The Use of Medical Hyperspectral Technology to Evaluate Microcirculatory Changes in Diabetic Foot Ulcers and Predict Clinical Outcomes

Received for publication 26 October 2006 and accepted in revised form 8 January 2007.

Short title: Hyperspectral Technology in Diabetic Foot Ulceration

Lalita Khaodhiar, MD,
Thanh Dinh, DPM,
Kevin T Schomacker, PhD,
Svetlana V Panasyuk, PhD,
Jenny E Freeman, MD,
Robert Lew, PhD,
Tiffany Vo,
Alexander A. Panasyuk,
Christina Lima, BA, CCRC,
John M Giurini, DPM,
Thomas E Lyons, DPM,
Aristidis Veves, MD

Joslin-Beth Israel Deaconess Foot Center and Microcirculation Laboratory (LK, TD, TV, CL, JMG, TEL, AV), Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts and HyperMed Inc (KTS, SVP, JEF, AAP) Waltham, MA, Department of Biostatistics, Boston University (RL).

Corresponding author: Aristidis Veves, MD., Microcirculation Lab., Palmer 321A, Beth Israel Deaconess Medical Center, West Campus, One Deaconess Road, Boston, MA, 02215. E-mail: aveves@caregroup.harvard.edu

Acknowledgments. The study was supported in part by a research grant by a National Institutes of Health grant (R41 DK69871) to JF.
ABSTRACT

Background: Foot ulceration (DFU) is a serious complication of diabetes and new techniques that can predict wound healing may prove very helpful. We tested the ability of Medical Hyperspectral Technology (HT), a novel diagnostic scanning technique which can quantify tissue oxy- and deoxyhemoglobin, to predict DFU healing.

Methods: Ten T1DM patients with 21 foot ulcer sites, 13 T1DM without ulcers and 14 non-diabetic controls were seen up to four times over a 6-month period. HT measurements of oxyhemoglobin (HT-Oxy), deoxyhemoglobin (HT-Deoxy) were performed at or near the ulcer area, and on the upper extremity and the lower extremity distant from the ulcer. A HT-Healing Index for each site was calculated from the HT-Oxy and HT-Deoxy values.

Results: Hyperspectral tissue oxygenation measurements observed changes in tissue immediately surrounding the ulcer when comparing ulcers that heal and ulcers that do not heal (p <.001). The sensitivity, specificity, positive predictive and negative predictive value of the HT Index to predicting healing were 93%, 86%, 93% and 86% when evaluated on images taken at the first visit. Changes in HT-Oxy among the three risk group were noted for the metatarsal area of the foot (p <.05) and the palm (p <.01). Changes in HT-Deoxy and HT Healing Index were noted for the palm only (p <.05 and p <.01, respectively).

Conclusions: HT has the capability to identify microvascular abnormalities and tissue oxygenation in the diabetic foot and predict ulcer healing. HT can assist in the management of foot ulceration.
INTRODUCTION

Diabetic foot ulceration (DFU) remains a serious problem as 15% of all diabetic patients are expected to be affected during their life span. The infected and/or ischemic DFU accounts for ~25% of all hospital days among diabetics while foot ulceration precedes 85% of lower extremity amputations. Currently, large multicenter studies have reported that the healing rate of DFU over a 12 to 20-week period lies between 30-60%. Early identification of the patients who will go on to fail to heal an ulcer can be of particular help as it can allow the physician to make the right choice of treatment path between conservative and aggressive. Pathways can be developed to streamline patient care and to apply new, expensive therapies only in patients who need them.

The evaluation of neuropathy, peripheral vascular disease, presence of infection and the depth of the ulcer are standard procedures for the management of DFU. However, none of the above measurements can predict wound healing. The only method that has previously been shown to predict wound healing is the measurement of changes in the ulcer area over a 4-week period of intensive care. However, the positive predictive value of this technique is only 58% while the negative predictive value is 91%. Additionally, using the measurement of change method requires sequential patient examinations and may delay the initiation of appropriate therapy. Therefore, new simple techniques which can provide immediate information with improved accuracy can be of great help in the effective management of DFU.

Medical HyperSpectral Technology (HT) provides a novel diagnostic tool that quantifies tissue oxygenation and presents it in an anatomically relevant map. HT has been shown to detect systemic and local microcirculatory changes associated with diabetes. We have employed HT to evaluate oxygen delivery and oxygen extraction of cutaneous tissue based on pixel by pixel measurements of oxy- and deoxyhemoglobin. In the present study, our main aim was to assess tissue oxygenation around DFU and assess the ability of this technique to predict DFU healing and track the progress of foot ulcers over a relatively long period of six months. A secondary aim was to establish baseline differences between type 1 diabetic patients with ulcers and type 1 diabetic patients and non-diabetic subjects without ulcers.

DESIGN

Patients

The study included three groups: healthy non-diabetic subjects, type 1 diabetic patients with no ulcer and type 1 diabetic patients with at least one foot ulcer at the beginning of the study. The diagnosis of type 1 diabetes was established according to the recommendations of the American Diabetes Association (ADA) Expert Committee. Exclusion criteria included peripheral arterial occlusive disease (PAD) that was severe enough to require surgical bypass operation, heart failure that resulted in lower extremity edema, stroke or transient ischemic attack with residual nerve dysfunction, uncontrolled hypertension, end stage renal disease, any other serious chronic diseases can affect wound healing, treatment with systemic glucocorticoids or antineoplastic
medications and pregnant or lactating women. The study protocol was approved by the Beth Israel Deaconess Medical Center Institutional Review Board. All participants gave written informed consent.

**Procedures**

The type 1 diabetic subjects were seen at baseline, 6 weeks, 3 months and 6 months, while normal subjects were seen only twice, at baseline and 6 months. Clinical evaluation included age, sex, weight, height, body mass index (BMI), history of alcohol consumption, type and duration of diabetes, and presence of other micro- and macrovascular complications. The presence of diabetic peripheral neuropathy was defined according to the principles of the San Antonio Consensus criteria. For this, the Neuropathy Symptom Score (NSS) and the Neuropathy Disability Score (NDS), the Vibration Perception Threshold (VPT), using a biothesiometer (Biomedical Instruments, Newbury, Ohio) and the Cutaneous Pressure Perception Threshold (CPPT), using Semmes-Weinstein monofilaments were determined as previously described. Ulcers were classified into two groups: ulcers that healed or ulcers that did not heal. Ulcers with complete reepithelialization and no exudates at the fourth visit (6-months) were considered healed. The healing status of ulcers from subjects that failed to return for the fourth visits was determined from a phone interview by a surgical physician placed at the end of the study. Due to the small size of this study, this criterion was chosen to minimize the loss of subjects due to a failure to complete the study. Patients received regular care by their physicians and were selected from a large number of practices treating T1DM patients. No criteria for wound size or duration were used to select patients. Physicians caring for the diabetic subjects were blinded as to the data and measurements collected in the study.

**HT Evaluation of Oxy- and Deoxy-Hemoglobin**

Data were collected with a HyperMed CombiVu-R System (HyperMed, Inc., Waltham, MA) as previously described. In brief, Medical HT is a method of “scanning spectroscopy” based on local chemical composition. HT employs a spectral separator to vary the wavelength of light admitted to a digital detector to provide a spectrum for each pixel – a hyperspectral scan. Tissue spectra are compared to standard spectra for oxyhemoglobin and deoxyhemoglobin and tissue oxyhemoglobin (HT-Oxy) and tissue deoxyhemoglobin (HT-Deoxy) determined for each pixel. HT-Oxy and HT-Deoxy units represent values for oxyhemoglobin and deoxyhemoglobin found in the tissue volume measured by HTcOM. For this study, a 30 second tissue scan was obtained at a 12-inch focal distance and ratioed to a calibration scan obtained using a calibrator (Check Pad, HyperMed, Inc.). The spatial resolution of the HT images was 60 microns. A picture of the HT system used in the study is provided in Figure 1.

Subjects were studied in a standard reclining chair. HT scans were obtained from the plantar aspect of feet, the palm and the area around the ulcer if present. Data were analyzed off line using spectral decomposition, two dimensional scan processing, and scan registration techniques. Mean HT-Oxy and HT-Deoxy values were obtained from a one inch diameter circle placed at the central region of the scans of the palm and soles. The images were taken from regions that are
relatively flat and the distance between the camera and tissue surface was also fixed to minimize variability in the measurements. Additionally, all foot ulcer scans were overlaid with a pattern of 25 concentric rings spaced by 1 mm and divided into 8 (45 degree) pie segments thereby forming 200 sectors per ulcer region. The positioning of the pattern was determined in each case on the first subject visit and then replaced in a similar anatomic position in subsequent visits. The center of the concentric ring was placed at the center of the ulcer for ulcers smaller than 25 mm in any direction (Figure 2). For one very large ulcer (> 18 square centimeters at maximum), the center of the circular pattern was also placed at 4 equally spaced points along the quadrants of the ulcer border. This led to the analysis of 21 ulcer sites describing 17 ulcers.

HT-Oxy and HT-Deoxy values were obtained for each pixel in the scan. The ulcer margins were outlined from color images prior to HT analysis. Mean HT-Oxy and HT-Deoxy values were obtained from each of the sectors covering intact skin outside the ulcer. Hemoglobin oxygen saturation (HT-Sat) values were calculated from HT-Oxy and HT-Deoxy values as previously described. A HT Healing Index was also calculated to provide a single quantitative measurement to use in the prediction of ulcer healing. Mathematically, the HT Healing Index is a simple algorithm defined as the distance between the point defined by HT-Oxy and HT-Deoxy and a discriminant line that best separated healing and nonhealing ulcers. HT-Oxy, HT-Deoxy and HT Healing Index are all reported in HT units. Colorized scans were created to demonstrate tissue oxygenation. HT-Oxy levels are associated with different colors and HT-Deoxy with different levels of brightness (intensity) as depicted on the color bar provided along side each image.

Healing for the ulcers was determined at the fourth (6 month) visit and ulcers were considered healed if the wound was completely re-epithelialized. Healing for 3 ulcers was determined from a phone interview due to a missed fourth visit (2 healed, 1 did not heal).

Laser Doppler Blood Flow Measurements (LD)

Endothelium-dependent vasodilatation in the cutaneous microcirculation was measured by laser Doppler flowmetry. The measurement was performed on the dorsum of both feet and volar aspect of the forearm as previously described. Laser Doppler images at each site were obtained with a Laser Doppler Perfusion Imager (Lisca PIM 2.0, Lisca Development AB, Linkoping, Sweden).

Transcutaneous Oxygenation Monitor Measurements (TCOM)

Transcutaneous oxygen pressures were measured on the dorsum of the left and right foot using a transcutaneous oxygen monitoring system (model PF5040, PeriMed, Inc.). Oxygen partial pressures measured in mmHg were recorded from the sites 20 minutes after attaching the probes onto the skin and equilibrium was established.

Statistical analysis

The statistical analysis was performed by a professional biostatistician (RL). All multivariate analyses were carried out using analysis of variance (ANOVA) and standard extensions of ANOVA such as mixed effects ANOVA. In these analyses the continuous outcomes were biometric
indices: HT-Oxy, HT-Deoxy, HT-Sat, HT Healing Index, TCOM, LD, ABI, NSS, and NDS. The independent variable of primary interest was the level of risk: no diabetes, T1DM without ulcers, T1DM with ulcers, or a subset of these categories such as healing versus non-healing ulcer. Covariates included body site and clinic visit. The compound variable subject*ulcer_segment was the random effect used in the mixed effects models of HT measurements at each ulcer site. This conservative method accounted for the large of number of repeated measures made at each ulcer site.

Analyses that report on pairwise differences used the standard t-tests obtained after an ANOVA that set the standard error equal to the root of the mean squared error. We distinguish between the raw unadjusted mean values and the ANOVA-balanced 'least square means' obtained after fitting an ANOVA model.

The discrimination analysis was obtained with linear discriminant analysis. The HT healing index is defined as the distance between each HT measurement point (HT-Oxy, HT-Deoxy) and the discriminant boundary that separates the healing ulcer sites from the non-healing ulcer sites.

RESULTS

The study included 10 T1DM patients with foot ulceration, 13 T1DM without ulcers and 14 non-diabetic controls. All groups were matched for age and gender. Details about the subjects' characteristics are given in Table 1. Based on the TCOM and ABI baseline measurements, all patients with ulcers had typical neuropathic ulcers in the absence of peripheral arterial disease.

Comparison between healing and non-healing ulcers and a symmetrical area of intact skin at the contralateral foot

For this analysis, we compared measurements from hyperspectral scans taken from intact tissue immediately surrounding the ulcer using a radial circle grid. The group estimates, which were based on all visits, were evaluated and are shown in Table 2.

There were 21 ulcer sites studied in 10 diabetic patients. Twelve ulcer sites were located on the plantar foot surface, six were located on the dorsal area of the foot and three were located around the ankle. Initial ulcer size range from 0.1 cm$^2$ to 6.5 cm$^2$. Initial size was not associated with non-healing ulcers. Seven ulcer sites from 3 subjects failed to heal and data from 27 visits were included for the analysis. Fourteen ulcer sites from 9 subjects completely healed and data from 39 visits were included in the analysis. Three sites: feet with healing ulcers, feet with non-healing ulcers, and contralateral non-ulcer feet, were treated as separate groups, in part because one subject has an ulcer on one foot that healed and an ulcer on the other foot that did not heal. As a result, the analysis was done at the foot level and not the subject level. All HT measured values were significantly different among all three groups (p <.0001). HT-Oxy measurements were lowest in the skin area around the ulcers that did not heal when compared to those that did heal (p <.01) and to measurements from the contralateral limb (p <.0001). There were also differences noted between skin around ulcers that did heal and the contralateral limb (p <.01). The results of HT-Deoxy measurements and the HT Healing Index were similar to the HT-Oxy results and the same differences existed among all three groups, being lowest in the skin area around the
ulcers that did not heal when compared to those that did heal and the intact skin of the contralateral limb. No differences were observed between any groups using measurements of the resting skin blood flow obtained by laser Doppler flowmetry or measurements of the skin oxygenation levels obtained by TCOM.

Scans and numerical data from a subject with one ulcer on each foot, one of which healed and one of which extended and led to amputation are provided in Figure 3.

Identifying healing ulcers with hyperspectral tissue oxygenation measurements

The HT Healing Index was determined at 21 ulcer sites in tissue immediately surrounding the ulcer (Figure 4). Ulcer sites that had healed at 6 months (n=14) were compared to ulcer sites that did not heal (n=7). HT Healing Index data collected at the first visit are reported in Figure 4A. The HT Healing Index was determined to best separate healing from non-healing ulcers. Sites having a positive HT Healing Index were predicted to heal while sites with a negative HT Healing Index were predicted to not heal. The sensitivity, specificity, positive predictive and negative predictive value of the HT Healing Index to predicting healing were 93% (95% CI: 66-100%), 86% (95% CI: 42-100%), 93% (95% CI: 66-100%) and 86% (95% CI: 42-100%), respectively. In Figure 4B, the same HT Healing Index was collected from ulcer sites at the first, second and third visits. The sensitivity, specificity, positive predictive and negative predictive value for healing were 86% (95% CI: 70-95%), 86% (95% CI: 64-97%), 91% (95% CI: 76-98%) and 78% (95% CI: 56-93%) when evaluated on data from the first three visits. The reported values for PPV and NPV are reasonable approximation for this small population because the prevalence of ulcers that heal is close to 50%, which is the assumption made when using the equations to calculate these values. In all cases healing was defined at the end of the 6 month study.

Comparison of HT measurements among the three groups of subjects.

We measured HT-Oxy and HT-Deoxy and calculated the HT Healing Index at ulcer free locations on the upper and lower extremities (mid-palm and metatarsal foot sole). Both of these tissue regions are covered by glabrous skin that is rich in arteriovenous anastomosis and allows extensive a-v shunting. Glabrous skin in general has higher levels of oxygenation and greater reactivity than skin from other parts of the body.

Changes in HT-Oxy among the three risk groups were noted for the metatarsal area of the foot (p <.05) and the palm (p <.01). Differences among the three risk groups were also noted at the palm site for HT-Deoxy (p <.05) and HT Index (p <.01). Pairwise differences are presented in Figure 5.

DISCUSSION

In the present study we have shown that tissue oxygenation measurements over a 6-month period, using Medical Hyperspectral Technology (HT), a novel technique, can satisfactorily identify ulcers that progress to complete healing and ulcers that fail to heal. This was achieved by performing HT measurements in skin tissue immediately surrounding ulcers. Furthermore, differences were also observed in HT measurements from healthy, non-ulcerated skin tissue at the
contralateral limb and measurements at skin tissue surrounding healing or non-healing ulcers.

Wounded tissue has a greater oxygen demand during the healing process and this requires greater oxygen extraction and greater oxygen delivery. HT measurements of the area around a wound or ulcer provide an indication of oxygen delivery by providing a measurement of oxyhemoglobin (HT-Oxy) and an indication of oxygen extraction by providing a measurement of deoxyhemoglobin (HT-Deoxy). As expected, in the present study, there are increased levels of both HT-Oxy and HT-Deoxy in wounds that heal versus those that do not. Whether the primary culprit is macrovascular disease or microvascular disease, tissue with vasculature that is incapable of supplying sufficient blood and oxygen simply will not heal. In addition to the HT-Oxy and HT-Deoxy measurements, an HT Healing Index has been developed to incorporate information derived from these two measurements to provide a single number for easy clinical use. In general if the HT Healing Index is greater than zero, it is likely that a given wound or ulcer will heal. If the HT Healing Index it is below zero, it is likely that the ulcer will not heal.

As mentioned in the introduction, the best previously available technique to predict healing in DFU is the assessment of ulcer size reduction over a 4-week period. Of note, this technique is characterized by a strong negative predictive value, therefore identifying the ulcers that are not going to heal, but has only a moderate positive predictive value. In contrast, HT measurements were shown to have high both positive and negative predictive values. Importantly, these highly sensitive and highly specific HT values were based on data collected at a single visit. Thus, in our first analysis, the HT data collected at the first visit led to predictions of healing at 6 months. Furthermore, the technique held up, with similarly high predictive values, if the results of the first three visits were analyzed.

These findings may have important clinical implications as they suggest that one HT measurement at the first visit of the patient or HT measurements every few months in slowly healing or non-healing ulcers can assist the physician with diagnosis, choice of therapy and therapeutic monitoring. At the initial visit, HT measurements may improve diagnosis and guide the physician toward earlier aggressive evaluation and therapy. Sequential HT measurements may then lead the physician to tailor and better individualize a patient’s therapeutic regimen to follow a conservative or a more aggressive path. Throughout the treatment process, quantitative HT measurements may provide the physician with better information with which to decide whether intensive treatment with expensive new therapeutic modalities is required. HT also may provide a therapeutic monitoring tool to help the physician better evaluate the response to such treatment.

The intent of the present study was to perform an observational study that tracks the association between HT-measurements and wound healing. It was not designed to test the efficacy of a particular treatment of the wound. Patients received regular care by their physicians and were selected from a large number of practices treating T1DM patients. No criteria for wound size or duration were used to select patients. Although these choices may introduce other confounding factors, these factors may affect the progress of wound healing but by no means affect the association
between wound healing and HT measurements.

Previous studies in our unit have also shown that tissue oxygenation, assessed with HT at the forearm and the dorsum of the foot, is reduced in the skin of diabetic patients and that this impairment is accentuated in the presence of neuropathy at the foot level. In the present study, we compared differences in non-ulcerated tissue in the lower and upper extremities. For our HT measurements, we examined the plantar metatarsal area of the foot and the palm that are covered by glabrous skin that is rich in arteriovenous anastomosis and has increased blood flow. We chose these areas because foot ulcers mainly occur in this type of skin and because previous studies have indicated different mechanisms of vasodilation in this area when compared to hairy skin that has considerably less arteriovenous anastomosis. The observed results in glabrous skin areas were similar to HT results we previously observed at the dorsum of the foot and the forearm, areas of hairy skin. Therefore, these results suggest that HT measurements are useful to identify differences in both glabrous and hairy skin. The fact that the differences were more pronounced at the palm, an area with high blood flow further support the fundamental principle that HT is measuring tissue oxy- and deoxyhemoglobin. The current data support previous findings that HT can demonstrate local, regional and systemic changes associated with progression of diabetes.

Further to the above, we also believe that the small size of the three compared groups was the main reason for the lack of statistical significance in all of the comparisons among all three subject groups. However, it should be noted that the measurement of HT tissue oxygenation in these areas in the present study was mainly undertaken to obtain baseline measurements in glabrous areas of the two extremities and to show that these measurements are parallel to those observed in the hairy skin. We did not intend to replicate the results of a previous study in larger numbers of subjects. This is the main reason for the lack of statistical power to identify differences in every measured area among all three studied groups. Despite this, we believe that the observed results allow us to satisfactorily address our hypotheses. Clearly, there were statistically significant differences in the upper extremity measurements between subjects with ulcers and nondiabetic subjects and differences between subjects with ulcers and controls. The current data supports previous findings that upper extremity HT measurements are altered in diabetes and further altered in subjects with more advanced disease as manifest by complications.

As we have seen before, upper extremity HT measurements can be seen to be reflective of the systemic microvasculature, since the upper extremity is traditionally not differentially affected by diabetic microvascular or macrovascular disease to the extent of the lower extremities (14). Measurements at the non-ulcerated foot sole surfaces, in turn, provide regional information, that can be considered indicative of the combination of both microvascular and macrovascular changes associated with atherosclerotic disease in large vessels exacerbated by diabetes. Most importantly here, the HT technique can be used to investigate the area around an ulcer, which is subjected to a combination of local, regional and systemic pathophysiology. The capability of a wound to heal is clearly influenced by all of these factors, and the
anatomically relevant HT measurement at the wound site, demonstrates the end effect of multiple factors on the wounded area.

The present study has its limitations. One limitation is that it included a relatively small number of subjects and, when present, multiple ulcers from the same subjects, or ulcer sites were entered in the analysis. The main reason for this is that this study was designed to provide proof of concept that HT can be used to predict DFU healing. The fact that we were able to obtain statistically relevant data from this relatively small sample size speaks to the power of the technique and its clinical relevance. Another factor to be considered is the limitation of the study to patients with exclusively type 1 diabetes. This decision was due to the fact that the work was supported by an NIH-NIDDK award which had been earmarked for research in type 1 diabetes. However, given the current consensus that the pathogenesis, natural history and healing rates of DFU are similar in both type 1 and 2 diabetes, we believe that the exclusive evaluation of type 1 diabetic patients does not affect the applicability of the results for the whole diabetic population with DFU (6). Further studies to provide data on larger numbers of patients and also to include patients with type 2 diabetes are underway.

In summary, in the present study we have tested the ability of Medical Hyperspectral Technology to predict diabetic foot ulcer (DFU) healing and track the progress of foot ulcers over a relatively long period of six months. Our results provide proof of concept that the technique can satisfactorily predict ulcer healing and has the capability to assist in the management of DFU. Use of HT to improve diagnosis can lead to implementation of early interventions and have important effects on clinical management of the diabetic foot.
References

7. Sheehan P, Jones P, Caselli A, Giurini JM, Veves A. Percent change in wound area of diabetic foot ulcers over a 4-week period is a robust predictor of complete healing in a 12-week prospective trial. *Diabetes Care* 2003;26:1879-82.
Table 1: Demographics and clinical characteristics of participants.

<table>
<thead>
<tr>
<th></th>
<th>T1DM with Foot Ulcer (DFU)</th>
<th>T1DM without Foot Ulcer (T1DM)</th>
<th>Control Group (C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total subjects</td>
<td>10</td>
<td>13</td>
<td>14</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51 (38-64)</td>
<td>48 (24-68)</td>
<td>41 (23-70)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6 / 4</td>
<td>8 / 5</td>
<td>6 / 8</td>
</tr>
<tr>
<td>Body mass index</td>
<td>29 ± 7</td>
<td>28 ± 4</td>
<td>28 ± 7</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>31 ± 12</td>
<td>26 ± 13</td>
<td>---</td>
</tr>
<tr>
<td>Systolic BP (Arm, mmHg) *</td>
<td>133 ± 20</td>
<td>124 ± 16</td>
<td>113 ± 9</td>
</tr>
<tr>
<td>Diastolic BP (Arm, mmHg)</td>
<td>76 ± 8</td>
<td>76 ± 10</td>
<td>72 ± 7</td>
</tr>
<tr>
<td>Ankle Brachial Index</td>
<td>1.14 ± 0.19</td>
<td>1.14 ± 0.16</td>
<td>1.24 ± 0.14</td>
</tr>
<tr>
<td>TCOM (mmHg)</td>
<td>46 ± 16</td>
<td>54 ± 11</td>
<td>52 ± 18</td>
</tr>
<tr>
<td>Laser Doppler (Arb. Units of Flux)</td>
<td>116 ± 18</td>
<td>112 ± 22</td>
<td>120 ± 50</td>
</tr>
<tr>
<td>Neuropathy Symptom Score *</td>
<td>5 ± 3</td>
<td>4 ± 3</td>
<td>1 ± 1</td>
</tr>
<tr>
<td>Neuropathy Disability Score §</td>
<td>15 ± 8</td>
<td>3 ± 3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Vibration Perception Threshold (Volts) §</td>
<td>44 ± 10</td>
<td>18 ± 10</td>
<td>10 ± 7</td>
</tr>
<tr>
<td>Semmes-Weinstein Filaments (marking number) §</td>
<td>6.2 ± 0.9</td>
<td>4.4 ± 0.8</td>
<td>3.6 ± 0.7</td>
</tr>
</tbody>
</table>

Mean ± SD, *: DFU, T1DM vs C, p <.05, §: DFU vs T1DM vs C, p <.05.
Table 2: Assessment of tissue oxygenation and perfusion by multiple techniques comparing ulcers that heal, ulcers that do not heal, and the contralateral site without an ulcer. Values represent group estimates (±SEM) and (n/N) refer to number of measurement sites and number of measurement site visits.

<table>
<thead>
<tr>
<th>Variable</th>
<th>T1DM with non-Healing Ulcers (7/27)*</th>
<th>T1DM with Healed Ulcers (14/39)</th>
<th>T1DM Ulcers Foot (7/22)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT-Oxy (arb units)</td>
<td>38 ± 2</td>
<td>50 ± 3</td>
<td>62 ± 4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HT-Deoxy (arb units)</td>
<td>26 ± 3</td>
<td>49 ± 2</td>
<td>71 ± 4</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>HT Index (arb units)</td>
<td>-8.5 ± 2.9</td>
<td>16 ± 3</td>
<td>38 ± 5</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>TCOM (mmHg)</td>
<td>58 ± 4</td>
<td>53 ± 3</td>
<td>48 ± 3</td>
<td>0.2021</td>
</tr>
<tr>
<td>Laser Doppler (arb flux units)</td>
<td>122 ± 10</td>
<td>112 ± 6</td>
<td>113 ± 7</td>
<td>0.6706</td>
</tr>
<tr>
<td>ABI</td>
<td>1.2 ± 0.05</td>
<td>1.1 ± 0.03</td>
<td>1.2 ± 0.03</td>
<td>0.2132</td>
</tr>
</tbody>
</table>
Figure 3
Figure 4
Figure 5