Skin Intrinsic Fluorescence Correlates With Autonomic and Distal Symmetrical Polyneuropathy in Individuals With Type 1 Diabetes

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OBJECTIVE—To determine whether skin intrinsic fluorescence (SIF) was associated with autonomic neuropathy and confirmed distal symmetrical polyneuropathy (CDSP) in 111 individuals with type 1 diabetes (mean age 49 years, mean diabetes duration 40 years).

RESEARCH DESIGN AND METHODS—SIF was measured using the SCOUT DM device. Autonomic neuropathy was defined as an electrocardiographic abnormal heart rate response to deep breathing (expiration-to-inspiration ratio <1.1). CDSP was defined using the Diabetes Control and Complications Trial clinical exam protocol (the presence of two or more of the following: symptoms, sensory and/or motor signs, and/or reduced/absent tendon reflexes consistent with DSP) confirmed by the presence of an abnormal age-specific vibratory threshold (using a Vibratron II tester).

RESULTS—The prevalence of autonomic neuropathy and CDSP were 61 and 66%, respectively. SIF was higher in those with autonomic neuropathy ($P < 0.0001$). In multivariable analyses controlling for age and updated mean (18-year average) HbA_{1c}, and allowing for other univariately and clinically significant correlates of autonomic neuropathy, each SD change in SIF was associated with a 2.6-greater likelihood of autonomic neuropathy ($P = 0.006$). Receiver operating characteristic (ROC) analyses revealed that SIF and updated mean HbA_{1c} accounted for 80 and 57%, respectively, of the area under the curve (AUC) for autonomic neuropathy. SIF also was higher in those with CDSP ($P < 0.0001$) and remained so in multivariable analyses (odds ratio 2.70; $P = 0.005$). ROC analyses revealed that SIF and updated mean HbA_{1c} accounted for 78 and 59%, respectively, of the AUC for CDSP.

CONCLUSIONS—SIF, a marker of dermal advanced glycation end products, appears to be more strongly associated with the presence of both CDSP and autonomic neuropathy than mean HbA_{1c}.

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Diabetic neuropathy, one of the most common of the late complications of diabetes (1), and the leading cause of nontraumatic amputations (1), is thought to play a role in the pathogenesis of the other late complications of diabetes (2) and carries a high risk of mortality (3). The formation of advanced glycation end products (AGEs) on nerve tissue has been postulated to play a major role in the pathogenesis of diabetic neuropathy (4). AGEs are derivatives of the Maillard reaction of reducing sugars and protein and typically are characterized by their ability to form cross-links with other proteins. Certain AGEs fluoresce when excited with near-ultraviolet and blue light (5). AGEs, increased in diabetes as a result of both hyperglycemia and oxidative stress, are observed in both myelinated and unmyelinated fibers of sural, peroneal, and saphenous nerves of individuals with diabetes (6,7) and may contribute to the segmental demyelination of diabetic neuropathy (8). AGEs also have been shown to interact with nitric oxide, leading to neuronal apoptosis (9).

In a substudy of the Diabetes Control and Complications Trial (DCCT), levels of skin collagen AGEs were increased in those with clinical neuropathy (10). Because certain dermal collagen AGEs, such as pentosidine and cross-lines, contain fluorescent cross-links (10), skin intrinsic fluorescence (SIF) can be quantified and act as a novel maker of AGE accumulation. This led us to investigate the associations of SIF with neuropathy, both autonomic and confirmed distal symmetrical polyneuropathy (CDSP), and how this compares with cumulative glycemic exposure as determined by measures of HbA_{1c} over the previous 18 years of follow-up.

RESEARCH DESIGN AND METHODS

—The Epidemiology of Diabetes Complications (EDC) cohort is a well-defined population ($n = 658$) with type 1 diabetes diagnosed before the age of 17 years at the Children's Hospital of Pittsburgh (11,12). Participants have been followed since 1986–1988, when mean age and diabetes duration were 28 and 19 years, respectively. A convenience sample of 111 participants (96% Caucasian) from the EDC study participated in this cross-sectional study of noninvasively measured SIF, which occurred ~2 years after the 18-year follow-up exam. All study procedures were approved by the University of Pittsburgh Institutional Review Board.

Medical exams took place during the 18-year follow-up. Blood samples were assayed for lipids, lipoproteins, glycated

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hemoglobin, and creatinine. HDL cholesterol was determined by a heparin and manganese procedure, a modification of the Lipid Research Clinics method (13). Cholesterol was measured enzymatically. Non–HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Stable glycosylated hemoglobin A_1 (HbA_1) was originally measured in saline-incubated samples by microcolumn cation–exchange chromatography (Isolab, Akron, OH). On 26 October 1987, the method was changed to high-performance liquid chromatography (Diamat; Bio-Rad Laboratories, Hercules, CA). The two methods were highly correlated (r = 0.95; Diamat HbA_1 = 0.18 ± 1.00 Isolab HbA_1). Beginning in 1998, HbA_{1c} was measured using the DCA2000 analyzer. Original HbA_1 (1986–1998) and A_{1c} (1998–2004) were converted to DCCT-aligned HbA_{1c} values using regression formulas derived from duplicate analyses (DCCT HbA_{1c} = [0.83 × EDC HbA_1] + 0.14; DCCT HbA_{1c} = [EDC HbA_{1c} − 1.13]/0.81). Cumulative glycemic exposure was determined using updated mean HbA_{1c}, calculated by taking the sum of an individual's HbA_{1c} values over the 18 years of follow-up and dividing it by the number of HbA_{lc} measurements taken on the individual. (During the nine biennial exams over the 18-year follow-up period, there were an average of eight measurements.) Urinary albumin from timed urine collections was determined immunonephelometrically. Overt nephropathy was defined as an albumin excretion rate (AER) >200 μg/min or a history of dialysis/kidney transplantation. Height was measured with a stadiometer and weight with a balance-beam scale. BMI was calculated (kg/m^2). Blood pressure was measured by a random-zero sphygmomanometer according to a standardized protocol (14) after a 5-min rest period and analyzed using the mean of the second and third readings.

CDSP was defined using the DCCT clinical exam protocol (15), i.e., at least two of the following three criteria present and not attributed to a nondiabetic cause: symptoms consistent with distal symmetrical polyneuropathy (DSP); decreased (requiring reinforcement) or absent tendon reflexes; and signs of sensory loss confirmed by the presence of an abnormal age-specific vibratory threshold (Vibratron II tester) (coefficient of variation was previously determined to be 8% [16]). Autonomic neuropathy was defined as an abnormal heart rate response to deep breathing, i.e., an expiration-to-inspiration (E/I) ratio <1.1 in six paced episodes (17). The clinical exam for CDSP was performed by a trained internist, and autonomic neuropathy and vibratory threshold testing was performed by a trained research assistant.

SIF was noninvasively measured from the skin of the left volar forearm using a SCOUT DM (VeraLight, Albuquerque, NM) skin fluorescence spectrometer (18,19). SIF was excited with an LED centered at 405 nm and was detected over the emission range of 441–482 nm. The skin reflectance was measured over both the excitation and emission regions and was used to compensate for absorbance caused by melanin and hemoglobin (5). The intrinsic fluorescence correction is expressed in Eq. 1:

$$f_{xm} = \frac{F_{xm}}{R_x^{k_x} R_m^{k_m}}$$

where measured fluorescence, F_{xm}, is divided by reflectance values at the excitation and emission wavelengths, R_x and R_m, respectively. The reflectance values are adjusted by the dimensionless exponents, k_x and k_m. For these analyses, k_x was set to 0.9 and k_m was set to 0.0. The resulting intrinsic fluorescence, f_{xm}, was integrated over the 441- to 496-nm spectral region to give the SIF sum. The intrasubject skin variation in SIF assessed by the SCOUT DM had been previously determined in a large diabetes screening study of 2,589 subjects at risk for developing type 2 diabetes (19). The interday Hoorn coefficient of variation (fasting vs. nonfasting) was 6.9% for SCOUT DM–measured SIF.

As a corollary to this primary study, a similar investigation was performed with collaborators at the MedStar Health Research Institute Diabetes Clinic to evaluate the ability of noninvasively measured SIF to distinguish the presence or absence of DSP during routine clinical assessment of patients with type 1 diabetes. The MedStar Health Research Institute Diabetes Clinic was a small, group-practice setting with three endocrinologists who mainly saw patients with type 1 and type 2 diabetes. Patients who primarily were seen and followed by a single clinician (R.E.R.) were invited to participate in this pilot study. Eligible participants were seen for a minimum of 4 years prior to the index visit when the SIF measurement was performed. Patients in this clinic were seen over a mean of 10 years. All procedures were approved by the MedStar Health Research Institute Office of Research Integrity/Institutional Review Board. Informed consent was obtained prior to any research-related procedure. Routine assessments independently coded in the research chart included detailed history (sex, ethnicity, history of diabetes-related complications, and categories of current therapies), physical examination (blood pressure, heart rate, and clinical evaluation for sensory loss consistent with peripheral neuropathy using the 5.07 [10 g] Semmes-Weinstein monofilament on the plantar surface of the feet [20]), and laboratory assessments (HbA_{1c} using the DCCT-aligned DCA 2000+ urine albumin-to-creatinine ratio and lipid panel performed at the local laboratory). In addition, medical charts were reviewed to obtain historical HbA_{1c} values.

The Student t test and χ^2 test were used to examine univariate correlates of autonomic neuropathy and CDSP. Pearson correlations were used to determine the relationship between SIF and continuous measures of the E/I ratio and toe vibratory thresholds. Logistic regression analysis with stepwise selection was used to determine the independent association of SIF with the prevalence of autonomic neuropathy and CDSP. Odds ratios are expressed as per SD change in continuous variables. Receiver operating characteristic (ROC) curves were used to determine the discriminative ability of SIF and mean HbA_{1c} to detect autonomic neuropathy or lower-limb CDSP. Aikeke information criterion was used to determine which models better accounted for the presence of autonomic neuropathy or CDSP. Statistical analysis was conducted using SAS version 9.1 (Cary, NC).

RESULTS—Characteristics of the individuals who participated in this SIF substudy versus the 18-year follow-up exam participants who did not are presented in Supplementary Table 1. Those participating in the SIF substudy were slightly older but of similar diabetes duration. They were also in marginally better control (updated HbA_{1c} 8.4 vs. 8.6%) but had similar rates of autonomic neuropathy (59.8 vs. 56.6%) and CDSP (64.7 vs. 54.2).

Characteristics of the SIF study participants by autonomic neuropathy status are presented in Table 1. Sixty-six participants had been previously diagnosed with autonomic neuropathy at the time of SIF measurement. Participants with

Table 1—*Characteristics of EDC participants by autonomic neuropathy and CDSP status*

Characteristic	Autonomic neuropathy			CDSP		
	Cases	Noncases	*P*	Cases	Noncases	*P*
n	66	43		73	38	
SIF (AU)*	0.0098 (0.0024)	0.0075 (0.0015)	<0.0001	0.0097 (0.0025)	0.0075 (0.0015)	<0.0001
Age at SIF measurement (years)	51.3 (7.0)	44.4 (5.9)	<0.0001	51.2 (7.0)	43.7 (5.4)	<0.0001
Duration at SIF measurement (years)	41.9 (6.9)	36.8 (6.2)	0.0001	42.4 (7.2)	35.5 (4.3)	<0.0001
Sex (female)	59.1 (39)	51.2 (22)	0.42	56.2 (41)	55.3 (21)	0.93
Height (cm)	167.6 (10.2)	168.7 (10.6)	0.58	168.1 (10.0)	167.8 (11.0)	0.91
Pulse (bpm)	75.0 (11.2)	71.6 (13.1)	0.16	74.2 (12.0)	72.6 (12.3)	0.52
R-R interval (ms)*	1.06 (0.04)	1.24 (0.11)	<0.0001	1.08 (0.09)	1.21 (0.12)	<0.0001
BMI (kg/m^2)	26.1 (5.0)	26.2 (3.2)	0.96	26.0 (4.9)	26.3 (3.0)	0.76
HbA_{1c} (%)	7.7 (1.7)	7.4 (1.3)	0.46	7.7 (1.7)	7.4 (1.1)	0.24
Updated mean HbA_{1c} (%)	8.5 (1.1)	8.2 (0.89)	0.17	8.5 (1.1)	8.2 (0.89)	0.09
AER (μg/min)*	18.2 (5.6–116.5)	4.6 (3.2–7.0)	<0.0001	18.2 (6.0–102.0)	4.1 (2.9–5.6)	<0.0001
Serum creatinine (mg/dL)*	1.0 (0.9–1.2)	1.0 (0.8–1.1)	0.0004	1.0 (0.9–1.2)	0.9 (0.8–1.0)	0.08
Systolic blood pressure (mmHg)	117.6 (15.7)	109.0 (10.5)	0.001	116.9 (15.5)	109.3 (10.9)	0.005
Diastolic blood pressure (mmHg)	62.9 (10.2)	66.4 (10.6)	0.10	63.3 (10.5)	66.3 (10.2)	0.17
Blood pressure medication use	28.1 (18)	4.9 (2)	0.004	22.9 (16)	13.9 (5)	0.27
HDL cholesterol (mg/dL)	62.6 (18.3)	55.9 (16.2)	0.06	61.3 (18.8)	57.4 (15.4)	0.29
Non–HDL cholesterol (mg/dL)	107.4 (26.3)	113.8 (31.1)	0.26	109.6 (28.6)	110.6 (28.4)	0.87
ACE inhibitor medication use	60.9 (39)	46.3 (19)	0.14	58.6 (41)	47.2 (17)	0.27
History of smoking	39.7 (25)	17.5 (7)	0.02	36.8 (25)	22.2 (8)	0.13
CDSP or autonomic neuropathy	90.9 (60)	25.6 (11)	<0.0001	84.5 (60)	15.8 (6)	<0.0001

Data are means (SD), median (interquartile range), or % (*n*), as appropriate. *Naturally log-transformed before analysis.

autonomic neuropathy were older (aged 51.3 vs. 44.4 years), were of longer diabetes duration (41.9 vs. 36.8 years), and had greater renal damage as measured by AER (18.2 vs. 4.6 μg/min). They also were more likely to have a history of smoking (39.7 vs. 17.5%). CDSP also was a comorbid complication in the majority (90.9%) of those with autonomic neuropathy. Finally, SIF was greater in those with autonomic neuropathy.

Characteristics of the SIF study participants by CDSP status also are presented in Table 1. There were 73 participants with CDSP. As with autonomic neuropathy, SIF was higher in those with CDSP, and correlates of CDSP were similar to those for autonomic neuropathy with the exception of serum creatinine, blood pressure medication use, and a history of smoking.

SIF was correlated with most of the participant characteristics presented in Table 1 but appeared to demonstrate the strongest associations with the E/I ratio of the R-R interval and with the toe vibratory threshold (Supplementary Table 2). The correlation between the natural log-transformed SIF and the E/I ratio was −0.47 ($P < 0.0001$), i.e., the higher the SIF, the more abnormal the E/I ratio. With the toe vibratory threshold, the SIF correlation was $r = 0.50$ ($P < 0.0001$).

Figure 1*A* compares the discriminative ability of SIF and updated mean HbA_{1c} to detect those with autonomic neuropathy as depicted in ROC curves. The area under the curve (AUC) for SIF and autonomic neuropathy was 0.80, whereas updated mean HbA_{1c} had an AUC of 0.57. SIF demonstrated good discrimination between those with and those without autonomic neuropathy, whereas the discriminative ability of updated mean HbA_{1c} was slightly better than chance. Figure 1*B* compares the discriminative ability of SIF and updated mean HbA_{1c} to detect those with CDSP. The AUC for SIF was 0.78 versus an AUC of 0.59 for updated mean HbA_{1c}. SIF demonstrated a superior ability to detect CDSP relative to updated mean HbA_{1c}.

Table 2 shows the independent association of SIF and updated mean HbA_{1c} with autonomic neuropathy and CDSP. In multivariable regression analyses with stepwise selection allowing for univariately

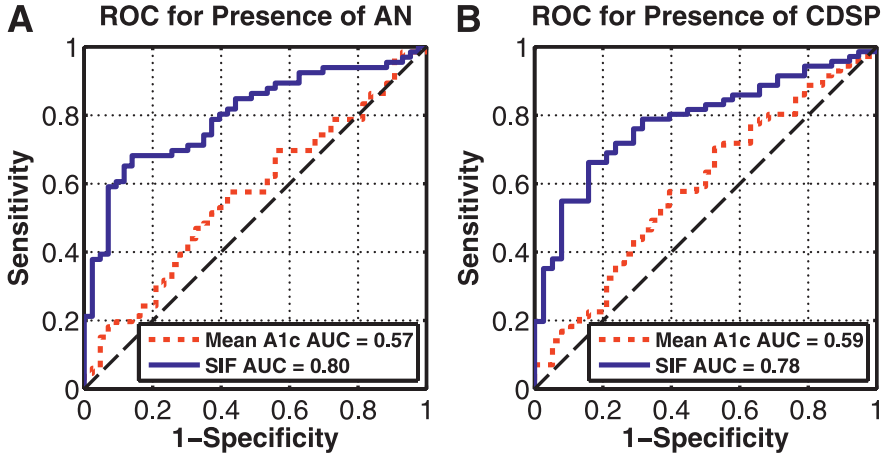

Figure 1—*ROC curves for the cross-sectional association of SIF and mean HbA_{1c} to autonomic neuropathy* (A) *and CDSP* (B).

Table 2—*Multivariable correlates of autonomic neuropathy and CDSP in EDC participants*

	Autonomic neuropathy		CDSP	
Variable	Model 1 Odds ratio (95% CI)	Model 2 Odds ratio (95% CI)	Model 1 Odds ratio (95% CI)	Model 2 Odds ratio (95% CI)
SIF*	3.54 (1.95–6.43)	2.62 (1.31–5.23)	3.52 (1.92–6.46)	2.70 (1.35–5.43)
Age at SIF measurement		2.72 (1.40–5.28)		3.50 (1.71–7.15)
Updated mean HbA_{1c}		NS		NS
AER*		3.95 (1.77–8.81)		3.63 (1.56–8.45)
HDL cholesterol		2.05 (1.10–3.84)		NS
Aikeke information criterion	113.485	98.560	111.754	98.226

Odds ratios are expressed as per SD change continuous variables. Model 1, univariate analysis; model 2, multivariable analysis, using stepwise selection. Stepwise selection models allowed for age at SIF measurement, SIF, updated mean HbA_{1c}, heart rate, height, serum creatinine, albumin excretion rate, HDL cholesterol, non–HDL cholesterol, systolic blood pressure, diastolic blood pressure, blood pressure medication use, and a history of smoking. NA, not allowed; NS, not selected. *Naturally log-transformed before analyses.

and clinically significant correlates of autonomic neuropathy, updated mean HbA_{1c} was not significantly associated with autonomic neuropathy. Independent correlates of autonomic neuropathy were SIF, age, AER, and HDL cholesterol. Each SD change in SIF was associated with a 2.6-greater likelihood of having autonomic neuropathy. For CDSP, multivariable regression analyses with stepwise selection, allowing for univariately and clinically significant correlates of CDSP, revealed that updated mean HbA_{1c} was not significantly associated with CDSP; however, each SD change in SIF was associated with a 2.7-greater likelihood of CDSP. Because SIF exhibited a strong independent relationship with overt nephropathy in this population (odds ratio 3.17 [95% CI 1.38–7.28]) and given the known relationship between renal disease and AGEs, in order to further control for potential confounding by kidney damage, analyses were rerun excluding individuals with a history of overt nephropathy. The strong independent relationship between SIF and autonomic neuropathy (2.22 [1.08–4.56]) and CDSP (2.53 [1.22–5.21]) remained.

To determine whether the time interval between the EDC clinical exam and the measurement of SIF affected the findings, analyses were rerun with participants stratified by the median time interval (1.8 years). The mean difference in SIF levels by autonomic neuropathy status was similar in those with a longer interval between the exam and the SIF measurement to those with a shorter interval. The same was true for CDSP. These results can be found in Supplementary Table 3.

In the corollary study at the MedStar Health Research Institute Diabetes Clinic to evaluate the clinical utility of SIF in a small pilot population, data from 58 individuals with type 1 diabetes were available for evaluation. Of the 58 individuals, 16 had clinical evidence of DSP, and 42 did not. The naturally logarithmically transformed SIF showed a trend for being higher in cases with clinical evidence of DSP ($P = 0.07$). In comparison, there was no significant difference in updated mean HbA_{1c} between the cases (8.1%) and noncases (7.8%) ($P = 0.30$). Additional data on this cohort is available in Supplementary Table 4.

CONCLUSIONS—Glycation of peripheral nerve fibers is thought to play a role in the development of diabetic neuropathy. We have shown that SIF, a marker of AGE accumulation, demonstrates a strong association with both autonomic and confirmed DSP. We have additionally shown that these associations are independent of traditional risk factors for neuropathy, such as age, glycemic control, renal function, and smoking, and that SIF was a better indicator of neuropathy than cumulative glycemic exposure, as reflected by updated mean HbA_{1c}.

Hypothesized pathogenic mechanisms of peripheral neuropathy include ischemic effects caused by vascular abnormalities, disruption of neuronal metabolism, axonal transport mechanisms and repair capabilities, glycation of peripheral nervous system connective tissue, and glycation of Schwann cells or extracellular matrix (7). Segmental demyelination is characterized by breakdown and loss of myelin over a few segments of the axon and results in decline in conduction velocity and conduction block. Because myelin facilitates conduction, we speculate that AGE accumulation within myelin may interfere with conduction. AGE formation in the endoneurium, cytoskeleton, plasma membrane, Schwann cells, or extracellular matrix may interfere with axonal elongation and regeneration, resulting in distal axonal atrophy or degeneration (7).

Our results demonstrating a cross-sectional association of SIF with diabetic peripheral neuropathy are consistent with reports of glycosylated proteins and AGEs in nerve tissue, particularly in those with diabetes. In eight type 2 diabetic patients with polyneuropathy, AGEs were found localized in the endoneurium, perineurium, and microvessels of peripheral nerve fibers (21). AGE deposition also was observed in the "cytoplasm of endothelial cells, pericytes, axoplasm, and Schwann cells of both myelinated and unmyelinated fibers" in a case-control study of five type 2 diabetic subjects and five nondiabetic control subjects, with greater intensity in the diabetic subjects; higher levels of AGEs correlated with reduced density of myelinated fibers (6). In elderly individuals with type 2 diabetes, Ryle and Donaghy (7) observed that compared with nondiabetic control subjects, pentosidine levels were increased in the cytoskeletal and myelin nerve fractions. There also were more cross-linked proteins in the cytoskeletal fraction of the nerves in those with diabetes relative to control subjects (7).

In our population, we observed that SIF had a much stronger association with both autonomic neuropathy and CDSP than cumulative 18-year glycemic exposure. In a substudy of the DCCT (10), measures of glycated collagen demonstrated a strong association with clinical neuropathy and, in the conventional-treatment

arm of the trial, were more strongly correlated with clinical neuropathy than was cumulative HbA_{1c} or the most recent HbA_{1c}. Glycated collagen explained 36 and 16%, respectively, of the variability of confirmed clinical neuropathy and nerve conduction velocity in the intensive-treatment arm and 51 and 31%, respectively, in the conventional-treatment arm. After additional adjustment for HbA_{1c}, this variability was 37 and 14%, respectively, for confirmed clinical neuropathy and nerve conduction velocity in the intensive-treatment arm and 67 and 18%, respectively, in the conventional-treatment arm (10).

To our knowledge, only one other study has investigated the relationship between skin fluorescence and diabetic neuropathy. Meerwaldt and collegues (22) observed a relationship between skin autofluorescence, using the AGEReader, and clinical diabetic neuropathy. Autofluorescence was higher in those with diabetes (40% with type 1 diabetes) and, among those with diabetes, even higher in those with a history of neuropathic foot ulceration. It also correlated with the Wagner score for severity of foot ulceration. Irrespective of neuropathy status, autofluorescence was inversely correlated with heart rate variability, baroreflex sensitivity, and nerve conduction velocity and amplitude.

A major limitation of this study was that SIF measurements were taken, on average, 2 years after clinical assessment for neuropathy in the EDC population. Unfortunately, the SCOUT technology became available during a phase of EDC when only surveys were being collected. Because the independent variable of interest, SIF, was measured approximately 2 years after the other clinical data, including the outcome variables of interest, autonomic neuropathy and DSP, we cannot rule out reverse causation, although this would seem very unlikely because AGEs have long been thought to be one of the major etiologic factors in diabetic neuropathy. The possibility that some participants have developed clinical neuropathy in the intervening period who did not have it previously is more likely. This effect would tend to bias against our findings, thus our associations could be considered conservative. When we reanalyzed the mean difference in SIF by stratification according to the median length of the interval between the last EDC clinical exam and the SIF measurement, we found similar results for those with a longer (>1.8 years) and those with a shorter (≤1.8 years) time interval. Another limitation of these analyses is that our population was composed of middle-aged adults of long diabetes duration, and, thus, these results are not generalizable to children, young adults, or individuals with short diabetes duration. Finally, some limitations arise from the measurement of SIF itself. In a study of 2,589 subjects at risk for type 2 diabetes, the interday Hoorn coefficient of variation was 6.9% (19). A factor that contributes to this measurement to measurement variation is skin heterogeneity from freckles, hair follicles, sweat glands, and wrinkles. Skin pigmentation differences between patients and within a patient over time (more or less tan) are mitigated by measuring the reflectivity of the skin and using the measured reflectance to correct the distortion of the melanin. It also is possible that diet could influence the skin AGE concentration, and this was not controlled for in the study. Small studies have shown a relationship between dietary AGEs and serum markers of inflammation (23). Inflammatory mediators are induced by dietary glycotoxins, a major risk factor for diabetes angiopathy (24). At this point in time, the role of exogenous AGEs in skin AGE formation has not been well established.

The corollary pilot of investigating the utility of skin fluorescence in a clinic population of patients with type 1 diabetes followed primarily by a single clinician investigator demonstrates the potential applicability of this novel technology in a clinical environment. Because of the differences in ascertainment (e.g., the research environment of the EDC included additional measures to confirm diagnosis of CDSP), the results from one site cannot be generalized to the other but rather side by side give a comparative insight into the real-world clinical environment where measures to diagnose clinical neuropathy are less sensitive and specific. Additional studies in the clinical environment of a broader demographic and with greater numbers of individuals are required to generalize the findings.

In conclusion, we have demonstrated a strong association between SIF and peripheral neuropathy in middle-aged individuals with type 1 diabetes. These findings support the hypothesis that AGEs play a role in the development of diabetic neuropathy. Prospective studies are needed to demonstrate the potential role of SIF in the prediction, assessment, and monitoring of neuropathy as well as in the efficacy of therapy for diabetic neuropathy.

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B.N.C. wrote the manuscript and collected and analyzed data. V.R.A. collected data, reviewed the manuscript, and contributed to discussion. J.D.M. reviewed and edited the manuscript. N.M. analyzed data, contributed to the methods, and reviewed and edited the manuscript. S.F. analyzed data. R.E.R. collected data and reviewed the manuscript. T.J.O. conceived of the study, collected data, directed the analyses, reviewed and edited the manuscript, and contributed to discussion.

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