30) participants with intermediate hyperglycaemia (Fasting Blood Sugar Level- FBS >100 mg/dL [10]), age >18 years, and no amputation were recruited at a Militar Geriatrico in Lima, Peru to participate in this study following a full informed consent. The demographic and diabetes related blood biochemicals are highlighted in Table 1. In addition, the participants were divided into two groups of age and BMI matched including: Diabetes (FBS>125mg/dl) and Prediabetes (125 mg/dl >FBS >100mg/dl) (Table 1).

Table 1 appears here

Data Acquisition

Data acquisition was performed with the participant lying in a supine position on a hospital bed with their feet positioned at the edge of the bed (Figure 1). The locations imaged for this study were the 1st metatarsal head (1st MTH), 3rd metatarsal head (3rd MTH), and the heel pad as the soft tissue directly over the apex of the calcaneus (Heel) as previously reported [17]. All images were collected by a trained sonographer using a linear array model L7-4, ATL, Bothell, WA, USA) controlled by a Verasonics ultrasound system (Vantage-64TM, Verasonics, Kirkland, WA, USA).

Figure 1 appears here:

Ultrasound coupling gel was applied directly to the participant's foot along with the standoff material (Figure 2). To image the 1st MTH, the ultrasound probe was placed perpendicular to the plantar surface of the foot imaging the 1st MTH in the frontal plane. The probe was manoeuvred so as the lateral sesamoid was located at the centre of the B-Mode image (Figure 2). To measure the 3rd MTH, the ultrasound probe was positioned so that the 3rd MTH was in the centre of the B-Mode image in the frontal plane. Finally, for the heel, the ultrasound probe was positioned so as that the apex of the calcaneus, in the frontal plane, was visible and at the centre of the B-Mode image in line with the previously developed method [17]. Since the amount of compression could affect the stiffness hence the shearwave speed in the tissue, the thickness of the standoff was used to assess and normalise the amount of applied force to the tissue [17]. A normalised thickness was calculated as the ratio of soft tissue thickness (Ttis) over the thickness of the interface to account for the effect of loading magnitude on the thickness of soft tissue [17].

Figure 2 appears here

To perform the Reverberant shear wave measurements, a customised Graphic User Interface (GUI) was implemented for B-mode imaging and plane wave acquisition in MATLAB software 2019b (The MathWorks, Natick, Massachusetts, USA). Once the correct anatomical landmarks had been identified by the sonographer, a set of passively driven speakers (MISCO, Minneapolis, MN, USA) were placed onto the medial and lateral malleoli of the foot being measured. The speakers were powered using an external amplifier (DENON, New Jersey, USA). When a satisfactory B-Mode image was achieved, the custom MATLAB script was started and the volume of the amplifier was turned up to a pre-specified value. The speakers emitted an audio track that consisted of tones of the following frequencies: 400 Hz, 450 Hz, 500 Hz, 550 Hz, and 600 Hz. Once the acquisition time finished the volume of the speakers was turned down. The frequency spectrum of 6 points symmetrically spaced between the gel pad and the tissue was shown to confirm the presence of each sinusoidal signal in the temporal-frequency domain and to provide real-time feedback of the quality of the acquisition. Three trials were performed per location per foot and the average of extracted values for the three trials was used in data analyses. Average error was calculated for test-retest repeatability based on the values extracted from these three trials. In-phase and quadrature (IQ) signal data was saved for post processing.

Data Processing

Particle displacements were calculated by using Loupas algorithm [25]. A fixed amount of signals with 10 periods were extracted pixel by pixel. After that, a bandpass temporal frequency filter centred at each vibration frequency with a bandwidth of 20 Hz was applied. Additionally, a spatial frequency domain filtering was applied considering wavenumbers limits setting minimum and maximum values of expected shear wave speed values (0.7m/s and 5 m/s respectively). Phase information was extracted and analysed following the method we previously reported [26] . RSWE estimator was performed using a 7.7 x 15.4 mm^2 kernel size to evaluate the spatial autocorrelation of particle velocity complex matrix. The real part of normalized autocorrelation was calculated, then the lateral profile was extracted ($\Delta z = 0$). Local wavenumber was estimated using a curve fitting to the theoretical autocorrelation function in the lateral direction. Each pixel generated a R^2value that was compared with a 0.7 as a minimum threshold. Pixels with lower cut-off values were not considered. Finally, the shear wave speed (SWS) was calculated by the following equation:

(1): $c_s = \frac{2\pi f_v}{k}$

where c_s is the SWS, f_v the vibration frequency and k the wavenumber.

Statistical Analysis

Coefficient of variation was calculated as the ratio between standard deviation and average based on the parameters calculated during the three data collection trials for each site. Shapiro-Wilk test indicated a non-normal distribution (p<0.05).

Spearman's rank order correlation (rho) test was used to assess the association between the parameters that indicate severity of diabetes and the tissue stiffness measured using reverberant shear-wave elastography (RSWE) approach.

In addition the groups were divided into age and BMI matched Diabetes (Fasting Blood Sugar-FBS >125mg/dl) and Prediabetes (125 mg/dl >Fasting Blood Sugar-FBS >100mg/dl) and Mann-Whitney U was used to assess the differences between the plantar soft tissue mechanical properties between the two groups for both left and right feet separately. All statistical analyses were conducted using commercially available software (IBM® SPSS®v.24).

c. Results

The repeatability for test-retest was presented by the average coefficient of variation are shown in Table 2.

Significant ($p<0.05$) positive correlations were observed between the fasting blood sugar-FBS level and the plantar soft tissue's shear wave speed at the 1st MTH of the left at all tested frequencies as follows: r= 0.402 (@ 400 Hz), r= 0.373 (@450 Hz), r= 0.474 (@500 Hz), r= 0.395 (@550 Hz) and r= 0.326 (@600 Hz) (Table 3). A significant ($p<0.05$) positive correlation was also observed between the FBS level and the plantar soft tissue's shear wave speed at the 1st MTH of the right foot r=0.364 (@450Hz) (Table 4). There was no other significant correlation observed between the shear wave speeds measured at different frequencies for any sites and any other parameter that indicated the severity of diabetes (Table 3 and 4).

In addition, the test of difference indicated a significantly ($P<0.05$) higher shear wave speed in the plantar soft tissue at the 1st MTH of the left foot at all tested frequencies as follows with the effect sizes: r= 0.297 (@450 Hz), r= 0.345 (@500 Hz), r= 0.322 (@550 Hz) and r= 0.275 (@600 Hz) in Diabetes (FBS >125mg/dl) as compared to the Prediabetes (125 mg/dl >FBS >100mg/dl) group (Table 5). Moreover, the diabetes group showed to have a significantly ($P<0.05$) higher shear wave speed in the plantar soft tissue at the 1st MTH of the right foot at 400 Hz, with the effect size: r=0.286 (Table 6). No other significant differences in the shear wave speed measured at different frequencies for any sites between the diabetes and prediabetes group (Table 5 and 6).

d. Discussion

The purpose of this study was to investigate the association between the mechanical properties of the plantar soft tissue and the measures associated with diabetes severity using reverberant shear wave ultrasound elastography approach. The method when applied to the foot showed to produce repeatable results for test-retest with low coefficient of variation (average ± standard deviation) as 1.82± 0.15% for mean R^2, and acceptable coefficients of variation for Shear wave speed (6.74 ± 0.42 %), tissue thickness (6.59 ± 3.51 %) and standoff thickness (5.64 ± 0.66%) (Table 2).

The shearwave speed at the 1st MTH was found to be significantly associated with the fasting blood sugar level. Since the higher shearwave speed can be associated to the higher stiffness of the tissue, the result of this study are in line with the results of previous investigations on the heel pad where significant association between Fasting Blood Sugar level and tissue stiffness [9]. Indeed the elevated glycaemic gap was found to predict adverse outcome of diabetic patients with necrotizing fasciitis (Chen et al, 2019) [27].

This is the first study that has utilised ultrasound reverberant shear wave elastography to characterise the mechanical properties of the plantar soft tissue of the foot. In addition, this study is unique as for the first time the mechanical properties of the plantar soft tissue in people with diabetes was assessed using shear wave elastography.

There is a clear trend that as the vibration frequency increase, there is generally an increase in the shear wave speed (Figure 3). This in line with the previous literature, using Reverberant shear wave elastography in which the shearwave speed were found to increase with an increase in frequency in the lower limb muscles [28]..

Interestingly Pai and Ledoux [8] have also shown similar results when plantar soft tissue was tested invitro using compression testing at low frequencies (1Hz -10Hz).

Figure 3 appears here

The values found in the current study for the shear wave speed at the heel (2.29-2.44 m/s) are comparable to the mechanical properties of the heel pad of healthy participants that were reported using shear wave elastography in a previous study [22]. That study report an average maximum shear wave speed of 2.8m/s (calculated based on reported value of 23.5kPa) and a minimum value of 1.35m/s (calculated based on reported value of 5.46 kPa) [22]. However, it should be noted that limited information is available about the frequency of the generated shear waves in a commercial ultrasound machines. Hence a direct comparison of the shear wave speed reported in previous studies and the values obtained in this study is not possible

The relationships between tissue thickness and the measured shear wave speeds were general observed to be inconsistent (Table 3 and 4). There were a few significant negative correlations between the shear wave speed and tissue thickness at the 1st MTH and Heel in both feet (Table 3 and 4), however only in the left foot a significant positive correlation were observed between the tissue thickness and shear wave speed at the 3rd MTH (Table 3). To eliminate the possible effect of compression on the measured shear wave speed, the relationship between the normalised tissue thickness only showed negative significant correlations with tissue thickness at the heel at few frequencies, while there was no significant relationship observed for either the 1st or the 3rd MTH at any site for either feet (Tables 3 and 4). This indicated that the normalised thickness of tissue does not affect the amount of measured shear wave speed at the 1st and 3rd MTH. However at the heel the normalised thickness plays a role and a heel with higher thickness seem to be affiliated with lower reverberant shear wave speed. This can be related to the fact that vibration a thicker tissue would be more difficult using the external vibrators.

In this study the shear wave speed was observed to be higher at the heel compared to the 1st MTH that is in line with the results of our previous study in which strain elastography was used to assess the mechanical properties of the soft tissue of participants with diabetes [17].

The distinct differences between diabetes and pre-diabetes groups indicate that the transition to Diabetes status is associated with a significant increase in tissue stiffness at specific sites where there is more vulnerability to diabetic foot ulcers.

This can have practical applications in early diagnosis of stiffening of the plantar soft tissue as a result of diabetes and can inform appropriate foot care in these groups of patients. The results of the current study are particularly important and have clinical implications as the general positive trend towards the increase in the plantar soft tissue stiffness and the fasting blood sugar level.

Comparing the diabetic and pre-diabetes groups used in this study, the results show a higher shear wave speed values for the plantar soft tissues of the foot in those with diabetes when compared to participants with pre-diabetes status. In the absence of previous studies in which the shear wave speed of plantar soft tissue was compared between the diabetes and prediabetes group, no direct comparison with the literature could be made.

While in previous studies prediabetes status has been associated with higher arterial stiffness [29] and with liver stiffness [30], the current study is the first to investigate the mechanical properties of the soft tissue in people with prediabetes and to compare that against people with diabetes. The observed association between the shear wave speed and the fasting blood sugar reported in the current study can be linked to the effect of glycation on the plantar soft tissue.

Glycation is the non-enzymatic bonding of a sugar molecule to a protein or lipid molecule that occurs as a result of prolonged increase in blood glucose levels (hyperglycaemia) and is linked to the structural and functional changes in the soft tissue [31].

In this study a correlation was found between Fasting Blood Sugar Level and the shear wave speed at different frequencies, given that HbA1c represents more long term effect of glycation, a similar if not stronger correlation would have been expected between HbA1c and shear wave speed. The lack of such relationship can be related to the fact that the standardisation of HbA1c assay is poor while the standardization of glucose assay is easier to implement. Inaccuracies in measurement and poor standardization of HbA1c measurements are still a common problem [32]. Although the standardization for plasma glucose is not perfect, the implementation of FBS could be more easily aligned to a standard than A1C. This could have been the main reason behind not observing any association between A1c and the plantar shear wave speed in this study.

The plantar fat pad is characterized by a honeycomb configuration in which fibrous septa (composed by collagen and elastin fibres) envelope adipose compartments[33]. Fibrous septa provide constraint to the deformation of adipose chambers that are made of closely packed fat cells [34]. . The results of the current study in which shear wave speed is correlated with the fasting blood sugar level is in line with the In-vitro studies of cadaveric diabetic feet in which a thicker fibrous septa with an increase in elastin concentrations [35] was linked to the increased stiffness in the mechanical properties of plantar soft tissue in diabetes [36].

The observed discrepancies between the results of the left and right foot in the current study is in line with previous observations in a few biomechanical studies on diabetes population. For example, asymmetry was reported between ipsilateral and contralateral regions of the foot in a study of patients with diabetes [37]. Furthermore, in our previous studies we found differences in the mechanical properties between ulcerated and non-ulcerated patients only in the left and not in the right foot [17]. This could be the result of the asymmetry in loading during gait that was previously reported [38].

e. **Conclusion**

This study showed the ability of the ultrasound reverberant shear wave elastography approach in assessing the mechanical properties of plantar soft tissue in people with diabetes. A significant association was found between the shear-wave speed at the 1st MTH and the fasting blood sugar. The method also showed to be able to differentiate the mechanical properties of plantar soft tissue at the 1st MTH of people with diabetes against those with prediabetes status. The results of this study supports the notion that the biomechanical changes in the blood affect the mechanical properties of the soft tissue in people with diabetes. Furthermore, this study indicates a distinct and significantly higher stiffness at the 1st MTH stiffness in people with diabetes as compared to their BMI and age matched pre-diabetes counterparts. These indicate the proposed method can improve the ability of assessing the severity of diabetic foot complications and can have potential implications in patient stratification.

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	Pre-Diabetes (11)		Diabetes (40)		All (51)		Mann-Whitney	
	Median	Range	Median	Range	Median	Range	P value	Effect Size
Age (year)	68	33	68	40	68	40	0.678	0.058
Height (m)	1.58	0.28	1.6	0.43	1.59	0.43	0.866	0.023
Weight (Kg)	65	25	68	59	68	59	0.920	0.014
BMI (Kg/m^2)	26.71	10.10	26.53	13.89	26.56	13.89	0.823	0.031
~~Diab.~~ Duration*. (months)	96	599	108	396	108	599	0.884	0.020
HBA1c (%)	6.5	2.4	6.75	2.6	6.7	3.1	0.761	0.042
Blood Sugar (mg/dL)	130	106	170	90	170	121	**0.020**	0.322
FBS (mg/dL)	108	38	151	60	150	105	**0.000**	0.704

Table 1: The median (range) for demographic and diabetes related blood biochemicals. * since onset of intermediate Blood Glucose Level (FBS>100 mg/dL)

Coefficient of variation (%)	400		450		500		550		600		Mean R^2		SWS		Thickness	
	Mean R^2	SWS	Mean R^2	SWS	Mean R^2	SWS	Mean R^2	SWS	Mean R^2	SWS	Average	STDEV	Average	STDEV	Stand-off	Tissue
1st MTH Left	2.23	6.68	2.55	7.66	2.43	6.51	1.93	6.73	1.93	7.04	2.21	0.28	6.92	0.45	5.67	9.61
3rd MTH Left	1.51	5.19	1.83	5.79	2.04	5.26	1.54	4.87	1.63	6.17	1.71	0.22	5.46	0.52	5.60	4.42
Heel Left	1.34	6.07	1.36	7.30	1.56	6.06	1.41	7.96	1.33	9.40	1.40	0.10	7.36	1.40	4.58	4.15
1st MTH Right	2.35	7.90	2.33	6.70	2.54	6.99	2.06	6.17	2.17	6.45	2.29	0.18	6.84	0.66	6.63	12.27
3rd MTH Right	1.67	6.14	1.77	6.22	2.05	5.93	1.72	6.08	1.74	5.85	1.79	0.15	6.04	0.15	5.46	5.25
Heel Right	1.42	7.05	1.56	6.99	1.59	7.03	1.43	8.61	1.46	9.53	1.49	0.08	7.84	1.17	5.91	3.84
Average	1.75	6.50	1.90	6.78	2.03	6.30	1.68	6.74	1.71	7.41	**1.82**		**6.74**		5.64	**6.59**
Stdev	0.43	0.93	0.45	0.69	0.41	0.68	0.27	1.36	0.31	1.64	**0.15**		**0.42**		0.66	**3.51**

Table 2: The repeatability for test-retest presented by the average coefficient of variation for different parameters.

	Correlation Coefficient Left Foot	Thickness	Norm. thick.	Age	Height	Weight	BMI	Diab. Dur.	HBA1c	Blood sugar	FBS
	SWS @ 400 Hz	-.345*	-0.175	0.120	-0.010	-0.050	-0.139	0.041	-0.139	0.107	.402*
	SWS @ 450 Hz	-.465*	-0.264	0.010	0.056	-0.004	-0.132	-0.008	-0.219	0.134	.373*
	SWS @ 500 Hz	-.422*	-0.211	0.034	0.096	0.009	-0.132	0.082	-0.087	0.130	.474*
1st MTH	SWS @ 550 Hz	-0.265	-0.089	0.197	-0.120	-0.145	-0.160	0.047	-0.225	0.070	.395*
	SWS @ 600 Hz	-.335*	-0.216	0.029	-0.120	-0.109	-0.115	0.104	-0.198	0.012	.326*
	Thickness	1.000	.767*	-0.174	-0.048	0.046	0.174	-0.106	0.232	-0.090	-0.160
	Norm. thick.	.767*	1.000	-0.108	0.026	0.182	.292*	-0.188	0.166	-0.176	-0.114
	SWS @ 400 Hz	.354*	0.128	-0.131	0.117	.307*	.307*	-0.067	0.019	-0.190	-0.038
	SWS @ 450 Hz	.342*	0.185	-0.128	0.130	.301*	.292*	0.003	0.215	-0.008	0.123
	SWS @ 500 Hz	.313*	0.191	-0.017	0.051	.305*	.291*	0.173	0.218	-0.100	0.167
3rd MTH	SWS @ 550 Hz	.367*	0.183	0.022	0.070	0.203	0.173	0.131	0.187	-0.057	0.189
	SWS @ 600 Hz	0.187	0.123	0.093	0.073	0.127	0.074	0.234	0.260	-0.126	0.027
	Thickness	1.000	0.545*	-.312*	0.083	0.223	.305*	-0.180	-0.028	-0.151	-0.012
	Norm. thick.	0.545*	1.000	-0.159	0.214	.380*	.318*	-0.028	-0.110	-0.261	0.005
	SWS @ 400 Hz	-0.245	-.392*	-0.056	-0.183	-0.076	0.071	-0.003	0.057	0.011	0.141
	SWS @ 450 Hz	-0.094	-0.089	-0.185	-0.012	0.034	0.078	-0.162	0.009	0.083	0.100
	SWS @ 500 Hz	-.304*	-.289*	-0.218	0.040	0.015	-0.041	-0.013	-0.019	0.015	0.068
Heel	SWS @ 550 Hz	-.372*	-.461*	-0.167	0.016	-0.089	-0.133	-0.065	-0.001	-0.107	0.009
	SWS @ 600 Hz	-.338*	-.370*	-0.094	0.084	-0.023	-0.113	0.028	-0.013	-0.030	0.029
	Thickness	1.000	.792*	0.065	0.013	0.199	.323*	0.148	-0.152	-0.139	-0.111
	Norm. thick.	.792*	1.000	0.051	0.080	.285*	.330*	0.091	-0.201	-0.245	-0.199

Table 3: Correlation (rho) shear wave speeds at different frequencies and sites vs the parameters indicating diabetes status at the left foot. * p<0.05

	Correlation Coefficient Right Foot	Thickness	Norm. thick.	Age	Height	Weight	BMI	Diab. Dur.	HBA1c	Blood sugar	FBS
1st MTH	SWS @ 400 Hz	-0.223	-0.151	0.017	0.128	-0.001	-0.141	-0.183	-0.148	0.004	0.236
	SWS @ 450 Hz	-.308*	-0.021	0.199	0.249	0.190	-0.051	-0.014	-0.084	0.074	.364*
	SWS @ 500 Hz	-.287*	-0.130	-0.042	0.243	0.175	-0.052	-0.054	-0.175	-0.065	0.264
	SWS @ 550 Hz	-0.212	-0.243	-0.070	0.192	0.089	-0.094	-0.094	-0.152	-0.042	0.198
	SWS @ 600 Hz	-0.269	-.336*	-0.101	0.199	0.011	-0.213	-0.018	-0.059	0.095	0.234
	Thickness	1.000	.578*	-0.231	0.158	.306*	.312*	-0.054	0.202	-0.117	-0.007
	Norm. thick.	.578*	1.000	-0.152	0.252	.400*	.326*	-0.091	0.076	-0.087	0.046
3rd MTH	SWS @ 400 Hz	0.112	0.016	-0.201	0.023	0.110	0.144	-0.055	0.131	-0.039	-0.058
	SWS @ 450 Hz	0.038	0.059	-0.132	-0.012	0.033	0.087	-0.073	0.112	-0.050	0.049
	SWS @ 500 Hz	0.115	-0.030	-0.023	0.075	0.047	0.003	0.098	0.061	-0.036	0.074
	SWS @ 550 Hz	0.187	0.095	-0.029	0.112	0.155	0.152	0.092	0.105	-0.024	0.040
	SWS @ 600 Hz	0.105	-0.040	0.040	-0.115	0.015	0.151	0.065	-0.043	-0.165	0.039
	Thickness	1.000	.413*	-.305*	0.215	.283*	0.253	-0.125	-0.064	-0.229	-0.038
	Norm. thick.	.413*	1.000	-0.056	.358*	.471*	.311*	0.025	-0.185	-0.178	0.046
Heel	SWS @ 400 Hz	-.292*	-.312*	-0.081	0.142	0.063	-0.075	-0.016	0.175	-0.050	0.089
	SWS @ 450 Hz	-.414*	-0.177	0.033	0.087	0.031	-0.084	-0.138	0.062	0.066	0.134
	SWS @ 500 Hz	-.351*	-0.188	0.014	0.090	-0.058	-0.193	-0.121	0.032	-0.001	0.071
	SWS @ 550 Hz	-.489*	-.297*	0.133	0.114	-0.134	-.316*	0.066	0.021	0.082	0.113
	SWS @ 600 Hz	-.593*	-.442*	0.072	-0.113	-.298*	-.331*	-0.049	0.023	-0.027	0.064
	Thickness	1.000	.683*	-0.132	0.055	.307*	.406*	0.008	-0.062	0.018	-0.004
	Norm. thick.	.683*	1.000	0.046	0.137	.300*	0.276	0.077	-0.186	-0.034	0.006

Table 4: Correlation (rho) shear wave speeds at different frequencies and sites vs the parameters indicating diabetes status at the right foot. * $p<0.05$

Left Site	Foot Tissue characteristics	Pre-Diabetes (11) Median	Range	Diabetes (40) Median	Range	All (51) Median	Range	Mann-Whitney P value	Effect Size
1st MTH	SWS @ 400 Hz (m/s)	2.227	0.743	2.422	1.456	2.356	1.456	0.136	0.207
	SWS @ 450 Hz (m/s)	2.296	0.809	2.492	1.369	2.453	1.369	**0.032**	0.297
	SWS @ 500 Hz (m/s)	2.272	0.896	2.596	0.883	2.535	0.991	**0.013**	0.345
	SWS @ 550 Hz (m/s)	2.357	0.850	2.654	1.315	2.603	1.315	**0.020**	0.322
	SWS @ 600 Hz (m/s)	2.458	0.933	2.759	1.201	2.722	1.201	**0.047**	0.275
	Thickness (mm)	6.300	5.986	4.327	11.684	4.856	11.684	**0.003**	0.406
	Ttis/Tstn	1.371	0.857	1.119	2.559	1.222	2.559	**0.037**	0.289
3rd MTH	SWS @ 400 Hz (m/s)	1.969	1.133	1.997	0.883	1.990	1.334	0.902	0.017
	SWS @ 450 Hz (m/s)	2.123	1.405	2.192	0.673	2.184	1.542	0.982	0.003
	SWS @ 500 Hz (m/s)	2.229	0.997	2.259	0.779	2.259	1.121	0.670	0.059
	SWS @ 550 Hz (m/s)	2.327	1.160	2.327	0.753	2.327	1.231	0.814	0.033
	SWS @ 600 Hz (m/s)	2.464	1.450	2.407	0.765	2.410	1.450	0.439	0.107
	Thickness (mm)	9.035	5.040	8.767	7.928	8.971	7.928	0.148	0.200
	Ttis/Tstn	2.052	1.309	1.881	1.842	1.930	1.842	0.297	0.144
Heel	SWS @ 400 Hz (m/s)	2.217	1.111	2.296	1.918	2.295	1.918	0.638	0.065
	SWS @ 450 Hz (m/s)	2.298	1.815	2.402	1.745	2.368	2.089	0.614	0.070
	SWS @ 500 Hz (m/s)	2.379	1.006	2.378	1.856	2.379	1.856	0.911	0.016
	SWS @ 550 Hz (m/s)	2.264	1.470	2.412	1.658	2.411	1.862	0.991	0.002
	SWS @ 600 Hz (m/s)	2.413	1.907	2.454	2.109	2.448	2.570	0.823	0.031
	Thickness (mm)	13.775	8.553	13.268	12.410	13.648	12.410	0.426	0.110
	Ttis/Tstn	3.333	2.850	3.184	5.279	3.199	5.279	0.235	0.165

Table 5: Shear wave speed measured at different frequencies and the thickness of the tissue at different sites for the left foot, and difference. *p<0.5

Right Foot Site	Tissue characteristics	Pre-Diabetes (11) Median	Range	Diabetes (40) Median	Range	All (51) Median	Range	Mann-Whitney P value	Effect Size
1st MTH	SWS @ 400 Hz (m/s)	2.293	1.750	2.341	1.628	2.330	1.767	0.277	0.151
	SWS @ 450 Hz (m/s)	2.330	1.831	2.444	1.127	2.420	1.831	**0.039**	0.286
	SWS @ 500 Hz (m/s)	2.448	1.573	2.546	1.451	2.525	1.573	0.095	0.231
	SWS @ 550 Hz (m/s)	2.446	1.231	2.567	1.233	2.541	1.251	0.175	0.188
	SWS @ 600 Hz (m/s)	2.504	1.199	2.652	1.064	2.620	1.199	0.065	0.256
	Thickness (mm)	6.747	5.236	5.099	7.309	5.454	7.412	**0.013**	0.343
	Ttis/Tstn	1.671	1.553	1.434	2.587	1.485	2.587	0.104	0.225
3rd MTH	SWS @ 400 Hz (m/s)	2.115	0.904	2.044	1.533	2.057	1.533	0.382	0.121
	SWS @ 450 Hz (m/s)	2.192	1.548	2.213	2.163	2.208	2.163	0.695	0.054
	SWS @ 500 Hz (m/s)	2.315	0.855	2.271	1.578	2.271	1.596	0.937	0.011
	SWS @ 550 Hz (m/s)	2.279	0.858	2.288	0.893	2.282	0.893	0.622	0.068
	SWS @ 600 Hz (m/s)	2.394	1.023	2.443	0.779	2.440	1.075	0.937	0.011
	Thickness (mm)	8.558	5.148	8.256	7.383	8.335	7.383	0.175	0.188
	Ttis/Tstn	2.010	1.212	2.123	2.376	2.081	2.376	0.614	0.070
Heel	SWS @ 400 Hz (m/s)	2.411	1.020	2.388	1.997	2.388	1.997	0.902	0.017
	SWS @ 450 Hz (m/s)	2.548	1.153	2.503	2.275	2.511	2.275	0.902	0.017
	SWS @ 500 Hz (m/s)	2.561	0.462	2.447	1.427	2.461	1.427	0.884	0.020
	SWS @ 550 Hz (m/s)	2.378	0.655	2.469	1.433	2.448	1.433	0.487	0.096
	SWS @ 600 Hz (m/s)	2.443	0.812	2.524	2.046	2.492	2.046	0.762	0.042
	Thickness (mm)	13.173	6.949	12.968	11.222	13.173	11.222	0.487	0.096
	Ttis/Tstn	3.376	3.380	3.422	6.578	3.376	6.578	0.955	0.008

Table 6: Shear wave speed measured at different frequencies and the thickness of the tissue at different sites for the right foot, and difference. *p<0.5

Figure 1. The data collection setup and the positioning of the speakers adjacent to the foot. The probe is shown to be in contact with the plantar skin at the heel through the stand-ff material.

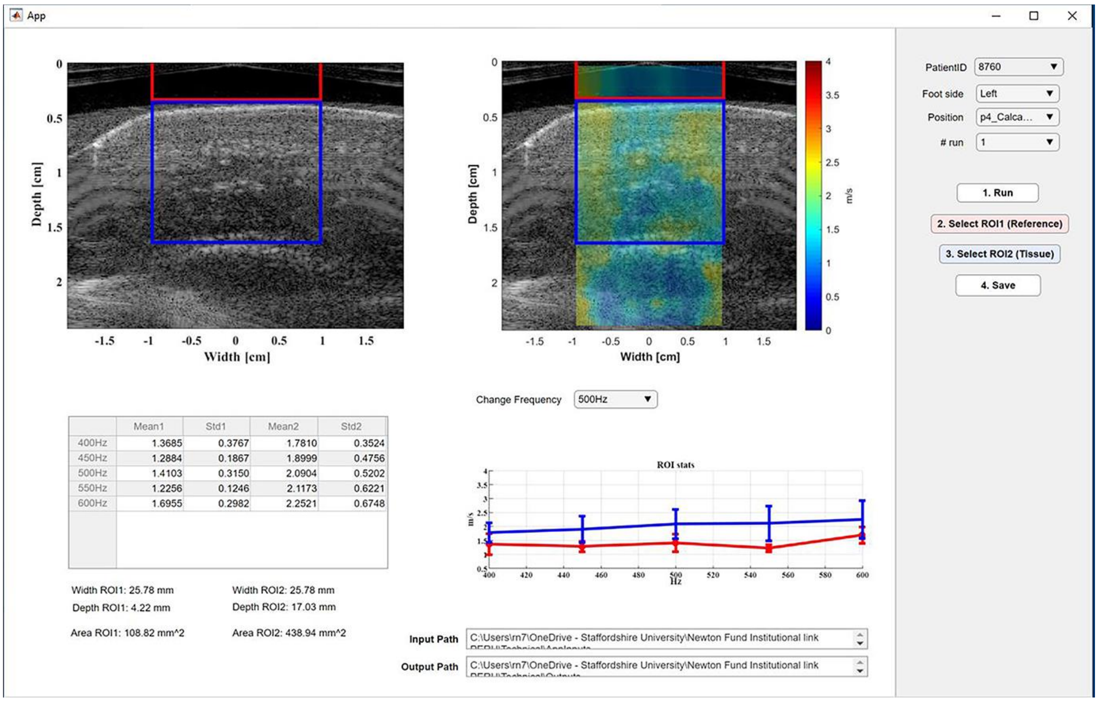

Figure 2. Left side- B Mode image showing the regions of interest including the Heel pad Tissue (blue box) and the standoff material (red box). The regions of interest in the tissue are defined as the area confined between the skin and the underlying bone (calcaneus in this case). Right side - Elastography image obtained with the RSWE approach superimposed on the B-mode image.

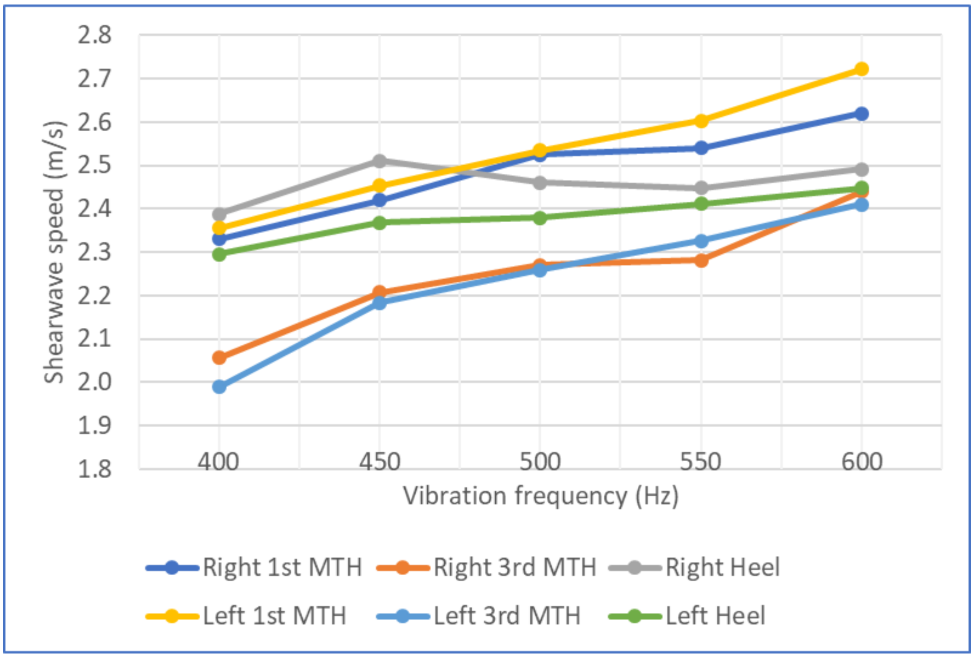

Figure 3: Median shear wave speed at different frequencies at different sites.