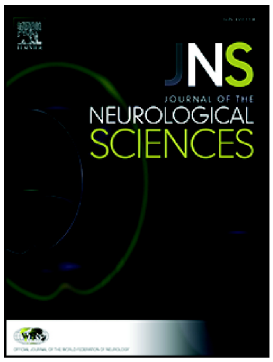

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In-Vivo Reflectance Confocal Microscopy of Meissner's Corpuscles in Diabetic Distal Symmetric Polyneuropathy

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Key Words: reflectance confocal microscopy, Meissner's corpuscle, diabetes, peripheral neuropathy, epidermal nerve fiber density

ABSTRACT

Objective: To evaluate in-vivo reflectance confocal microscopy (RCM) of Meissner's corpuscles (MC) in diabetic distal symmetric polyneuropathy (DSP).

Methods: Forty-three adults with diabetes and 21 control subjects underwent RCM of MC density at the fingertip of digit V, thenar eminence (TE), and arch of the foot, ankle skin biopsy

for epidermal nerve fiber density (ENFD), electrophysiological studies, monofilament threshold testing, and timed vibration at the toe. Subjects with diabetes were subdivided into groups with and without clinical DSP using the American Academy of Neurology (AAN) case definition and neuropathy outcomes were compared across groups.

Results: Both diabetic groups (with and without AAN clinical DSP criteria) had objective evidence of peripheral sensory involvement using conventional sensory measures, although those with clinical DSP criteria had greater abnormalities. MC densities were lower in the entire diabetic group at the TE and digit V relative to controls. MC densities at all imaging sites were associated with corresponding conventional sensory measures. MC densities were reduced in subjects without AAN clinical DSP criteria at the TE and digit V compared to controls whereas conventional upper limb sensory measures did not differ between these groups.

Conclusions: In-vivo RCM of MC density at digit V is a non-invasive, painless, objective marker in diabetes that offers a window into early large fiber sensory nerve terminal loss. Further studies are needed to determine whether RCM of MCs can identify quantitative changes in DSP associated with disease progression or treatment.

GLOSSARY

AAN = American Academy of Neurology, **AMP** = Amplitude, **ANCOVA** = analysis of covariance, **CMAP** = compound motor action potential, **CMT** = Charcot-Marie-Tooth, **CTSA** = Clinical and Translational Science Award, **CV** = conduction velocity, **ENFD** = epidermal nerve fiber density, **ICC** = intraclass correlation coefficient, **JDRF** = Juvenile Diabetes Research

Foundation, **MC** = Meissner's corpuscle, **MDNS** = Michigan Diabetic Neuropathy Score, **MF** = monofilament, **NAP** = nerve action potential, **NCS** = nerve conduction studies, **RCM** = reflectance confocal microscopy, **SNAP** = sensory nerve action potential, **TE** = thenar eminence

INTRODUCTION

Distal symmetric polyneuropathy (DSP) is a common complication of diabetes mellitus, leading to impairment in quality of life and morbidity including limb loss.[1, 2] Despite numerous clinical trials, intensive glycemic control remains the only proven disease-modifying therapy for diabetic DSP.[3-5] Possible reasons for this include a lack of effective compounds, enrollment of subjects with advanced DSP in clinical trials, and suboptimal measures of DSP.[6-9]

Various clinical scales have been used as DSP trial endpoints; however, significant placebo effects have been observed despite progression on nerve conduction studies (NCS).[6-9] Nerve conduction studies yield validated measures of DSP progression, but are not sensitive to changes in peripheral sensory nerve terminals that may occur early in the course of DSP.[6-9] Epidermal nerve fiber density (ENFD) on skin biopsy is a sensitive, validated DSP measure, but only provides a window into one component of DSP (small fibers).[10-12] Additional non-invasive and objective measures of DSP are needed, both clinically and for therapeutic trials.[9]

Meissner's corpuscles are the main touch-pressure sensory receptor in glabrous skin (hands, feet).[13, 14] Glabrous skin biopsy studies have identified MC density (MC/mm^2) at the fingertip as a sensitive measure of diabetic DSP.[11, 13-17] However, MC density has not been widely used as a DSP marker, as glabrous skin biopsies are invasive, and not suitable for serial monitoring.

In-vivo reflectance confocal microscopy (RCM) of skin is a rapid, non-invasive way to quantify MC density and appears to be a sensitive objective measure of HIV associated DSP and Charcot-Marie-Tooth (CMT) neuropathy, at a time when sensory NCS are less informative.[18-21] However, the role of RCM of MC density as an objective measure of diabetic DSP has not been established.

This study aimed to: 1) compare MC density by RCM, and standard peripheral neuropathy measures, between healthy control subjects and patients with diabetes, with and without clinical DSP; and 2) determine the relationships between MC density by RCM and standard peripheral neuropathy measures.

METHODS

Subjects

Twenty-one healthy control subjects and 43 subjects with known diabetes mellitus were recruited to participate in a cross sectional study. All subjects provided written informed consent under a Research Subjects Review Board approved protocol. Subjects with diabetes were recruited through two parallel but separately funded protocols, one through the Juvenile Diabetes Research Foundation (JDRF) and the other through a University of Rochester Clinical Translational Science Award (CTSA). The protocols ran concurrently and had identical eligibility criteria, investigators, clinical evaluators, and study procedures except that the CTSA protocol did not include a skin biopsy.

Inclusion criteria for the diabetes group were: age 18 – 65 years, known type 1 or type 2 diabetes based upon American Diabetes Association criteria[22], and able to walk. Inclusion criteria for the control group were: age 18 – 65 years with no symptoms or signs of peripheral

neuropathy, no history of diabetes, and a normal fasting glucose and hemoglobin A1c. Exclusion criteria for both groups were: a history of another systemic condition or neurotoxin exposure that predisposes to peripheral neuropathy; laboratory evidence of abnormalities in serum vitamin B12, thyroid stimulating hormone, protein electrophoresis (SPEP), immunofixation, or creatinine; a history or examination (foot deformities) suggestive of a hereditary neuropathy; signs or symptoms of a myelopathy or compression mononeuropathy; a history of limb amputation; or poorly controlled peripheral vascular disease.

Subjects recruited to the diabetes group were subdivided into two groups using the American Academy of Neurology (AAN) research definition for DSP based on symptoms and signs.[23] In order to meet the definition for clinical DSP, one must have at least neuropathic symptoms, decreased ankle reflexes, and decreased distal sensation. Using this definition, subjects with diabetes were classified as either having or not having clinical DSP. This subgroup categorization was done using clinical criteria (signs and symptoms) alone to allow for useful comparisons of peripheral nerve measures (NCS and ENFD) with MC density.

MC densities and touch-pressure thresholds for the control group have been previously described in part.[18, 19]

Clinical Evaluation

Neurological assessments included inquiry regarding negative and positive sensory symptoms. Sensory examination included assessment of pinprick, vibration, and light touch (using a 10 g monofilament), and was assessed as normal, reduced or absent at the dorsum of the great toe. Timed vibratory sensation in seconds was tested at the 1st interphalangeal joint of the great toe

using a 128 hertz tuning fork. Muscle strength, tone and bulk, and deep tendon reflexes were also assessed. The Michigan Diabetic Neuropathy Score (MDNS) was calculated for each patient with diabetes as a measure of clinical severity of DSP.

In-vivo RCM of MC density

In-vivo RCM of MCs was performed on the palmar surface of the distal phalanx of digit V, the thenar eminence, and medial sole (arch) on the non-dominant side.[20] MC imaging was performed by a single trained microscopist using the Vivascope 1500's VivaScan™ operating software (Lucid. Inc., Rochester, NY).[18-20] Eight 6 x 6 mm mosaic images covering a depth of 160 μm below the basal skin layer were acquired at each imaging site and MCs were counted using systematic random sampling by a blinded observer as previously described.[18-20] MC density was expressed as MCs/mm^2. The reliability of MC counts with RCM has previously been evaluated with an intrarater intraclass correlation coefficient (ICC) of 0.98 and an interrater ICC of 0.91.[20]

Touch-pressure sensory thresholds (monofilaments)

Monofilament touch-pressure sensory thresholds were assessed at each RCM imaging site using a series of 9 monofilaments of logarithmic increasing bending force (0.02 grams to 26 grams). A 4-2-1 testing algorithm was used with variable null stimuli as previously described.[18, 19] The touch-pressure threshold at each site was defined by the monofilament that was correctly detected at least 50% of the time.

Electrophysiologic studies

Each subject underwent NCS of the upper and lower limbs ipsilateral to the RCM imaging. The amplitudes (uV) and conduction velocities (CV) (m/s) were measured in the sural sensory, medial planter, ulnar motor, and ulnar sensory nerves. All studies were performed by a technologist certified by the American Association of Electrodiagnostic Technicians. Limb temperature was maintained above 32º C.

Skin biopsy (epidermal nerve fiber density)

All control subjects and 27 subjects with diabetes recruited under the JDRF protocol underwent a 3-mm skin biopsy 10 cm above the lateral malleolus on the leg ipsilateral to the RCM imaging. Biopsies were processed and immunostained with polyclonal antibodies to the panaxonal marker, protein gene product 9.5, and the ENFD was quantitated by a blinded observer according to previously published methods.[24, 25]

Statistical analysis

Demographic and clinical characteristics were compared between groups in a pairwise fashion using the Wilcoxon rank sum test or Fisher's exact test as appropriate.

Analysis of covariance (ANCOVA) based on ranks was used to compare MC density between healthy control subjects and the entire diabetes group at each imaging site with a two-tailed significance level of 5%.[26] Secondary analysis with ANCOVA based on ranks was used to compare MC density between healthy controls, patients with diabetes without clinical DSP, and patients with diabetes with clinical DSP in a pair-wise fashion using a Bonferroni-adjusted significance level of 1.7% (two-tailed) at each imaging site. Exploratory comparison of MC density between patients with type 1 and type 2 diabetes was conducted using a significance

level of 5% (two-tailed) at each imaging site. Models for digit V were adjusted for age, sex, weight, and hand surface area; those for thenar eminence were adjusted for age, sex, and weight; and those for the arch were adjusted for age, sex, weight, and height.[18]

Sural and medial plantar nerve action potential (NAP) amplitudes, ENFD, and touch-pressure thresholds were compared between healthy control subjects, patients with diabetes without clinical DSP, and patients with diabetes with clinical DSP using rank ANCOVA with a Bonferroni-adjusted significance level of 1.7% (two-tailed).[26] The remaining nerve conduction study outcomes and vibratory sensation were compared between the three groups using standard ANCOVA with a Bonferroni-adjusted significance level of 1.7% (two-tailed). The MDNS was compared between patients with diabetes without clinical DSP and patients with diabetes with clinical DSP using ANCOVA and a 5% significance level (two-tailed). Nerve conductions study measurements, ENFD, touch-pressure thresholds, and vibratory sensation were compared using ANCOVA and a 5% significance level (two-tailed) between patients with type 1 and type 2 diabetes. Ranked data were used where appropriate. Covariates in the ANCOVA models included age, sex, and weight.

Associations between MC densities at each imaging site and nerve conduction study outcomes, ENFD, touch-pressure thresholds, and vibratory sensation in all subjects, and MDNS in only subjects with diabetes, were examined with Spearman's rank correlation coefficients using a two-tailed significance level of 5%.

RESULTS

Demographic and clinical features

Forty-three patients with diabetes (14 with type 1 and 29 with type 2) and 21 healthy control subjects were enrolled (Table 1). Among those with diabetes, 15 subjects met AAN clinical criteria for DSP, while the remaining 28 subjects did not. Control subjects were younger than the entire diabetes group as well as the subgroups of patients with diabetes with and without clinical DSP. The controls weighed less on average than the entire diabetes group and the group with clinical DSP, but not those without clinical DSP. The distribution of height was comparable among the groups. Men and women were approximately equally represented among the controls and those with DSP while 68% of those without DSP were women, but the group differences were not statistically significant.

MC density analyses

MC densities (MC/mm^2) were lower in subjects with diabetes compared to control subjects at the fingertip and thenar eminence, but not the arch (median (interquartile range); fingertip: 1.95 (0.89, 3.39) vs. 5.28 (2.72, 8.72), $p = 0.001$; thenar eminence: 1.17 (0.61, 1.97) vs. 1.83 (1.5, 3.11), $p = 0.001$; arch: 0.11 (0, 0.36) vs. 0.72 (0.28, 1.39), $p = 0.47$; Figure 1). Among subjects with diabetes who met clinical criteria for DSP, MC densities at the fingertip ($p = 0.002$) were lower than those of controls (Table 2, Figure 2). MC densities in patients with diabetes without clinical DSP were lower than those of control subjects at the fingertip ($p = 0.008$) and the thenar eminence ($p = 0.005$), but not the arch (Table 2, Figure 2).

Epidermal nerve fiber density analyses

Mean epidermal nerve fiber density (ENFD) at the ankle was lower in the patients with diabetes without clinical DSP ($p = 0.006$) and with clinical DSP ($p < 0.0001$) compared to that of control subjects (Table 2).

Electrophysiologic analyses

In the upper extremity (Table 2), patients with diabetes with clinical DSP had slower ulnar sensory and motor CV ($p < 0.0001$ for each) and lower ulnar compound motor action potential (CMAP) amplitudes ($p = 0.001$) than those of control subjects. Patients with diabetes without clinical DSP had slower ulnar motor CV ($p < 0.0001$) than controls; however, the ulnar motor and sensory amplitudes and the ulnar sensory CV did not differ between subjects with diabetes without clinical DSP and control subjects.

In the lower extremity (Table 2), patients with diabetes with clinical DSP had slower sural CV ($p = 0.003$) and lower amplitude sural and medial plantar NAP ($p < 0.0001$ for each) compared to control subjects. Patients with diabetes without clinical DSP had slower medial plantar CV ($p < 0.0001$) and lower sural and medial plantar NAP amplitudes ($p = 0.012$ and $p = 0.005$, respectively) compared to control subjects.

Comparison of additional sensory measures

Monofilament touch-pressure thresholds were higher at the arch in patients with diabetes with clinical DSP than those in control subjects ($p = 0.003$); however, touch-pressure thresholds did not significantly differ between control subjects and the diabetic group with clinical DSP at the fingertip or thenar eminence, nor between control subjects and patients with diabetes without clinical DSP at any of the three locations (Table 2). Timed vibration sensation at the great toe

was decreased in patients with clinical DSP ($p < 0.0001$) and without clinical DSP ($p < 0.0001$) compared to control subjects (Table 2). The MDNS was higher in patients with diabetes with clinical DSP compared to those in patients with diabetes without clinical DSP ($p < 0.0001$) (Table 1).

Associations between MC densities and neuropathy outcomes in all subjects

MC density at the fingertip was associated with MC density at the thenar eminence ($r = 0.41$, $p = 0.001$) and the arch ($r = 0.44$, $p < 0.001$). MC density at the fingertip was also associated with ulnar SNAP amplitude ($r = 0.60$, $p < 0.0001$), ulnar SNAP CV ($r = 0.41$, $p = 0.001$), ulnar CMAP amplitude ($r = 0.39$, $p = 0.001$), and ulnar motor CV ($r = 0.43$, $p < 0.001$), and was inversely associated with touch-pressure thresholds at the fingertip ($r = - 0.35$, $p = 0.005$) and MDNS ($r = -0.44$, $p = 0.003$) (Table 3). MC density at the thenar eminence was associated with MC density at the arch ($r = 0.54$, $p < 0.0001$) and ulnar SNAP amplitude ($r = 0.26$, $p = 0.04$), and was inversely associated with touch-pressure thresholds at the thenar eminence ($r = - 0.32$, $p = 0.01$). MC density at the arch was associated with ENFD at the ankle ($r = 0.33$, $p = 0.02$), medial plantar SNAP amplitude ($r = 0.29$, $p = 0.02$), and timed vibration sensation at the great toe ($r = 0.36$, $p = 0.005$), and was inversely associated with touch-pressure thresholds at the arch ($r = - 0.32$, $p = 0.02$). Focused analyses of the relationships between MC density at the fingertip (digit V) and the corresponding upper limb sensory measures in the diabetic cohort alone demonstrated an association with ulnar SNAP amplitude ($r = 0.60$, $p < 0.0001$).

Comparisons between patients with type 1 and type 2 diabetes

Subjects with type 2 diabetes were older (56 years vs. 46.9 years, $p < 0.0001$), weighted more (99.4 kg vs. 78.7 kg, $p = 0.005$), and had been diagnosed with diabetes for a shorter duration (11.6 years vs. 26.7 years, $p = 0.004$) than subjects with type 1 diabetes, although gender representation, height, hemoglobin A1c, MDNS, and percentage of patients with clinical DSP did not differ between the two groups. There were differences in several peripheral nerve markers including MC densities in the hand (digit V, $p = 0.014$ and thenar eminence, $p = 0.047$), ENFD ($p = 0.002$), sural amplitude ($p = 0.016$), and medial plantar amplitude ($p = 0.009$) with greater degrees of peripheral nerve abnormalities in patients with type 2 diabetes, when controlling for age, weight, and gender.

DISCUSSION

We undertook a cross sectional study evaluating the potential role of in-vivo imaging of MCs with RCM as a measure of diabetic DSP. MC imaging is painless, non-invasive, objective, and can be readily performed serially in the same location by a trained technician. We found that MC densities by RCM were reduced in subjects with diabetes at the distal phalanx of digit V and at the thenar eminence relative to those in control subjects. Additionally, MC densities at all imaging sites were associated with corresponding conventional sensory measures including NCS and monofilament touch-pressure thresholds. Furthermore, MC densities at digit V were inversely correlated with the MDNS, a clinical measure of DSP severity. These findings indicate that MC imaging can provide an objective marker of distal large fiber sensory involvement in patients with diabetes and are in line with previously published data supporting RCM of MC density as a measure of HIV associated DSP and sensory involvement in hereditary neuropathy.[18, 19, 27]

The measurable differences in MC densities at the hand of subjects with diabetes compared to controls prompted further evaluation of the relationships between MC densities at digit V and the corresponding ulnar nerve sensory measures in the diabetic cohort alone. MC densities at digit V were strongly associated with ulnar SNAP amplitude further supporting the role of MC density via RCM at digit V as an objective marker of distal sensory changes in patients with diabetes.

To further evaluate the utility of RCM of MC density as an early marker of peripheral sensory nerve terminal involvement, subjects with diabetes were subdivided into groups with and without clinically defined DSP using the AAN research definition for DSP based on symptoms and signs.[23] This definition was developed with a high threshold for inclusion as a way to increase specificity.[23] As a result, it distinguishes subjects with clinically evident DSP from those with possible subclinical DSP. The current study supports this concept. Both diabetic groups (with and without AAN clinical criteria for DSP) had objective evidence of sensory involvement as supported by lower ENFD, medial plantar SNAP amplitudes, and sural SNAP amplitudes compared to control subjects. However, patients with diabetes who met AAN clinical criteria for DSP had greater objective peripheral sensory abnormalities compared to those not meeting clinical DSP criteria.

Patients with diabetes meeting clinical DSP criteria had lower MC densities at the distal phalanx of digit V compared to control subjects. Moreover, MC densities were lower at the fingertip and thenar eminence of patients with diabetes who did not meet clinical criteria for DSP compared to control subjects. These data indicate that MC density imaging via RCM at digit V is a marker of early diabetic DSP at a time when the corresponding ulnar sensory nerve conduction studies and monofilament touch-pressure threshold testing at digit V are not able to detect

measurable changes. Furthermore, among all subjects with diabetes, only 3 had MC densities of 0 MC/mm^2 at digit V, despite 20 subjects with unobtainable medial plantar SNAPs and 9 with unobtainable sural SNAPs, suggesting that there is not likely to be a significant floor effect at this site.

Skin and sural nerve biopsy studies have demonstrated that both small unmyelinated fibers and myelinated fibers are affected early in diabetic DSP, prior to measurable changes on NCS or other quantitative sensory testing.[4, 10, 11, 17, 24, 28-32] The earliest neuropathic changes in diabetic DSP are seen in the skin at the distal nerve terminals, which explains why NCS are not optimally sensitive for the detection of early diabetic DSP.[8, 28, 29] Meissner's corpuscles are composed of axon terminals of A-ß (myelinated) and C fiber (unmyelinated) sensory afferents and play a role in touch-pressure perception and possibly mechanical nociception.[13, 14, 33, 34] Changes in MC densities correlate with changes in axon terminals of both myelinated and unmyelinated fibers.[11, 16, 17] Therefore, it stands to reason that MC density in the skin would be a useful marker of early diabetic DSP, a conclusion supported by biopsy studies and expanded upon with the present study.[11, 16, 17]

Our data, in contrast, do not support the utility of MC density via RCM at the arch of the foot as a useful marker of diabetic DSP. While MC density at the foot was associated with MC density at both hand imaging sites and with corresponding conventional lower extremity sensory measures, there were no significant differences between control subjects and the diabetes subgroups with respect to MC density at the foot. MC densities are low in the arch of healthy subjects at baseline and are known to decline with age.[15, 18, 19] In the present study, the mean MC density at the arch in controls was 1 MC/mm^2 and the number of subjects with 0 MC/mm^2 at the arch reached 33% in the diabetic group and 15% in controls. Consequently, age related

variability and a floor effect appear to limit the role of RCM of MC density at the arch as a marker of diabetic DSP.

Our study included participants with both type 1 and type 2 diabetes. Not surprisingly, patients with type 2 diabetes were older, weighed more, and had been diagnosed with diabetes for a shorter duration than patients with type 1 diabetes, although hemoglobin A1c, MDNS, and the percentage of patients with clinical DSP did not differ between the two groups. There were differences in several peripheral nerve markers between the two groups including MC densities in the hand, ENFD, and lower extremity SNAP amplitudes with greater degrees of peripheral nerve abnormalities in patients with type 2 diabetes, when controlling for age, weight, and gender. These findings are consistent with a recent study demonstrating a higher incidence of microvascular complications including peripheral neuropathy in patients with type 2 compared to type 1 diabetes.[35] However, our findings need to be interpreted cautiously given the small number of subjects in each group.

The present study had some limitations. As noted, MC density is influenced by age. Among our study sample, subjects with diabetes were older than control subjects. To overcome this limitation, we adjusted for age in our statistical models that compared subjects with diabetes and control subjects. While it is possible that this modeling did not completely remove confounding by age, we believe that any residual confounding is likely to be small. Notwithstanding, our findings are consistent with those of three previous studies evaluating the role of MC density by RCM imaging as a marker of peripheral neuropathy in other disorders.[18-20, 36]

Another potential limitation were the clinical criteria for DSP used to categorize subjects with diabetes. However, inclusion of NCS and ENFD in the DSP definition would have

precluded useful comparison of these measures to MC density. Furthermore, the AAN definition performed well in this study defining two distinct groups according to the degree of objective abnormality of the peripheral nervous system on ancillary peripheral nerve measures.

In summary, RCM of MC density at digit V is a non-invasive, painless, objective marker of early peripheral sensory involvement in diabetes that provides a unique window into large fiber sensory nerve terminals. Future longitudinal studies are needed to determine whether RCM of MCs can identify quantitative changes in DSP associated with disease progression or treatment.

AUTHOR CONTRIBUTIONS

Peter D. Creigh: drafting and revising the manuscript for intellectual content, analysis and interpretation of data. Michael P. McDermott: revising the manuscript for intellectual content, analysis and interpretation of data. Janet E. Sowden: acquisition of data, coordination of study, revising the manuscript for intellectual content. Michele Ferguson: acquisition of data, coordination of study, revising the manuscript for intellectual content. David N. Herrmann: design and conceptualization of the study, drafting and revising the manuscript for intellectual content, analysis and interpretation of data, supervision and coordination of study, obtaining funding.

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DISCLOSURE

Peter D. Creigh, Michael P. McDermott, Janet E. Sowden, and Michele Ferguson report no disclosures. David N. Herrmann is a clinical trial adjudication committee member for Medpace, and a consultant for Guidepoint Global, Acceleron, Flex Pharma and LAM Therapeutics, Inc.

Author Contributions:
Peter D. Creigh: drafting and revising the manuscript for intellectual content, analysis and interpretation of data.
Michael P. McDermott: revising the manuscript for intellectual content, analysis and interpretation of data.
Janet E. Sowden: acquisition of data, coordination of study, revising the manuscript for intellectual content.
Michele Ferguson: acquisition of data, coordination of study, revising the manuscript for intellectual content.
David N. Herrmann: design and conceptualization of the study, drafting and revising the manuscript for intellectual content, analysis and interpretation of data, supervision and coordination of study, obtaining funding.

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Peter D. Creigh – reports no disclosures.
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Michele Ferguson – reports no disclosures.
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Figure 1 Meissner's corpuscle densities for control subjects and subjects with diabetes

Meissner's corpuscle (MC) densities for control subjects and all subjects with diabetes at the fingertip (D5), thenar eminence (TE), and foot arch. Box plots illustrate median and quartiles, with the diamond representing the mean. P values (two-tailed) are derived from analysis of covariance based on ranks; see text for details.

Figure 2 In-Vivo Reflectance Confocal Microscopy of Meissner's corpuscles in control subjects and subjects with diabetes

Mosaic images obtained from the palmar surface of the distal phalanx of digit V using in-vivo reflectance confocal microscopy illustrating progressively lower Meissner's corpuscle (MC) density between (A) a control subject, (B) a subject with diabetes without DSP, and (C) a subject with diabetes with DSP. White arrows highlight MCs. Black arrows highlight the absence of MCs.

Table 1 Demographic and clinical characteristics of study subjects

		Diabetes		
	Controls	Combined	No DSP	DSP
N	21	43	28	15
Age (years)	42.7 (8.0)	53.3 (9.8)[d]	50.7 (10.6)[c]	58.2 (5.9)[d]
Gender (F/M)	11/10	26/17	19/9	7/8
Height (cm)	170 (11.8)	169 (10.1)	168 (9.5)	170 (11.6)
Weight (kg)	77.4 (16.6)	92.3 (25.8)[a]	89.8 (25)	97.2 (27.6)[b]
Hemoglobin A1c		7.67 (1.93)	7.62 (2.17)	7.76 (1.45)
Years since diabetes diagnosis		17.4 (14.6)	15.0 (13.9)	22.6 (15.4)
MDNS		8.6 (6.1)	5.7 (4.6)	14.0 (4.8)[e]

Abbreviations: MDNS = Michigan Diabetic Neuropathy Score, No DSP = Patients with diabetes without AAN research criteria for distal symmetric polyneuropathy, DSP = Patients with diabetes with AAN research criteria for distal symmetric polyneuropathy,

Values are mean (standard deviation).

P values represent pairwise comparisons between each of the diabetes groups and the control group using a Wilcoxon rank sum test or Fisher's exact test as appropriate: [a] $p = 0.05$, [b] $p = 0.04$, [c] $p = 0.005$, [d] $p < 0.0001$; and between the two diabetes groups (No DSP vs DSP) using ANCOVA with a significance level of 5% (two-tailed): [e] $p < 0.0001$

Table 2 Neuropathy outcomes

		Diabetes		Comparisons (p value)		
	Controls (1)	No DSP (2)	DSP (3)	1 vs. 2	1 vs. 3	2 vs. 3
N	21	28	15			
Upper limb						
MC density, digit V (MC/mm^2)	5.89 (3.64)	2.91 (2.00)	1.65 (2.16)	p = 0.008*	p = 0.002*	p = 0.52
MC density, TE (MC/mm^2)	2.25 (1.43)	1.42 (1.14)	1.46 (1.22)	p = 0.005*	p = 0.08	p = 1.00
Ulnar SNAP amplitude (uV)	33.6 (11.7)	25.2 (14.9)	14.6 (14.6)	p = 0.63	p = 0.06	p = 0.29
Ulnar SNAP CV (m/s)	55.7 (4.9)	52.4 (5.0)	47.4 (5.8)	p = 0.09	p < 0.0001*	p = 0.01*
Ulnar motor amplitude (uV)	11.7 (1.9)	10.5 (2.2)	9.0 (2.6)	p = 0.17	p = 0.001*	p = 0.11
Ulnar motor CV (m/s)	59.9 (3.5)	54.2 (5.3)	52.4 (5.8)	p < 0.0001*	p < 0.0001*	p = 0.78
MF threshold, TE (grams)	0.09 (0.05)	0.16 (0.12)	0.30 (0.31)	p = 0.33	p = 0.54	p = 1.00
MF threshold, digit V (grams)	0.07 (0.05)	0.07 (0.04)	0.11 (0.04)	p = 1.00	p = 0.3	p = 0.18
Lower limb						
MC density, arch (MC/mm^2)	1.01 (0.98)	0.52 (0.86)	0.28 (0.43)	p = 0.72	p = 1.00	p = 1.00
ENFD, ankle (fibers/mm)	13.70 (4.99)	8.63 (6.96)	1.52 (3.88)	p = 0.006*	p < 0.0001*	p = 0.07
Sural SNAP amplitude (uV)	23.1 (9.9)	13.7 (9.3)	6.0 (9.2)	p = 0.012*	p < 0.0001*	p = 0.02
Sural SNAP CV (m/s)	49.6 (5.4)	46.7 (5.6)	42.3 (7.2)	p = 0.09	p = 0.003*	p = 0.11
Plantar amplitude (uV)	10.4 (4.1)	5.7 (5.8)	2.3 (7.4)	p = 0.005*	p < 0.0001*	p = 0.01*
Plantar SNAP CV (m/s)	52.7 (4.9)	48.0 (5.3)	53.6 (7.4)	p < 0.0001*	p = 1.00	p = 0.21
MF threshold, arch (grams)	0.25 (0.28)	0.62 (0.66)	8.11 (11.2)	p = 0.58	p = 0.003*	p = 0.01*
Vibration, great toe (sec)	16.8 (4.38)	9.19 (4.90)	4.20 (4.18)	p < 0.0001*	p < 0.0001*	p = 0.11

Abbreviations: CV = conduction velocity, DSP = patients with diabetes with AAN research criteria for distal symmetric polyneuropathy, No DSP = patients with diabetes without AAN research criteria for distal symmetric polyneuropathy,

ENFD = epidermal nerve fiber density, MC = Meissner's corpuscle, MF = monofilament, SNAP = sensory nerve action potential, TE = thenar eminence

Values are mean (standard deviation)

* Bonferroni-adjusted significance level of 0.017 (2-tailed).

Table 3 **Correlations between MC density and conventional neuropathy outcomes**

MC Density, Digit V	
Ulnar SNAP amp*	0.60 (<0.0001)
Ulnar SNAP CV*	0.41 (0.001)
Ulnar motor amp*	0.39 (0.001)
Ulnar motor CV*	0.43 (<0.001)
MF threshold, Digit V*	- 0.35 (0.005)
MDNS*	-0.44 (0.003)
MC Density, TE	
Ulnar SNAP amp*	0.26 (0.04)
Ulnar SNAP CV	0.20 (0.13)
Ulnar motor amp	0.15 (0.25)
Ulnar motor CV	0.16 (0.21)
MF threshold, TE*	- 0.32 (0.01)
MDNS	- 0.15 (0.33)
MC Density, Arch	
ENFD, ankle*	0.33 (0.02)
Sural SNAP amp	0.23 (0.07)
Sural CV	- 0.04 (0.79)
Medial Plantar SNAP amp*	0.29 (0.02)
Medial Plantar CV	- 0.05 (0.77)
MF threshold, great toe*	- 0.32 (0.02)
Vibration, great toe*	0.36 (0.005)
MDNS	- 0.21 (0.18)

Abbreviations: Amp = amplitude (uV), CV = conduction velocity (m/s), ENFD = epidermal nerve fiber density, MC = Meissner's corpuscle, MDNS = Michigan Diabetic Neuropathy Score, SNAP = sensory nerve action potential

Values are Spearman's rank correlation coefficient (p – value)

*p < 0.05 (2-tailed)

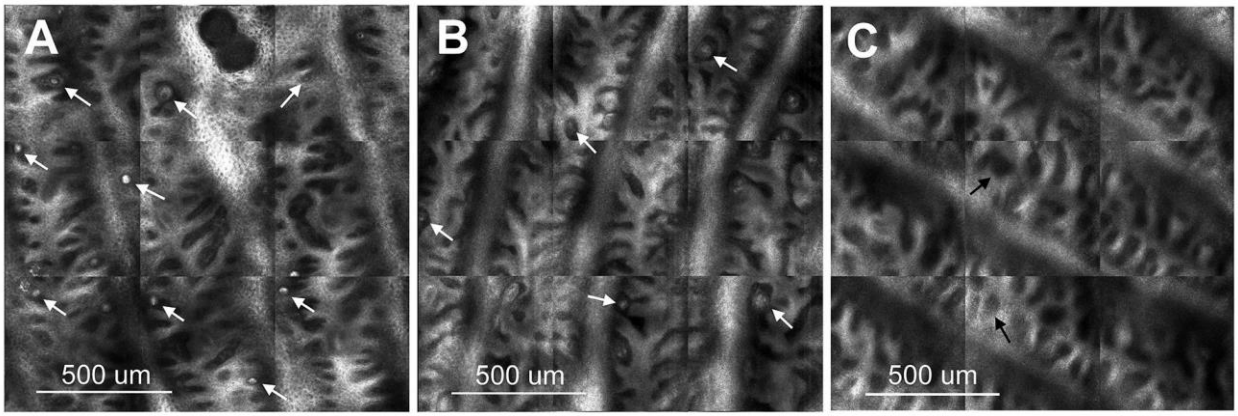

Figure 1

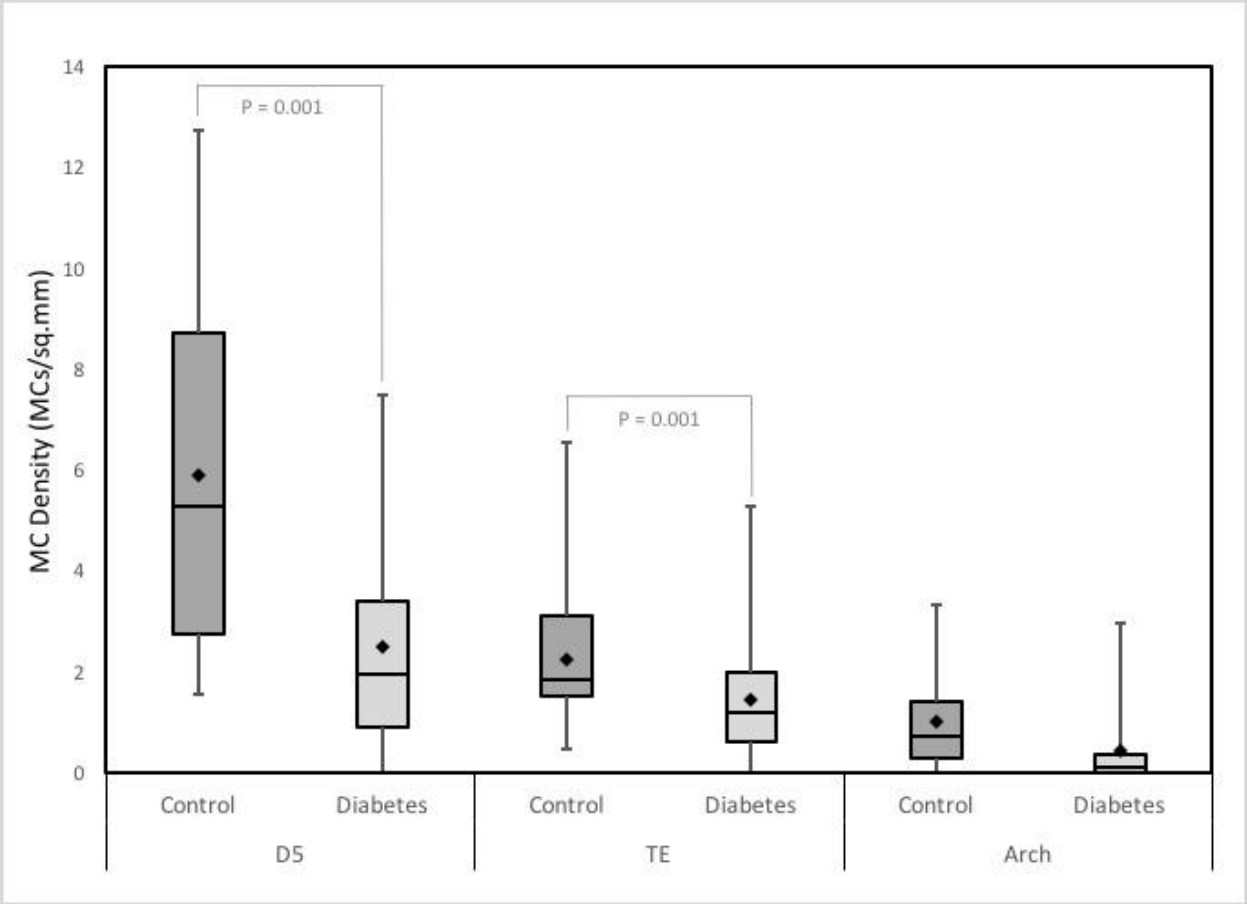

Figure 2

Highlights

1. In-vivo reflectance confocal microscopy (RCM) can non-invasively image skin
2. RCM of glabrous skin enables quantification of Meissner's corpuscle (MC) density
3. MC density via RCM is an objective marker of early sensory involvement in diabetes