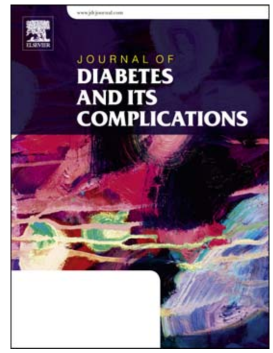
Accepted Manuscript



Differences in the mechanical characteristics of plantar soft tissue between ulcerated and non-ulcerated foot

Roozbeh Naemi, Panagiotis Chatzistergos, Lakshmi Sundar, Nachiappan Chockalingam, Ambadi Ramachandran

PII: S1056-8727(16)30183-0
DOI: doi: 10.1016/j.jdiacomp.2016.06.003
Reference: JDC 6761

To appear in: *Journal of Diabetes and Its Complications*

Received date: 9 April 2016
Revised date: 23 May 2016
Accepted date: 6 June 2016

Please cite this article as: Naemi, R., Chatzistergos, P., Sundar, L., Chockalingam, N. & Ramachandran, A., Differences in the mechanical characteristics of plantar soft tissue between ulcerated and non-ulcerated foot, *Journal of Diabetes and Its Complications* (2016), doi: 10.1016/j.jdiacomp.2016.06.003

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

ACCEPTED MANUSCRIPT

Title: Differences in the mechanical characteristics of plantar soft tissue between ulcerated and non-ulcerated foot

Running title: Ulceration and plantar soft tissue mechanics

Roozbeh Naemi [a*] PhD, Panagiotis Chatzistergos [a] PhD, Lakshmi Sundar [b] MD, Nachiappan Chockalingam [a] PhD, Ambadi Ramachandran [b] MD

a: Faculty of Health Sciences, Staffordshire University, Stoke on Trent, Staffordshire, UK
b: AR Diabetes Hospitals, Chennai, Tamil Nadu, India

*Corresponding Author: Roozbeh Naemi
Science Centre, Leek Road
Stoke on Trent ST4 2DF UK
Tel. +44 (0) 1782 295879
Fax. +44 (0) 01782 294321
Email: r.naemi@staffs.ac.uk

Abstract:

Aims: The purpose of this study was to investigate the differences in mechanical properties of the plantar soft tissue between the ulcerated and non-ulcerated feet in patients with diabetic neuropathy.

Methods: Thirty nine patients who met the inclusion criteria participated in this study. Ten out of 39 participants had an active ulcer at a site other than the plantar heel and the first metatarsal head. Real time ultrasound elastography was performed to measure the soft tissue thickness and stiffness of the heel pad and sub-metatarsal fat pad. To account for the qualitative nature of conventional real time elastography, relative tissue stiffness was assessed against that of a standardised ultrasound standoff material.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Results: The results indicated that the ulcerated group had a significantly lower heel pad relative stiffness (t (37) = 2.559, P = 0.015, η2 = 0.150) in the left foot.

Conclusions: The observed difference in the stiffness of the heel pad between the ulcerated and non-ulcerated feet indicates a possible link between tissue mechanics and ulceration. Further analysis of the data proposed in this study provided a quantitative assessment of plantar fat pad deformability which can contribute to understanding the role of tissue biomechanics in ulceration.

Key words: Biomechanics, Diabetic foot, Ultrasound, Stand-off, Imaging, Elastography, Stiffness

1. Introduction

The plantar tissue located between the foot skeleton and the ground, is an anatomically complex structure that consists of fatty cell components (Buschmann et al., 1995). This interface acts like an efficient shock absorber, with non-linear visco-elastic behaviour that is designed to dampen the effects of impact forces during gait. The mechanical properties of this interface can be altered as a result of changes in the structure of these fatty cells including septa in pathological conditions such as diabetes. For example collagen septa in diabetic heel fat pads are found to be thicker while the adipose cells were found to be smaller compared to the fat pads in non-diabetic people (Buschmann et al., 1995; Kao, Davis, & Hardy, 1999). It is also established that diabetes is associated with an increase of fragmentation that may affect the microscopic and macroscopic composition of the plantar soft tissues (Jahss et al., 1992; Kao et al., 1999). These changes in the mechanical properties of the soft tissue makes the plantar soft tissue more vulnerable to mechanical trauma and

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

tissue damage and ulceration. In a previous study the diabetic fat pad is reported to be less elastic and showed to have caused impaired cushioning effect in distributing pressure (Kao et al., 1999).

To investigate the effect of diabetes on the mechanical behaviour of the plantar soft tissue, a number of in-vivo studies performed comparing age-matched groups of non-diabetic and diabetic volunteers. These studies concluded that the plantar tissue in people with diabetes is thicker (Chao, Zheng, Huang, & Cheing, 2010), stiffer (Chao, Zheng, & Cheing, 2011; Klaesner, Hastings, Zou, Lewis, & Mueller, 2002), harder (Piaggesi et al., 1999) and also tend to have less energy return efficiency (Hsu et al., 2000).

The changes in the mechanical properties of plantar soft tissue in people with diabetes could be due to the histological changes inside the tissues as a result of glycation (Pai & Ledoux, 2010). In support of the later, Chatzistergos et al., (Chatzistergos, Naemi, Sundar, Ramachandran, & Chockalingam, 2014) reported a significantly higher stiffness during loading of heel pad in people with higher levels of Fasting Blood Sugar that is commonly recognised as a risk factor for ulceration.

While the altered mechanical properties of diabetic plantar soft tissue can reduce its capacity to uniformly distribute loads, the repetitive excessive loading that the patient does not recognise as a result of neuropathy can also make changes to the mechanical behaviour of the soft tissue and makes it vulnerable to overloading and trauma (Ulbrecht, Cavanagh, & Caputo, 2004). For example, it has been previously reported that people with heel pain syndrome, which is classically associated to an “overuse injury”, have a stiffer heel pad compared to their healthy counterparts (Rome, Webb, Unsworth, & Haslock, 2001).

This finding indicates that instead of just being a by-product or possibly a contributor to overloading and ulceration, an acute change in the mechanical properties of plantar soft

ACCEPTED MANUSCRIPT

tissues might also be an indicator of deep tissue trauma and an early indicator for ulceration. While mechanical trauma has been recognised as a cause of foot ulceration in diabetic neuropathic patients, there has been no study comparing the mechanical properties of the plantar soft tissue in ulcerated vs non-ulcerated feet. Such a study will directly verify the differences between the mechanical properties of soft tissue in an ulcerated vs non-ulcerated foot, and can provide an insight into the role of changes in the mechanical properties as a result of glycation and excessive loading.

The role of plantar soft tissue biomechanics in the complex and multifactorial process of ulceration is not yet understood and therefore it is ignored for the assessment of tissue viability and ulceration risk. One of the main reasons for this is the lack of established methods for the assessment of plantar soft tissue biomechanics in the clinic.

Real time strain ultrasound elastography is getting popular in the area of musculo-skeletal assessment offering a qualitative assessment of relative deformability of the imaged tissues (Lin, Lin, Chou, Chen, & Wang, 2015). Even though some preliminary studies have demonstrated the use of elastography in differentiating between the deformability of plantar soft tissue of diabetic and non-diabetic patients (Matteoli, Forzoni, Vannetti, Virga, & Corvi, 2015), the capability of the technique to differentiate between the deformability of plantar soft tissue in an ulcerated vs non-ulcerated foot hasn't yet been investigated. The overall aim of this study is to determine if a significant difference exists between the mechanical behaviour of the plantar soft tissue in the ulcerated vs non-ulcerated feet, with an objective of extending the current knowledge in this area and to develop a basis for a clinically relevant protocol to assess the mechanical characteristics of the plantar soft tissue.

ACCEPTED MANUSCRIPT

2. Subjects, Materials and Methods

2.1. Participant Characteristics

Thirty nine patients with diabetic neuropathy volunteered to participate in this study. Ethical approval was sought and granted by the Ethics committee and all volunteers provided full informed consent. Participants were recruited from an outpatient foot clinic at a specialist diabetes hospital in Chennai (India), between 11th and 30th June, 2015. Data were collected in a single session which took approximately 30 minutes per participant. Table 1 shows the demographic characteristics of the participants. Participants had no history of ulceration at the site of interest (i.e. first metatarsal head and the heel). All data were collected with participants laying on the coach in a supine position. Vibration Perception Threshold (VPT) was measured at the hallux, 1st metatarsal head and the heel using a biothesiometer, and participants with VPT scores more than 25 volts in these sites were included in the study. Ten out of 39 volunteers had an active ulcer at a site other than the plantar heel and the first metatarsal head. Six volunteers had ulcers only on the left foot, two volunteer had an ulcer only on the right foot and two volunteers had ulcers on both feet. The sites of ulcers were: Medial and lateral malleolus, hallux, and lateral aspect of the heel for the left foot, and right fifth toe, lateral and posterior aspects of the heel, second metatarsal head. The duration of ulcer was between 15 and 35 days. The ulcerated patients wore a standard half shoe, while the non-ulcerated patients used a standard sandal with a soft Microcellular Polymer flat insole. In addition both the ulcerated and non-ulcerated patients were on oral hypoglycemic agents with some being insulin dependent.

<Table 1 goes here>

ACCEPTED MANUSCRIPT

2.2.Equipment

Real time strain elastography (Esaote S.p.A., IT) was performed using a linear ultrasound probe (LA533, 13 MHz, Footprint: 53x11 mm) and a stand-off (Sonokit, Sonogel, Vertriebs, Gmbh, Sonic velocity 1405 m/s, absorption 0.09 dB/MHz.mm and Reflection: 2.4%) which according to the manufacturer had properties similar to human soft tissue. The interface contact area was 30 x 66 mm and the interface thickness was 11 mm.

2.3.Data Collection Procedure

Cyclic loading of the tissue was performed using a low amplitude pattern of loading and unloading and was adjusted to get the best elastography map as was instructed by the performance indicator in the software user interface. The plantar soft tissue was compressed between the interface and the bony prominences of calcaneus and first metatarsal heads for testing at the rear foot and forefoot regions respectively. According to the protocol suggested by the manufacturer all measurements were performed using images corresponding to the maximum deformation (Esaote, 2014). The regions of interest that is the area under compression between the probe and the bony prominence. Within this region the tissue and standoff zones were identified using two ellipses (Figure 1). The stand-off was used as reference to enable the quantitative comparison of plantar soft tissue deformability between subjects.

<Figure 1 goes here>

2.4.Data Extraction

The proprietary software associated with the ultrasound machine reported the areas of each measurement region (ellipse) together with the relative deformability as the ratio of average

ACCEPTED MANUSCRIPT

deformability in the interface region to the average deformability in the tissue region (Figure 1). Furthermore the area of the ellipse was used to calculate the thickness of the soft tissue and the interface.

The interface was used to enable comparison of strainability (Esaote, 2014) between different trials, with the relative values showing the ratio of strainability in the interface to the strainability in the soft tissue. The values more than unit indicating a tissue that is less deformable relative to the interface material. This relative strainability is referred here after as tissue relative stiffness (E_t). The relative stiffness (E_t) of the plantar soft tissue was measured at the forefoot (sub-first metatarsal head fat pad) and rear-foot (heel pad).

All measurements were carried out on three different images/frames captured at the instant of maximum compression, and were averaged to represent the value for each parameter. The mean standard error of these measurements for all participants were calculated as the average of standard errors (the ratio of standard deviation to average) of the three measurements and are presented in Table 2.

2.5. Pilot Study and Sample Size calculations

Due to the novelty of the method in assessment of mechanical characteristics of the plantar soft tissue, a small scale pilot test was performed to assess the repeatability of the testing protocol and to generate baseline measures to inform sample size calculations. More specifically a test re-test was performed in which the regions of interest were tested twice, the results of which are presented in Table 2 as a measure of repeatability. In addition, the average and standard deviation of the relative stiffness (E_t) of the heel pads of five participants were calculated as 1.29 ± 0.19. This indicated that to detect a 20% difference between the two groups with Alpha=0.05, Power=0.80, and considering the ulcerated vs non-

ACCEPTED MANUSCRIPT

ulcerated patient ratio in the clinic, a minimum sample size of 27 non-ulcerated vs 6 ulcerated participants were required.

2.6.Statistical Analysis

To account for the effect of different confounding variables on the measured relative stiffness and tissue thickness a number of indices were calculated, taking into account the correlations between the measured parameters. The aim of the correlation analyses was to get a deeper insight into the interplay between the measured parameters and inform a normalisation process and to help with interpretation of the results.

An independent samples T-test was used to assess the differences in the plantar soft tissue mechanical parameters between the ulcerated and non-ulcerated feet for the left and right foot separately.

3. Results

Test re-test and the calculation of average standard deviation between the three measurements performed on a frame verified that the testing process is repeatable with low intra-subject variability. Indeed the differences between successive tests were lower than 2.4% for all measured quantities (Table 2). Also measurements were done on three different images/frames captured at the instant of maximum compression, revealed that the average standard error was lower than 11% (Table 2).

< Table 2 goes here>

The mechanical parameters for the rear-foot and forefoot plantar soft tissue are presented in Figure 2 and Figure 3 for the right and left foot respectively together with the results from an independent sample T-test. The results indicated that the ulcerated group had a significantly

ACCEPTED MANUSCRIPT

lower heel pad relative Stiffness (t (37) = 2.559, $P = 0.015$, $\eta 2 = 0.150$) on the left foot (Figure 3), while this value for the right foot was not significantly different between the two groups (Figure 2). Furthermore there was no significant difference in the sub-metatarsal fat pad relative stiffness between the two groups (Figure 2 & 3). There was no significant difference in the thickness of the plantar soft tissue at either sites for either the left or right foot, but a significantly higher interface thickness (t (37) = 2.642, $P = 0.012$, $\eta 2 = 0.159$) was found for tests done on the left heel in the ulcerated vs non-ulcerated group (Figure 3).

Considering that the initial interface thickness is always the same, the difference in deformed interface thickness indicates a difference in applied force under which tissue thickness was measured. To account for the effect of loading magnitude on the thickness of soft tissue, a normalised thickness was calculated as the ratio of soft tissue thickness (T_t) over the thickness of the interface (T_i). However no significant difference was found for the either fat pad underneath the first sub-metatarsal or for the heel pad normalised thicknesses between the two groups (Figure 2) for either left or right feet.

As a significant ($p<0.01$) positive correlation was observed between relative tissue stiffness (E_t) and the tissue thickness (T_t) ($r=0.429$) at the heel, the ratio of the two (E_t/T_t) was calculated to normalise for this effect (Figure 3). No significant differences for this normalised parameter was observed in either of the tested sites between the ulcerated and non-ulcerated groups.

There was also a significant ($p<0.01$) negative correlation observed between the relative stiffness (E_t) and interface thicknesses ($r=-0.578$) which highlights the effect the magnitude of applied force on the measured relative stiffness. To account for this effect another normalised parameter was calculated as the interface thickness multiplied by tissue relative stiffness ($T_i\ .E_t$) (Figure 2, 3) When this normalisation was performed a lower normalised

ACCEPTED MANUSCRIPT

heel pad relative stiffness (t (37) = 2.429, P = 0.020, η2 = 0.137) was observed for the ulcerated group on the left foot (Figure 3), while such difference was not observed for the right foot. Similarly no significant difference in this parameter was found for fat pad underneath the first metatarsal heads on either left or right foot.

To account for the combined effect of tissue and interface thicknesses on the relative stiffness, the parameter ($T_i.E_t/T_t$) was calculated, and a significant difference (t (37) = 2.426, P = 0.031, η2 = 0.120) for the left heel pad, while there was no significant difference observed for this parameter in the right heel pad or in the 1st sub metatarsal pad between the ulcerated and non-ulcerated groups (Figure 2 and 3).

Additionally the VPT Scores for ulcerated feet was found to be significantly higher compared to non-ulcerated ones for both the heel (t (37) = 5.459, P = 0.000, η2 = 0.446) and the sub 1st Metatarsal areas (t (37) = 5.339, P = 0.000, η2 = 0.435) on the right foot. Indeed for the right foot the average (±STDEV) VPT score for ulcerated and non-ulcerated feet was 52.7 V ± 2.8 V and 40.2 V ± 10.6 V in the heel area and 52.72 V± 2.8 V and 39.9 V ± 11.3 V in the sub 1st Metatarsal area.

No significant difference in the VPT scores were observed for the left foot on either the heel or in the sub 1st Metatarsal area, although a trend toward lower VPT score was observed in the ulcerated foot for both tested sites.

<Figure 2 goes here>

ACCEPTED MANUSCRIPT

4. Discussion

4.1.Mechanical Properties

The results clearly show a clear trend towards lower relative stiffness of the heel pad in ulcerated feet compared to those with no ulceration. Although there has been a scarcity of studies investigating the mechanical properties of ulcerated and non-ulcerated feet, previous studies have reported a thicker (Chao et al., 2010), and stiffer (Chao et al., 2011; Klaesner et al., 2002) plantar soft tissue in people with diabetes when compared to their healthy counterparts. Assuming that the mechanical properties of the plantar soft tissue changes towards ulceration, during the course of the disease, one can expect a thicker and stiffer heel pad in the ulcerated foot when compared to the non-ulcerated foot. Interestingly, the results of this study contradicts this expected trend, by indicating that the heel pad in an ulcerated foot is less stiff for the loading range applied within that area.

The observed discrepancy could partly be due to the fact that ultrasound indentation and compression (Chao et al., 2011; Chatzistergos et al., 2014; Erdemir, Viveiros, Ulbrecht, & Cavanagh, 2006; Hsu et al., 2000) which are among the most commonly used techniques measure tissue stiffness for significantly higher loads compared to the forces applied to the tissue during real time elastography. Indeed, in the case of real time elastography, the applied force is minimal indicating that the measured stiffness corresponds to the initial part of the tissues' stress/strain behaviour. While the indentation and compression technique can assess the mechanical behaviour of plantar soft tissue systematically, their use in the clinic is restricted by safety considerations and by logistical requirements. Whereas the ultrasound elastography does not have the limitations mentioned above, making it as a favourable technique to enhance the understanding of the tissue properties.

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Furthermore, it was previously indicated that the increase in the plantar soft tissue hardness was correlated to the increase of the power ratio that is the ratio of high-frequency power to the total power in the power spectrum of plantar pressure during walking (Charanya, Patil, Narayanamurthy, Parivalavan, & Visvanathan, 2004). Charanya and co-workers (Charanya et al., 2004) related this to the possibility of development of plantar ulcers while assuming that the increase in the soft tissue hardness increase the soft tissue stiffness.

The observed relationship was further iterated when the shore hardness to the texture characteristics of the skin was studied using a wavelet transformation on ultrasound images of the plantar soft tissue as a feature that encodes the internal state of the sole of the foot (Puri, Patil, Balasubramanian, & Narayanamurthy, 2005). While a strong correlation between the changes in the internal arrangement of the tissue and skin hardness was reported, the ulcerated group appeared to have a varied relative plantar soft tissue skin hardness (66.7-200%) compared to the non-ulcerated group (Puri et al., 2005).

Narayanamurthy and colleagues concluded that other influential parameters interplay in the relationship between ulceration and the mechanical properties of the soft tissue and tissue hardness cannot independently predict the risk for ulcer in diabetic patients (Narayanamurthy, Poddar, & Periyasamy, 2014).

4.2.Novel assessment technique and its usefulness

Ultrasound elastography in general and real time elastography in particular can potentially offer new insight on the link between planar soft tissue biomechanics and ulceration. Real time elastography is qualitative in nature enabling the assessment of the relative deformability of imaged tissues in single ultrasound image/trial. Hence, as it stands comparison between different images or patients is not possible. To overcome this limitation

ACCEPTED MANUSCRIPT

in this study a stand-off material was used and the relative deformability of plantar soft tissues was calculated against it. Considering that the stand-off stiffness remains constant for the duration of the study this method enabled the comparison of soft tissue stiffness between subjects, through introducing a parameter defined here as relative stiffness (i.e. the ratio of tissue stiffness to standoff stiffness).

The results obtained in the current study indicated a significant difference in the stiffness of the heel pad in the ulcerated vs non-ulcerated foot with strong effect size (0.15± 0.02). From these results one can conclude that the parameters related to the mechanical properties of plantar soft tissue have the potential to be directly used to assess the soft tissue status with regards to its vulnerability to mechanical trauma and ulceration.

While the elastography technique used in this study shows potential for clinically relevant assessment of relative tissue stiffness, the implications of applying different loads on the measured parameters need to be taken into serious consideration. In this study the use of a standoff and the measurement of its thickness under maximum compression enabled the indirect assessment of differences in applied compressive load between subjects. It appears that significantly less force was applied to the heel pad of the ulcerated group (evidenced by the observed higher interface thickness in the ulcerated compared to non-ulcerated group). This could be indirectly related to the operator being more cautious with the ulcerated patients to avoid any possible damage to the soft tissue that may cause further trauma.

In an attempt to minimise the effect of loading on the results tissue thickness and relative stiffness were normalised using the values of standoff thickness. In the case of tissue thickness after normalising for the effect of different loading it was found that the compressed heel pads of ulcerated group were not significantly thinner. On the contrary in the case of relative stiffness, despite the significantly lower relative heel pad stiffness in

ACCEPTED MANUSCRIPT

ulcerated vs non-ulcerated group, when the effect of different applied force was taken into account significant differences were still evident.

Two more normalisation methods were also used to account for the effect of tissue thickness and the combined effect of loading and tissue thickness on relative stiffness. When the heel relative stiffness was normalised to tissue thickness, there was no significant difference observed in the ulcerated vs non-ulcerated group.

At this stage it needs to be emphasised that the measured tissue thickness corresponds to the deformed tissue, hence influenced by the applied load. While the load can be estimated by the interface thickness, a normalised thickness is deemed to be more representative of the tissue thickness. When this normalisation was performed significant differences in the ratio of relative stiffness to normalised thickness at the heel region for the left foot between the ulcerated and non-ulcerated feet was observed.

Furthermore the fact that no significant difference in any of the thickness and stiffness parameters for the first sub first metatarsal fat pad was observed show that some other mechanisms may play role in changing the mechanical properties of the plantar soft tissue in this region compared to the heel. This may also be due to differences in the mechanical properties and histological characteristics between the two regions, but further studies are needed to investigate the observed difference.

It needs to be mentioned that despite the similar trend of differences in stiffness of heel pad that was observed between the ulcerated and non-ulcerated group in the right foot, because of a lower number of ulceration in the right feet the results were not significant for the right foot,. However for the stiffness of fat pad underneath the first metatarsal the trend of difference between the ulcerated and non-ulcerated foot was not consistent between left and right foot for either left or right foot.

ACCEPTED MANUSCRIPT

Furthermore the significantly VPT score observed in ulcerated vs Non-ulcerated right foot and the similar trend (but not significant) for the left foot highlights again the significant role of the lack of sensation play in diabetic foot ulceration.

Also part of the difference in the mechanical properties of the ulcerated vs non-ulcerated foot can be due to the difference in loading, as the patient can load the ulcerated foot differently compared to the non-ulcerated foot. Furthermore this could have been affected by the differences in the footwear between ulcerated and non-ulcerated patients (i.e. half shoe and diabetic sandals). However at this stage it is not possible to determine whether the differences observed between the ulcerated and non-ulcerated foot is the result of loading.

4.3.Relationship to previous studies

On the other hand, the results of the current study is in line with the previous in-vitro observations by Pai and Ledoux (Pai & Ledoux, 2010) in which the modulus of elasticity of the first metatarsal fat pad was reported to be higher compared to the heel pad (1547 kPa vs 846 kPa respectively). While the results of the current in-vivo study cannot be directly compared to what was reported by Pai and Ledoux (Pai & Ledoux, 2010) a similar trend was observed where the heel pad was found to have lower relative stiffness compared to the 1st sub metatarsal fat pad stiffness in the non-ulcerated group (1.304 vs 1.849) (Figure 3).

Furthermore it needs to be emphasised that the elastography technique due to low amplitude quantifies the initial slope of the stress-strain curve, while the in-vivo indentation technique (i.e. Chatzistergos et al., 2014) generates compression over a larger strain threshold. For this reason no direct comparison between the results of this study and those that used indentation can be made.   The results observed within this study are based on elastography assessment of

ACCEPTED MANUSCRIPT

plantar soft tissue in ulcerated foot and there is no other study investigating the mechanical properties of the ulcerated foot in such a way.

4.4.Clinical application

At this stage it is too early to conclude whether the observed differences in the mechanical properties of heel pad is a cause or consequence of the ulceration. Further structured prospective studies with a large populations are required to investigate the reported changes to identify this relationship. This will help to determine whether the differences observed is physiological changes that contribute to ulceration or pathophysiological changes that happen after ulceration as a result of altered loading or other pathophysiological phenomena.

Overall the results of this study and the ultrasound strain elastography modality along with the post-processing technique used in this study can potentially contribute to diagnosing tissue at risk and in predicting the onset of ulceration in diabetic neuropathic patients. Being able to load the imaged tissues in a reproducible way appears to be one of the main obstacles for a quantitative assessment of tissue relative stiffness using real time elastography. A software upgrade that enables tracking the deformation of a stand-off of known mechanical properties and provides visual feedback to the operator to apply optimal amount of force could resolve this issue. This would significantly enhance the clinical relevance and applicability of real time elastography data.

In future and upon further validation using prospective studies the technique used in this study can help in identifying a threshold for plantar soft tissue stiffness and thickness over which ulceration would be imminent. The adoption of such approach can help clinicians identifying the foot at risk of imminent ulceration and in taking appropriate preventive measures like offloading with much more efficient and effective treatment outcome prior to ulceration incident.

ACCEPTED MANUSCRIPT

5. Conclusion

The observed difference in deformability at the left heel between the ulcerated and non-ulcerated feet indicates a possible link between tissue mechanics and ulceration. Ultrasound strain elastography of the plantar soft tissue, when incorporated into the appropriate post processing analyses has the potential to serve as valuable tool for measuring the changes in the stiffness and thickness of the plantar fat pad. This can have major applications in further understanding the role of tissue biomechanics in ulceration.

Acknowledgement: Support from *DiaBSmart* that was funded by the European Commission under Industry Academia Partnerships and Pathways - Nov 2011-Oct 2015 - is acknowledged (FP7-PEOPLE-2011-IAPP, Grant Agreement Number 285985). www.diabsmart.eu

We declare that the funding body had no contribution to the study design; or influence in the collection, analysis and interpretation of data; or in the writing of the report; and in the decision to submit the article for publication. Technical support from Esaote S.p.A is acknowledged.

Author's Contributions: R.N. designed and conceived the study, collected and analysed the data and led the preparation of the manuscript; P.C. contributed to the research design and the preparation of the manuscript; L.S. and A.R contributed to patient recruitment and data collection. N.C. contributed to the overall study design and manuscript preparation.

ACCEPTED MANUSCRIPT

References

Buschmann, W. R., Jahss, M. H., Kummer, F., Desai, P., Gee, R. O., & Ricci, J. L. (1995). Histology and Histomorphometric Analysis of the Normal and Atrophic Heel Fat Pad. *Foot & Ankle International*, *16*(5), 254–258. http://doi.org/10.1177/107110079501600502

Chao, C. Y. L., Zheng, Y.-P., & Cheing, G. L. Y. (2011). Epidermal thickness and biomechanical properties of plantar tissues in diabetic foot. *Ultrasound in Medicine & Biology*, *37*(7), 1029–38. http://doi.org/10.1016/j.ultrasmedbio.2011.04.004

Chao, C. Y. L., Zheng, Y.-P., Huang, Y.-P., & Cheing, G. L.-Y. (2010). Biomechanical properties of the forefoot plantar soft tissue as measured by an optical coherence tomography-based air-jet indentation system and tissue ultrasound palpation system. *Clinical Biomechanics (Bristol, Avon)*, *25*(6), 594–600. http://doi.org/10.1016/j.clinbiomech.2010.03.008

Charanya, G., Patil, K. M., Narayanamurthy, V. B., Parivalavan, R., & Visvanathan, K. (2004). Effect of foot sole hardness, thickness and footwear on foot pressure distribution parameters in diabetic neuropathy. *Proceedings of the Institution of Mechanical Engineers, Part H: Journal of Engineering in Medicine*, *218*(6), 431–443. http://doi.org/10.1243/0954411042632117

Chatzistergos, P. E., Naemi, R., Sundar, L., Ramachandran, A., & Chockalingam, N. (2014). The relationship between the mechanical properties of heel-pad and common clinical measures associated with foot ulcers in patients with diabetes. *Journal of Diabetes and Its Complications*, *28*(4), 488–93. http://doi.org/10.1016/j.jdiacomp.2014.03.011

Erdemir, A., Viveiros, M. L., Ulbrecht, J. S., & Cavanagh, P. R. (2006). An inverse finite-element model of heel-pad indentation. *Journal of Biomechanics*, *39*(7), 1279–86. http://doi.org/10.1016/j.jbiomech.2005.03.007

Esaote. (2014). MyLabTwice and MyLabClass Family user manual- ElaXto OPTION, 359000800.

Hsu, T. C., Wang, C. L., Shau, Y. W., Tang, F. T., Li, K. L., & Chen, C. Y. (2000). Altered heel-pad mechanical properties in patients with Type 2 diabetes mellitus. *Diabetic Medicine : A Journal of the British Diabetic Association*, *17*(12), 854–9. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/11168328

Jahss, M. H., Michelson, J. D., Desai, P., Kaye, R., Kummer, F., Buschman, W., … Reich, S. (1992). Investigations into the fat pads of the sole of the foot: anatomy and histology. *Foot & Ankle*, *13*(5), 233–42.

Kao, P. F., Davis, B. L., & Hardy, P. A. (1999). Characterization of the calcaneal fat pad in diabetic and non-diabetic patients using magnetic resonance imaging. *Magnetic Resonance Imaging*, *17*(6), 851–857. http://doi.org/10.1016/S0730-725X(99)00019-3

Klaesner, J. W., Hastings, M. K., Zou, D., Lewis, C., & Mueller, M. J. (2002). Plantar tissue stiffness in patients with diabetes mellitus and peripheral neuropathy. *Archives of Physical Medicine and Rehabilitation*, *83*(12), 1796–801. http://doi.org/10.1053/apmr.2002.35661

Lin, C.-Y., Lin, C.-C., Chou, Y.-C., Chen, P.-Y., & Wang, C.-L. (2015). Heel Pad Stiffness in Plantar Heel Pain by Shear Wave Elastography. *Ultrasound in Medicine & Biology*, *41*(11), 2890–8. http://doi.org/10.1016/j.ultrasmedbio.2015.07.004

ACCEPTED MANUSCRIPT

Matteoli, S., Forzoni, L., Vannetti, F., Virga, A., & Corvi, A. (2015). Sonoelastography on diabetic heel pads : a feasibility study, *C-1005*, 1–14.

Narayanamurthy, V. B., Poddar, R., & Periyasamy, R. (2014). Biomechanical Properties of the Foot Sole in Diabetic Mellitus Patients: *International Journal of Biomedical and Clinical Engineering*, *3*(1), 1–17. http://doi.org/10.4018/ijbce.2014010101

Pai, S., & Ledoux, W. R. (2010). The compressive mechanical properties of diabetic and non-diabetic plantar soft tissue. *Journal of Biomechanics*, *43*(9), 1754–60. http://doi.org/10.1016/j.jbiomech.2010.02.021

Piaggesi, A., Romanelli, M., Schipani, E., Campi, F., Magliaro, A., Baccetti, F., & Navalesi, R. (1999). Hardness of Plantar Skin in Diabetic Neuropathic Feet. *Journal of Diabetes and Its Complications*, *13*(3), 129–134. http://doi.org/10.1016/S1056-8727(98)00022-1

Puri, M., Patil, K. M., Balasubramanian, V., & Narayanamurthy, V. B. (2005). Texture analysis of foot sole soft tissue images in diabetic neuropathy using wavelet transform. *Medical & Biological Engineering & Computing*, *43*(6), 756–763. http://doi.org/10.1007/BF02430954

Rome, K., Webb, P., Unsworth, A., & Haslock, I. (2001). Heel pad stiffness in runners with plantar heel pain. *Clinical Biomechanics (Bristol, Avon)*, *16*(10), 901–5.

Ulbrecht, J. S., Cavanagh, P. R., & Caputo, G. M. (2004). Foot problems in diabetes: an overview. *Clinical Infectious Diseases : An Official Publication of the Infectious Diseases Society of America*, *39 Suppl 2*(Supplement_2), S73–82. http://doi.org/10.1086/383266

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

	Age (years)	Total number (F/M)	DURATION OF DM (Y)	Height (cm)	Body mass (kg)	BMI (kg/m^2)
Non Ulcerated	57 ± 6	30(5/25)	14 ± 5	170 ± 9	79 ± 14	27.2 ± 4.2
Ulcerated	56 ± 11	9(1/8)	15 ± 7	168 ± 7	76 ± 16	26.8 ± 4.3

Table 1: The demographic characteristics of ulcerated and non-ulcerated group Mean ± Stdev

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

	Intra-subject Variability		Test retest Repeatability	
Error %	**Heel Average (± STDEV)**	**1st Met Average (± STDEV)**	**Heel Average**	**1st Met Average**
Tissue Area	3.13 (± 3.44)	3.39 (± 3.49)	2.05	0.82
Interface Area	1.62 (± 1.19)	1.74 (± 1.09)	2.35	1.07
Tissue Relative Stiffness	6.60 (± 4.41)	10.61 (± 7.27)	2.29	1.96

Table 2: The percentage error calculated based on the three different frames captured at the instant of maximum compression (intra-subject variability) together with the percentage error for test re-test (repeatability)

ACCEPTED MANUSCRIPT

ACCEPTED MANUSCRIPT

Figure 1. The B-mode ultrasound image of heel pad and stand-off under maximum compression (left). The elastography image superimposed over the same B-mode image (right). The ellipses used for measuring relative stiffness and thickness are also shown. In all cases the major axis of ellipse is equal to the width of the image (22.7 mm), with its minor axis adjusted to the thickness of the soft tissue and standoff respectively for zone 1 and 2. The area of each zone as the area of ellipses for zone 1 (A Z1) and Zone 2 (A Z2), together with the relative deformability of Zone 2 (standoff) to Zone 1 (tissue) appear at the left end of the screen (ELX2/1).

Figure 2: Top row: The thickness for tissue (T_t) and standoff (T_i) and normalised thickness (T_t/T_i) for the right foot - heel (left) and forefoot (right). Statistically significant differences ($P<0.05$) are marked with *. Bottom row: The Relative Stiffness (E_t) and three Normalised Stiffness accounting for the effect of stand-off thickness/ loading ($E_t .T_i$), the effect of tissue thickness (E_t/T_t) and the combined effect of the above ($E_t.T_i/T_t$) for the heel (left) and forefoot (right). Statistically significant differences ($P<0.05$) are marked with *.

Figure 3: Top row: The thickness for tissue (Tt) and standoff (Ti) and normalised thickness (Tt/Ti) for the left foot - heel (left) and forefoot (right). Statistically significant differences (P<0.05) are marked with *. Bottom row: The Relative Stiffness (Et) and three Normalised Stiffness accounting for the effect of stand-off thickness/ loading (Et .Ti), the effect of tissue thickness (Et/Tt) and the combined effect of the above (Et.Ti/Tt) for the heel (left) and forefoot (right). Statistically significant differences (P<0.05) are marked with *.

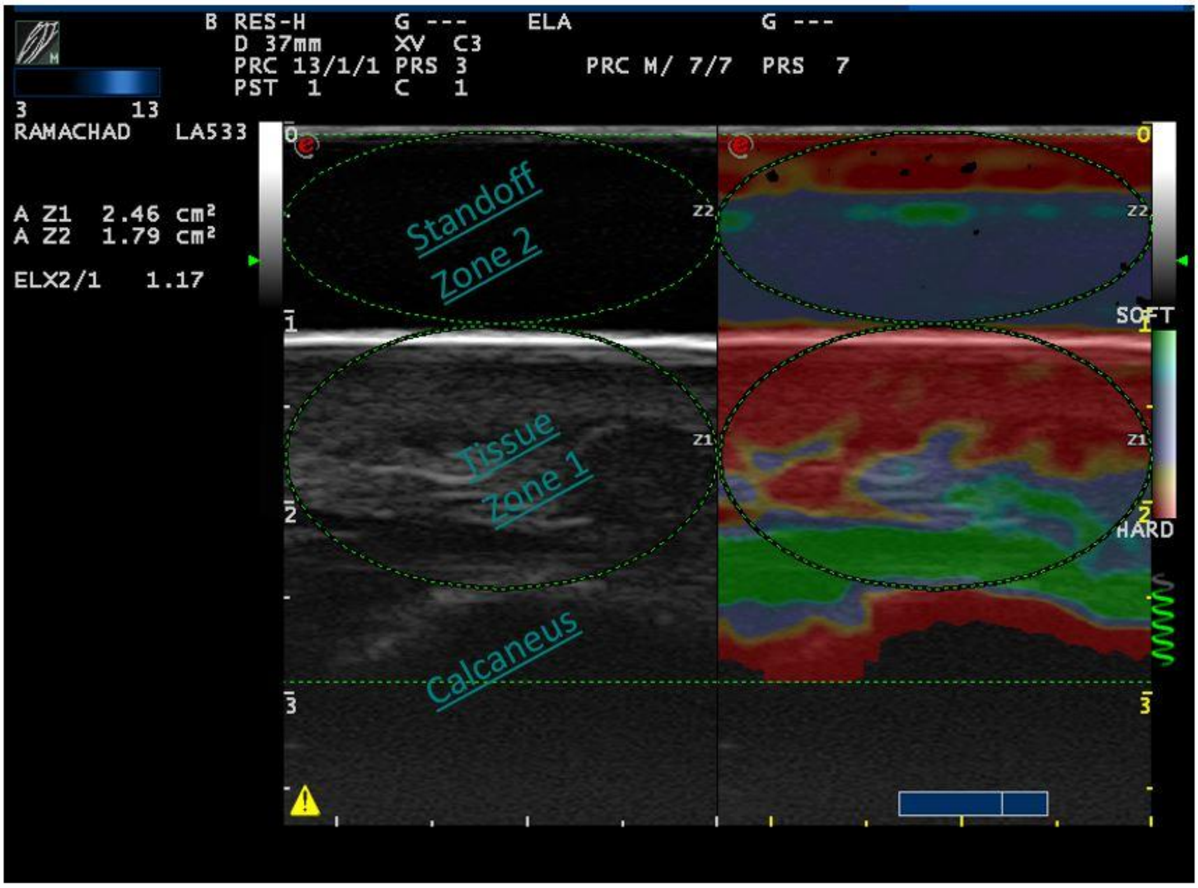

B RES-H
D 37mm
PRC 13/1/1
PST 1
G ---
XV C3
PRS 3
C 1
ELA
PRC M/ 7/7
G ---
PRS 7
3 13
RAMACHAD LA533
A Z1 2.46 cm²
A Z2 1.79 cm²
ELX2/1 1.17
Standoff
Zone 2
Tissue
Zone 1
Calcaneus
Z2
Z1
SOFT
HARD

Figure 1

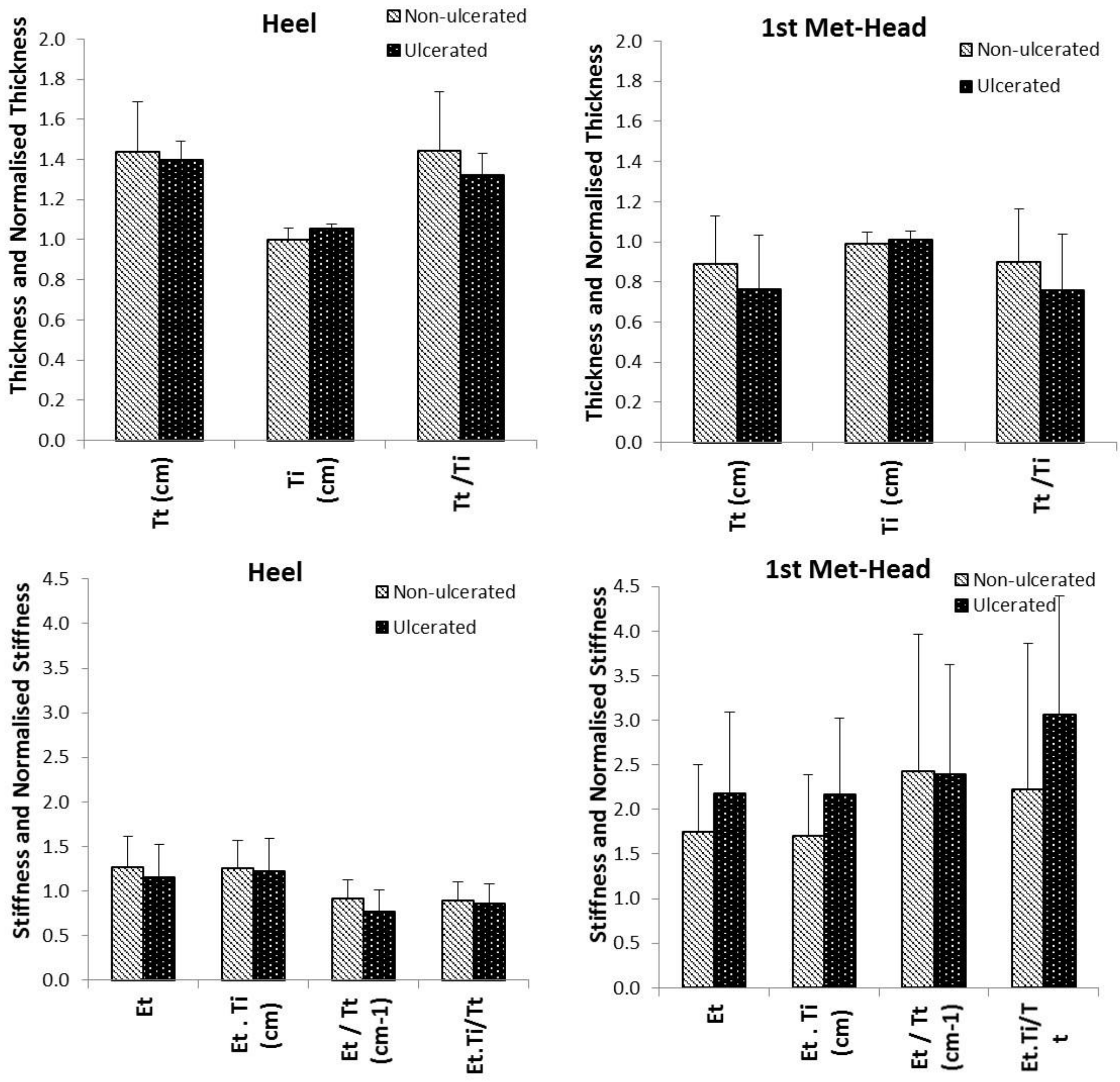

Heel
Non-ulcerated
Ulcerated
Thickness and Normalised Thickness
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Tt (cm)
Ti (cm)
Tt /Ti
1st Met-Head
Non-ulcerated
Ulcerated
Thickness and Normalised Thickness
Tt (cm)
Ti (cm)
Tt /Ti
Heel
Non-ulcerated
Ulcerated
Stiffness and Normalised Stiffness
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Et
Et . Ti (cm)
Et / Tt (cm-1)
Et.Ti/Tt
1st Met-Head
Non-ulcerated
Ulcerated
Stiffness and Normalised Stiffness
Et
Et . Ti (cm)
Et / Tt (cm-1)
Et.Ti/Tt

Figure 2

ACCEPTED MANUSCRIPT

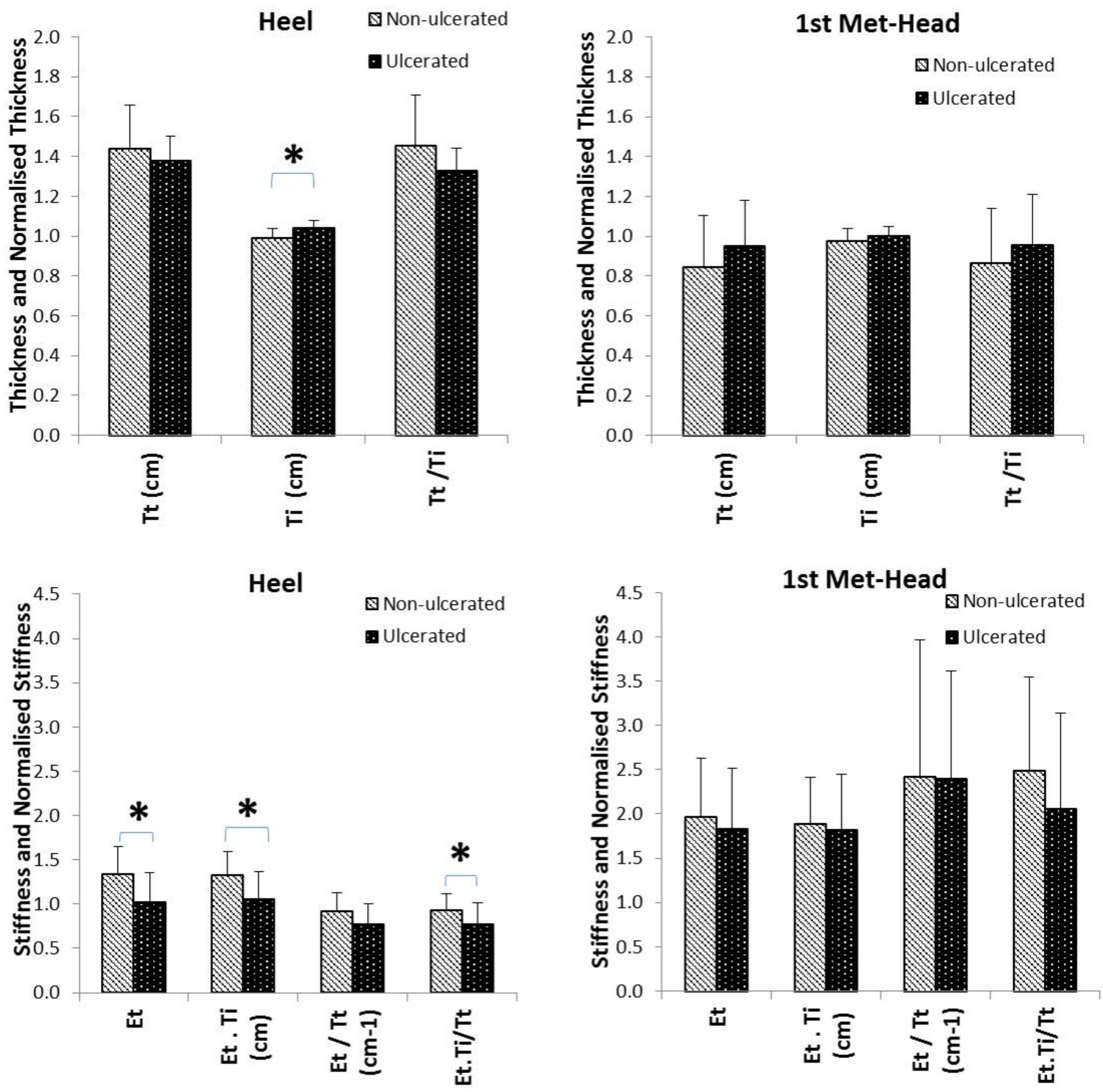


Figure 3