Title: The relationship between the mechanical properties of heel-pad and common clinical measures associated with foot ulcers in patients with diabetes

Authors:

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Abstract:

Aim: The present study aims at investigating the correlation between the mechanical properties of the heel-pad of people with type-2 diabetes and the clinical parameters used to monitor their health and also to assess ulceration risk.

Methods: A new device for the in-vivo testing of plantar soft tissues was built and pilot-tested. This device consists of an ultrasound probe connected in series with a dynamometer. Loading is applied manually using a ball-screw actuator. A total of 35 volunteers with type-2 diabetes were recruited and the thickness, stiffness of their heel-pads as well as the energy absorbed during loading was assessed. The participants with diabetes also underwent blood tests and measurements of Ankle Brachial Index and Vibration Perception Threshold.

Results: Pearson correlation analysis revealed strong correlations between triglycerides and heel-pad stiffness ($r=0.675, N=27, p<0.001$) and between triglycerides and energy ($r=-0.598, N=27, p=0.002$). A correlation of medium strength was found between Fasting Blood Sugar (FBS) and stiffness ($r=0.408, N=29, p=0.043$).

Conclusions: People with type-2 diabetes and high levels of triglycerides and FBS are more likely to have stiffer heel-pads. Increased stiffness could limit the tissues' ability to evenly distribute loads making them more vulnerable to trauma and ulceration.

Keywords: In-vivo tissue stiffness, diabetic foot, ulceration risk, ultrasound indentation, FBS, Hypertriglyceridemia.

1. Introduction

Recent reports indicate that approximately 15% of people with diabetes world-wide will at some stage develop diabetic foot ulceration (Boulton, 2000). Indeed diabetes mellitus (type-2) is the most frequent cause of non-traumatic lower-limb amputations. In the UK up to 100 people per week have a limb amputated as a result of diabetes even though up to 80% of these amputations could have been prevented with correct management (Diabetes UK, 2011). The severity of this fact becomes even more pronounced considering that eight out of ten people die within five years of having an amputation (Khanolkar et al., 2008).

Foot ulcers in people with Diabetes are multi-factorial and linked to a variety of risk factors like peripheral neuropathy, vascular insufficiency and physiological measures (Crawford et al., 2007). Whilst, some of the epidemiological studies demonstrate that the indicators of neuropathy like impaired sensation, Vibration Perception Threshold (VPT) are predictors of ulceration (Crawford et al., 2007; Frykberg et al., 1998), other studies show that peripheral vascular disease indicated by Ankle Brachial Index (ABI), glycohaemoglobin (HbA_{1c}) level and duration of diabetes are the main contributing factors to ulcers (Boyko et al., 2006). Although these major risk factors are known to contribute to foot ulceration, it is not completely understood how they affect the mechanical properties of plantar soft tissue.

From previous research, it is clear that diabetes can affect the internal structure and the mechanical properties of the plantar soft tissues. Some in-vivo studies performed with age-matched groups of non-diabetic and diabetic volunteers have found that diabetic plantar tissue tends to be thicker (Chao et al., 2011), stiffer (Chao et al., 2011; Klaesner et al., 2002), harder (Piaggesi et al., 1999) and shows higher energy dissipation ratios (i.e. the ratio of the energy-input over the energy-return after the end of a load/unload cycle) (Hsu et al., 2007, 2002, 2000). On the other hand Erdemir et al. (Erdemir et al., 2006) studied the mechanical behaviour of heel-pad using a novel methodology which combined in-vivo testing and finite element modelling to inverse engineer the tissue's material coefficients. The

authors of that study found no statistically significant stiffness or thickness difference between age-matched groups of people with diabetes and non-diabetic volunteers (Erdemir et al., 2006).

Despite this there is a clear paucity of studies which explore the relationship between the mechanical properties of plantar soft tissue and commonly employed clinical and biochemical measures. Hsu et al. (Hsu et al., 2000) found a weak correlation between the tissue's energy dissipation ratio and the patient's neuropathy score measured based on 10g monofilament test. These authors also indicated a strong correlation between energy dissipation ratio and the duration of diabetes.

The most popular technique for the in-vivo study of plantar soft tissues' mechanical behaviour is the combined use of ultrasonography and dynamometry. Previous research have developed different devices to perform in-vivo indentation (Chao et al., 2011; Erdemir et al., 2006; Hsu et al., 2007, 2000; Tong et al., 2003; Zheng et al., 1999) or bulk compression tests (Hsu et al., 2009; Rome et al., 1998; Zheng et al., 2012). Typically, during an indentation test the plantar soft tissue is compressed between the indenting device and a bony prominence. During the test the applied force is recorded using a load sensor (i.e. dynamometer or load cell) and tissue deformation is measured from the ultrasound images.

Given this background, the overall aim of the present study is to investigate if significant correlation exists between the mechanical properties of the heel-pad of people with diabetes and the clinical parameters used to monitor their health and ulceration risk.

2. Patients and method

A total of seventeen (17) volunteers with no known musculoskeletal disease or diabetes (Group 1) with average age 35.0(±5.8) years, average height 158.3(±9.8) cm and average body mass 65(±14) Kg were recruited to pilot-test the in-vivo loading procedure and produce reference data. Moreover, thirty five (35) volunteers with type-2 diabetes (Group 2) with average age 54.8(±9.1) years, average height

167.4(±9.3) cm, average body mass 73(±16) Kg and average duration of diabetes 13.9(±7.8) years were recruited at a diabetic referral centre to participate in this non-invasive study. The ethical approval was sought and granted by the Ethics committee and all volunteers provided full informed consent.

All volunteers were subjected to in-vivo mechanical tests to study the mechanical behaviour of their heel-pads. The participants with diabetes also underwent blood tests, ABI and VPT measurements. All tests were performed on the same day and besides the biomechanical measurements all clinical and biochemical tests were part of the normal treatment plan of each volunteer. The biochemical parameters measured from the blood tests included the levels of HbA_{1C}, Fasting Blood Sugar (FBS), Post Prandial Blood Sugar (PPBS), Serum Creatinine, Serum Cholesterol and triglycerides. All participants were screened for dry skin, callosity and ulceration and were excluded from this study if they had any signs of cutaneous conditions affecting the plantar surface of the foot.

The mechanical behaviour of the heel-pad was studied using a custom made loading device (Figure 1). This device consists of a 13 MHz linear array ultrasound probe connected in series with a dynamometer (500N Cytec, C.I.T. Technics, Centre for Innovative Technics, Netherlands) and mounted on a rigid metallic frame. The footprint-area of the ultrasound probe is equal to 12.7 mm × 47.1 mm. The ultrasound probe and the dynamometer are connected with a custom made probe holder which is capable of griping ultrasound probes of different sizes and shapes (Figure 1c). Different sites of the foot can be tested by changing the distance of the probe from the base of the frame while different planes can be imaged by rotating the probe around its central axis. Loading is applied manually with the help of a ball-screw actuator by rotating a hand wheel. A complete anti-clockwise revolution of the hand wheel generates 5 mm of forward movement. The metallic frame is also equipped with adjustable foot supports that can rigidly fix the subject's foot.

After fixing the subject's foot on the metallic frame the ultrasound probe was rotated and pressed against the plantar surface of the heel until the calcaneus was clearly visible. The calcaneus was

initially imaged in the antero-posterior (sagittal) plane. After identifying the medial process of the calcaneal tuberosity the height of the probe was modified to shift the apex of the calcaneus at the centre of the ultrasound image. The probe was then rotated by 90° and fixed to image the medio-lateral (frontal) plane.

The right heel of each volunteer was subjected to five preconditioning load/unload cycles followed by three measurement cycles to a maximum compressive force of 30 N. Given that the contact area between the ultrasound probe and the foot was equal to 6.58 cm^2 the maximum applied pressure was ≈55 kPa. Although this pressure may appear less than the heel pressures reported within the literature during walking, due to the nature of the static position of the foot and the way that the force was applied, and given that there are no baseline measurements on the direct application of any force in plantar surface of the foot in diabetic patients, this magnitude was considered to be sufficient for experimental purposes. The dynamometer readings and the ultrasound images were recorded during loading for the last three load cycles with a sampling rate of 20 Hz. The loading rate was controlled with the help of a metronome. The crank handle was rotated with an angular velocity ≈90 deg/sec moving the probe with a speed of ≈1.25 mm/sec. The speed of the ultrasound probe was the same during loading and unloading.

The value of the minimum bone to probe distance was measured after the end of the tests using video analysis software (Kinovea open source project, www.kinovea.org). The initial heel-pad thickness was measured from the first ultrasound image of the first load cycle after preconditioning (Figure 2a) while tissue deformation was calculated by subtracting the minimum measured probe to bone distance from the initial heel-pad thickness (Figure 2a-c). The measured forces and deformations were synchronized by matching the instances of maximum deformation with those of maximum force to create force/deformation curves for each one of the three cycles of loading per volunteer. The heel-pad's stiffness was calculated as the slope of the final part of the force/deformation curves. Furthermore the energy input during loading was calculated as the area below the force-deformation curves (Figure 2d). The normalized energy and stiffness over initial thickness were also calculated.

The reliability of the testing procedure was assessed for the non-diabetic volunteers. More specifically the deformation rate of each load cycle was measured and its average value and standard deviation was calculated. The reproducibility of the entire testing procedure was assessed for a single participant through a test-retest procedure. Moreover the impact of the heel-pad's loading history on its mechanical behaviour was assessed by comparing the results for the three load cycles using paired-sample t-tests.

To assess the ability of the followed methodology to differentiate between different groups of volunteers the statistical significance of the differences between the results of the two groups was assessed by independent-samples t-tests (statistical significance level = 0.05). The correlation between the measured mechanical parameters (i.e. stiffness, normalized stiffness, energy, normalized energy and thickness) and age or BMI was investigated for both groups of volunteers using Pearson correlation analysis. In the case of volunteers with diabetes the correlation between the mechanical parameters and the duration of diabetes, ABI, VPT, HbA_{1C}, FBS, PPBS, Serum Creatinine, Serum Cholesterol and triglycerides were also included into the correlation analysis. All statistical analyses were performed using IBM® SPSS®v.21.

3. Results

The average value of the deformation rate calculated during the pilot-testing of the device was equal to 0.96 mm/sec ± 0.14 mm/sec which results in an inter-subject variability of 14%. The average intra-subject variability of the deformation rate was equal to 7%. The reproducibility of the mechanical measurements assessed by a test-retest procedure was 5% for tissue thickness, 4% for tissue stiffness and 8% for energy. The comparison between the results from different load cycles showed that the mechanical behaviour of the heel-pad was not influenced by its loading history. No statistically significant difference was found between the results of different load cycles ($p>0.05$).

The results for both groups of volunteers are presented in table 1 while representative results in terms of force/normalised deformation curves are shown in figure 3. As it can be seen the heel-pads of volunteers of the two groups appear to exhibit different mechanical behaviour. In quantitative terms, the heel-pads of the second group had a significantly higher stiffness ($p=0.034$, two-tailed) and a significantly lower energy ($p=0.007$, two-tailed). Similar results were found for the normalised stiffness and energy ($p=0.001$ and $p=0.038$ respectively). The participants of Group 1 showed a 38% higher energy absorbed during loading (35% higher when normalised to the initial thickness) as compared to Group 2. Furthermore Group 2 showed 36% higher stiffness (38% higher normalized stiffness) as compared to Group 1. No statistically significant difference was found between the groups in terms of thickness ($p=0.985$, two-tailed).

The Pearson correlation analysis revealed a medium strength positive correlation between stiffness and FBS ($r=0.408$, $N=29$, $p=0.043$) (Figure 4a). Strong correlations were found between the level of triglycerides of the volunteers with diabetes and the mechanical measurements (Figure 4b and Figure 4c). More specifically strong positive correlations were found between triglycerides and stiffness ($r=0.675$, $N=27$, $p<0.001$) (Figure 4b) as well as normalized stiffness ($r=0.667$, $N=27$, $p<0.001$). On the other hand strong negative correlations were found between triglycerides and energy ($r=-0.598$, $N=27$, $p=0.002$) (Figure 4c) and between triglycerides and the normalized energy ($r=-0.599$, $N=27$, $p=0.002$).

No statistically significant correlation was found between the mechanical measurements and the volunteers' age or BMI for both groups. Moreover in the case of diabetic volunteers no correlation was found between the mechanical measurements and duration of diabetes, ABI, VPT and the remaining biochemical parameters.

4. Discussion

The present study aimed at investigating the correlation between the mechanical behaviour of heel-pad and common parameters measured routinely in a clinical practice (Table 1). For this purpose a custom ultrasound-based device was designed and built to load the foot and study the in-vivo mechanical behaviour of plantar soft tissues. A linear array ultrasound probe with a relatively big foot-print (≈6cm^2) was connected in series with a dynamometer and mounted on a rigid frame. The instrumented probe could be moved in a controlled manner with the use of a ball-screw actuator to load the foot.

Ultrasonography enables the in-vivo, non-invasive measurement of internal tissue deformations and therefore it is ideal for the study of plantar soft tissue biomechanics. So far ultrasonography has been used to perform indentation (Chao et al., 2011; Erdemir et al., 2006; Hsu et al., 2007, 2000; Tong et al., 2003; Zheng et al., 1999) and bulk compression tests (Hsu et al., 2009; Rome et al., 1998; Zheng et al., 2012). Ultrasound indentation tests are usually performed using cylindrical or prismatic indenters with footprint-area between 0.6 cm^2 (Chao et al., 2011; Zheng et al., 1999) and 4.7 cm^2 (Erdemir et al., 2006; Hsu et al., 2007). On the other hand for bulk compression tests the foot is loaded using a plate with dimensions similar or bigger that to the foot itself. The effect of the probe's footprint-area on the measured stiffness of the heel-pad was investigated numerically by Spears et al. (Spears and Miller-Young, 2006). They concluded that larger probes can produce more reliable and robust measurements of the stiffness of the heel-pad.

Ultrasound indentation is usually performed either with the use of hand held systems or with the use of custom made loading frames. In the latter, an instrumented ultrasound probe is moved either manually using a linear guide or an actuator ("assisted" loading) or with the use of motorized drive mechanisms ("automated" loading). The reliability of different loading techniques for ultrasound indentation tests was investigated by Kawchuk et al. (Kawchuk et al., 2006). The authors of that study concluded that the use of a rigid frame can significantly improve the reliability of the indentation procedure compared to the use of a hand held system. However, no significant difference was found

for the cases of "assisted" or "automated" loading. The main disadvantage of "assisted" loading is the poor control over the loading rate. Previous studies showed that this problem can be addressed using a metronome (Hsu et al., 2007, 2005). In the case of the present study the use of a metronome enabled the realization of load/unload cycles with a variability of 14% in terms of deformation rate.

The device presented here was used to measure the thickness and the force/deformation curve of the heel-pads of 17 volunteers with no known musculoskeletal disease or diabetes and 35 volunteers with type-2 diabetes. The slope of the force deformation curve as well as the area below the curve was calculated to quantify heel-pad's macroscopic response to loading. The statistical analysis of the results revealed statistically significant correlations between the mechanical and clinical parameters for the volunteers with diabetes. More specifically a strong positive correlation was found between the level of triglycerides and the stiffness of the heel-pad as well as the energy absorbed during loading. More specifically volunteers with high levels of triglycerides tended to have stiffer heel-pads that absorb less energy during loading. The stiffness of the heel-pads was also correlated to the FBS with higher FBS values linked to higher tissue stiffness. These results demonstrate for the first time that there is a link between the biochemical profile of a person with diabetes (type-2) and the mechanical behaviour of his/her plantar soft tissues. These results can have implications in assessing the risk of ulceration in people with diabetes and neuropathy.

The correlation between FBS and the heel-pad stiffness found in this study can be attributed to the effect of hyperglycaemia and the role of glycation on the collagen fibrils within the septal wall of the plantar soft tissue (Hsu et al., 2002). Through microscopic studies of the heel-pad fragmented and distorted collagen fibrils were found in the heel-pad structure of people with diabetes as compared to a parallel structure of collagen fibrils in the heel-pad of non-diabetic adults (Hsu et al., 2002). Although no previous study has investigated the correlation between hyperglycaemia and plantar heel-pad stiffness, the results of this study can be justified by the fact that high blood sugar levels can further attribute to non-enzymatic glycosylation that alter the soft tissue microscopic structure that result in a stiffer heel–pad (Paul and Bailey, 1996).

Hypertriglyceridemia on the other hand is commonly seen in patients with altered carbohydrate metabolism. In patients with hyperglycaemia, more so in Noninsulin-Dependent Diabetes Mellitus (NIDDM), there is elevated Free Fatty Acids (FFA) which increases the levels of Very-Low-Density-Lipoprotein Triglyceride (VLDL TG). Hypertriglyceridemia is an important risk factor for coronary artery disease since it increases the stiffness of the blood vessels (Koniari et al., 2011). Previous research has also demonstrated that the level of triglycerides affects the mechanical behaviour of tendons and is linked to the formation of Achilles tendon xanthomas and increased risk of rupture (Abboud et al., 2012; Beason et al., 2013). To the knowledge of the authors of the present study no correlation between triglycerides' level and the mechanical behaviour of plantar soft tissues has been found before. In the case of plantar soft tissues Hsu et al. (Hsu et al., 2000) found that their mechanical behaviour (i.e. energy dissipation ratio) is influenced by neuropathy and the duration of diabetes.

The comparison between the two groups of participants showed that Group 2 (i.e. the volunteers with type-2 diabetes) had significantly higher stiffness and absorbed less energy during loading. No statistically significant difference was found in terms of heel-pad thickness.

At this point it has to be stressed out that comparing between diabetic and non-diabetic populations was not the main objective of this study and therefore the two groups were not age-matched. Indeed the non-diabetic volunteers were recruited for the pilot testing of the device and the in-vivo measurement procedure. Based on that the differences observed in terms of stiffness and energy between the two groups could be caused either by aging or by diabetes or by a combination of both. Indeed the average age of the volunteers of Group 1 was significantly lower than Group 2. According to literature the stiffness of plantar soft tissues can increase either as a result of aging (Kwan et al., 2010) or because of diabetes (Chao et al., 2011; Klaesner et al., 2002; Pai and Ledoux, 2012, 2010). Similarly heel-pad thickness can also increase either as a result of ageing (Kwan et al., 2010) or diabetes (Chao et al., 2011).

Although the difference in age between the control and the experimental group could be perceived as a weakness of this study, based on the above the most interesting finding of the comparison between the two groups of volunteers is not the difference in terms of stiffness and energy but the lack of statistically significant difference in terms of thickness (even though both aging and diabetes are reported to increase thickness). Future studies could possibly look at substantiating these results using age/ gender matched cohort of participants.

The main limitation of the present study stems from the fact that loading was applied manually. The use of a metronome gave a satisfactory control over the deformation rate at low loading speeds but the application of more "dynamic" loading scenarios requires the use of an automated and motorised loading system. Regardless of this limitation the in-vivo measurement technique presented here was able to reveal for the first time a strong correlation between the biochemical profile of people with type-2 diabetes and the mechanical behaviour of their plantar soft tissues.

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Competing interests: None declared

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Tables:

Table 1: The size (N) of the two groups of volunteers, the average values and standard deviations of their age, body mass index (BMI), tissue stiffness, tissue thickness and energy absorbed during loading (energy). For group 2 the average values and standard deviations of the clinical parameters included into this study are also presented (if not otherwise indicated the sample size of a measurement is equal to group size).

	Group 1	**Group 2**	*P value*
Group size (M/F)	17(5/11)	35(27/8)	-
Age (y)	38.4 ± 8.7	54.8 ± 9.2	-
BMI	0.260 ± 0.055	0.262 ± 0.049	-
Duration of diabetes (y)	*N/A*	13.9 ± 7.9	-
ABI	*N/A*	1.18 ± 0.20	-
VPT (Volt)	*N/A*	37 ± 25	-
FBS (mg/dl)	*N/A*	180 ± 86 *(N=29)*	-
PPBS (mg/dl)	*N/A*	251 ± 99 (N=28)	-
HbA_{1C} (%)	*N/A*	9.1 ± 2.6 *(N=33)*	-
Serum Creatinine (mg/dl)	*N/A*	0.88 ± 0.29 *(N=32)*	-
Cholesterol (mg/dl)	*N/A*	164 ± 45 *(N=29)*	-
Triglycerides (mg/dl)	*N/A*	141± 72 *(N=27)*	-
Stored energy (mJ)	34.7 ± 9.8	25.1 ± 9.7	0.002
Norm. energy (mJ/mm)	1.76±0.44	1.30±0.45	0.001
Stiffness (N/mm)	17.1 ± 6.0	26.6 ± 16	0.022
Norm. Stiffness (N/mm^2)	0.89±0.37	1.43±0.97	0.038
Thickness (mm)	19.5 ± 4.7	19.4 ± 3.5	0.985

Figure legends:

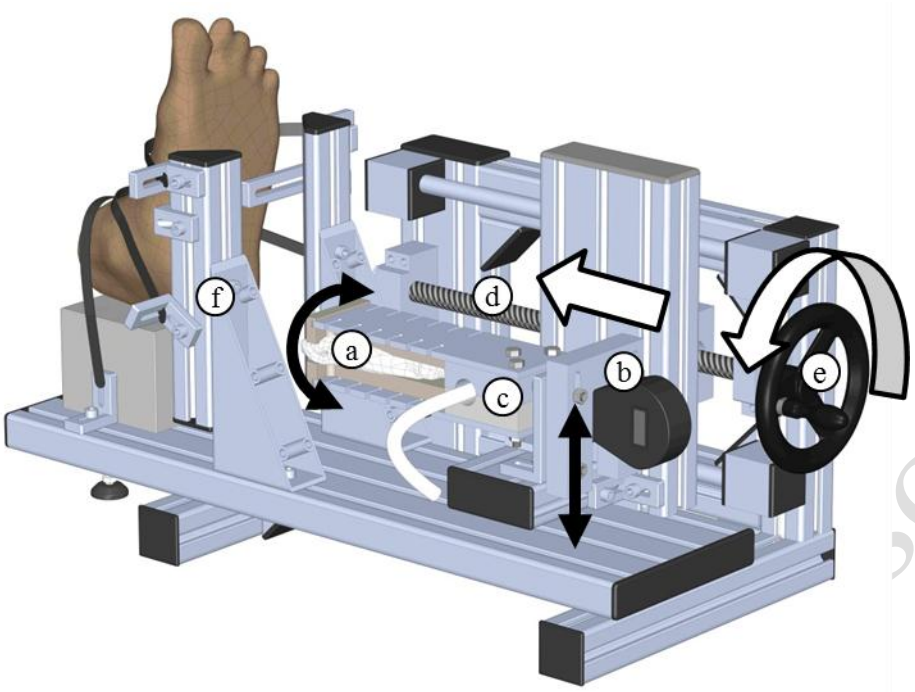
Figure 1: The custom made device used for the in-vivo loading tests. a) Ultrasound probe, b) dynamometer, c) probe holder, d) ball-screw actuator, e) hand wheel and f) foot support.

Figure 2: Representative ultrasound images for the measurement of heel-pad thickness (a) and deformation (a-c) and the results finally recorded after the end of each test (d), namely stiffness and energy absorbed during loading.

Figure 3: Representative force/ normalised-deformation curves for non-diabetic and diabetic tissues. The results of all three load cycles performed for each volunteer are shown.

Figure 4: Scatter-plots for the correlation between Stiffness and FBS (a) Stiffness and Triglycerides (b), and Energy and Triglycerides (c).

443 **Figure 1:**



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455 Figure 2:

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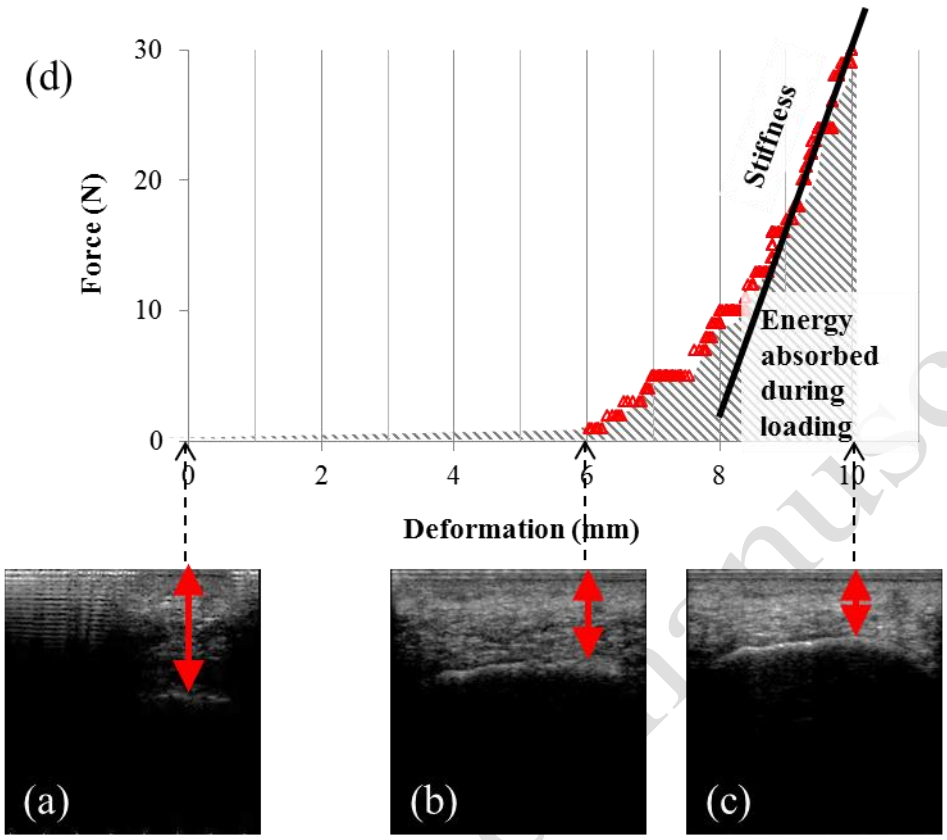

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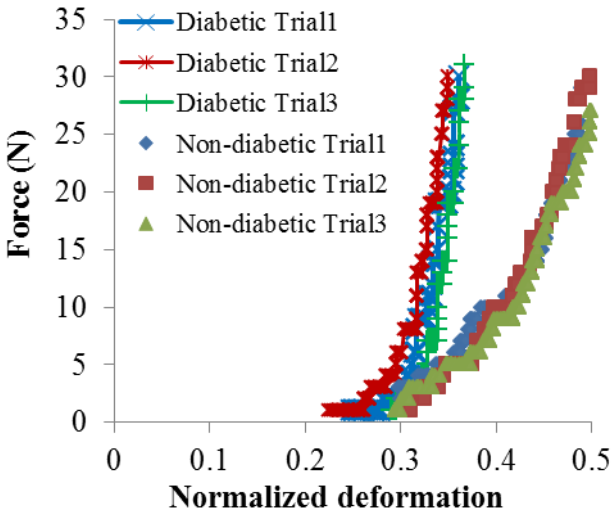
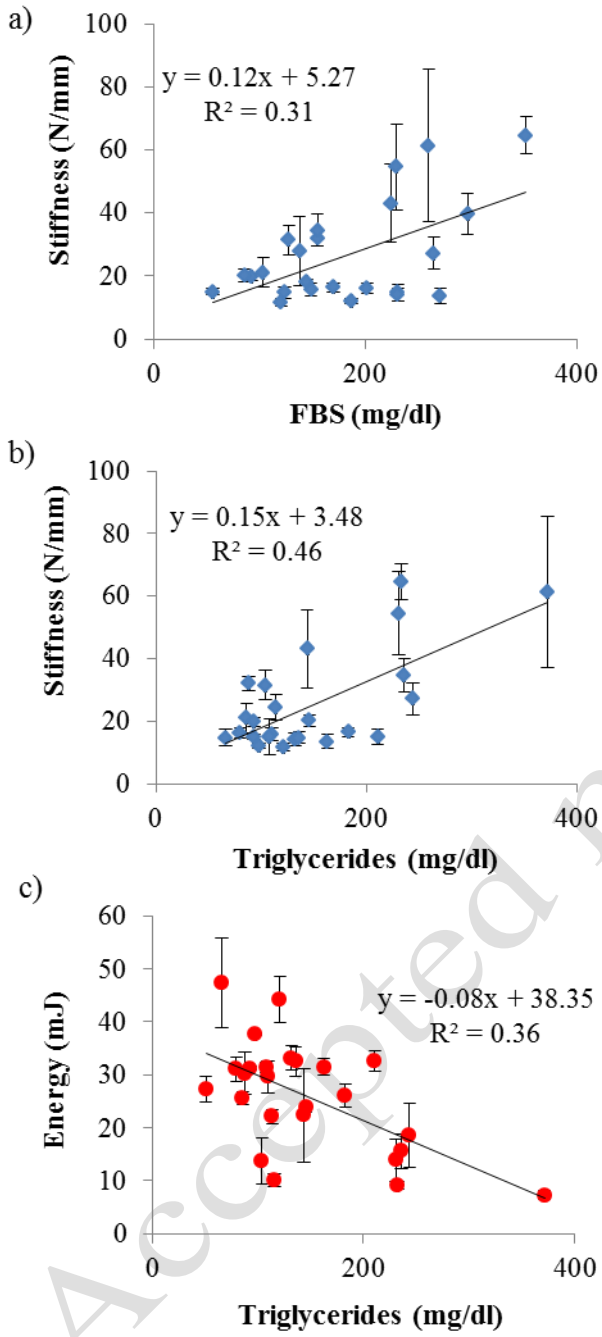
Figure 3:



478 **Figure 4:**



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