Clinical and technical factors associated with skin intrinsic fluorescence in subjects with type 1 diabetes from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications study

Patricia A. Cleary  
*George Washington University*

Barbara H. Braffett  
*George Washington University*

Trevor J. Orchard  
*University of Pittsburgh*

Timothy J. Lyons  
*University of Oklahoma Health Sciences Center*

John D. Maynard  
*VeraLight, Inc*

Recommended Citation


This Journal Article is brought to you for free and open access by the Biochemistry and Molecular Medicine at Health Sciences Research Commons. It has been accepted for inclusion in Biochemistry and Molecular Medicine Faculty Publications by an authorized administrator of Health Sciences Research Commons. For more information, please contact hsrc@gwu.edu.
Clinical and Technical Factors Associated with Skin Intrinsic Fluorescence in Subjects with Type 1 Diabetes from the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Study

Patricia A. Cleary, MS¹ Barbara H. Braffett, PhD¹ Trevor Orchard, MD² Timothy J. Lyons, MD³ John Maynard, MS⁴ Catherine Cowie, PhD⁵ Rose A. Gubitosi-Klug, MD, PhD⁶ Jeff Way, BS⁴ Karen Anderson, MS¹ Annette Barnie, RN, CDE⁷ Stephan Villavicencio, MS¹ and the DCCT/EDIC Research Group*

Abstract

Background: The Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) studies have established multiyear mean hemoglobin A1c (HbA1c) as predictive of microvascular complications in persons with type 1 diabetes. However, multiyear mean HbA1c is not always available in the clinical setting. Skin advanced glycation end products (AGEs) are thought to partially reflect effects of hyperglycemia over time, and measurement of skin AGEs might be a surrogate for multiyear mean HbA1c. As certain AGEs fluoresce and skin fluorescence has been demonstrated to correlate with the concentration of skin AGEs, noninvasive measurement by skin intrinsic fluorescence (SIF) facilitates the exploration of the association of mean HbA1c and other clinical/technical factors with SIF using the detailed phenotypic database available in DCCT/EDIC.

Subjects and Methods: Of the subjects, 1,185 (53% male) had measurements of SIF during years 16/17 of EDIC with mean age and diabetes duration of 51.5 and 29.8 years, respectively. SIF measurements were obtained on the underside of the forearm near the elbow using a skin fluorescence spectrometer. Demographic data and health history were self-reported, and an annual standardized examination measured clinical status. Linear regression models were constructed to identify significant clinical and technical factors associated with SIF, and the final models only used factors that were significant.

Results: SIF ranged from 8.7 to 54.0 arbitrary units and was log-normally distributed. Log(SIF) correlated more with mean HbA1c as the time period increased. In multivariate analyses log(SIF) was significantly associated with mean HbA1c, age, estimated glomerular filtration rate <60 mL/min/m², smoking status, skin tone, and clinic latitude <37°N.

Conclusions: SIF reflects age, mean HbA1c over time, smoking, and renal damage, which are known risk factors for diabetes complications.

Introduction

The Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Interventions and Complications (EDIC) study have extensively characterized a cohort of 1,441 subjects with type 1 diabetes mellitus (T1DM) over 28 years, resulting in a comprehensive phenotypic history of clinical risk factors, glycemic exposure, and development of micro- and macrovascular complications. The DCCT/EDIC studies have clearly demonstrated the causal role of glycemic exposure, as assessed by mean hemoglobin A1c (HbA1c), in development of diabetes-related microvascular...
complications. Skin advanced glycation end products (AGEs) are formed in part by exposure to glucose over time and have been shown to be independently associated with and predictive of the microvascular complications of diabetes.\(^3\,4\) Certain skin AGEs fluoresce (e.g., pentosidine, crosslines), facilitating noninvasive measurement\(^5\,6\) that could be used in clinical studies or settings.

Although the pathogenesis is poorly understood, hyperglycemia-associated tissue damage operates through formation and accumulation of AGEs.\(^8\,9\) AGEs are the products of free radical oxidation between reducing sugars and amino groups in proteins. AGE formation is considered a process that is enhanced in diabetes by elevated glucose concentrations and oxidative stress.\(^10\,11\) Studies have focused on AGE modification of skin collagen, which may reflect vessel wall stiffening, basement membrane thickening, and demyelination of nerve fibers.\(^11\,13\)

Skin AGE content accumulates with age, and this accumulation is accelerated in diabetes.\(^14\) In addition, studies have shown that AGE accumulation is higher with compromised kidney function because of reduced ability to clear AGEs from the body.\(^15\)

Skin intrinsic fluorescence (SIF) provides an opportunity for noninvasive measurement\(^3\,7\,16\) and enables larger studies than possible with biopsies and chemical assays of skin AGEs. SIF may be affected by technical factors such as environmental sun exposure and skin tone because the melanin and hemoglobin in skin can absorb the light used to excite skin fluorescence more strongly than the emitted fluorescence, leading to distortion of SIF.\(^5\,6\) The geographically diverse DCCT/EDIC study afforded the opportunity to examine the influence of these technical factors on SIF in concert with numerous clinical factors contained in the 28-year phenotypic history available in this cohort. The DCCT/EDIC study also facilitates examination of the association of SIF with time-weighted mean HbA1c to determine if SIF is an indicator of historic glycemic exposure that is often unavailable in normal clinical settings.

We therefore used the detailed phenotypic history available in the DCCT/EDIC study and measurements of SIF on 1,185 EDIC participants during years 16–17 to determine the association with SIF of glycemic exposure, other clinical factors, and technical factors that may impact the SIF measurement.

**Subjects and Methods**

**Study population**

The inclusion and exclusion criteria, treatment protocol, and baseline characteristics of the DCCT cohort have been previously described.\(^17\,18\) In brief, 1,441 subjects with T1DM who were 13–39 years old were recruited between 1983 and 1989. The primary prevention cohort consisted of 726 subjects who, at study baseline, had no retinopathy, a urinary albumin excretion rate (AER) of <40 mg/24-h period, and diabetes duration of 1–5 years. The secondary intervention cohort consisted of 715 subjects who had very mild to moderate nonproliferative retinopathy, urinary AER ≤200 mg/24-h period, and diabetes duration 1–15 years. Individuals with hypertension (defined by systolic blood pressure of ≥140 mm Hg or diastolic blood pressure of ≥90 mm Hg), a history of symptomatic ischemic heart disease, major electrocardiogram abnormalities, or severe hypercholesterolemia were excluded.

The 711 patients randomized to intensive treatment received either multiple daily insulin injections or continuous subcutaneous insulin infusion with external insulin pumps, along with frequent self-glucose monitoring. Conventional therapy patients (n = 730) were treated with one or two daily insulin injections and daily urine or blood glucose testing. The intensive and conventional treatment groups maintained median HbA1c levels of 7.0% and 9.0%, respectively, during the 6.5-year mean DCCT follow-up. In 1994, 1,375 subjects (96% of the surviving cohort) agreed to participate in the EDIC study to examine DCCT treatment effects on longer-term complications of diabetes.\(^2\) With the initiation of the EDIC study, conventional treatment participants were offered instruction in intensive therapy. EDIC participants were evaluated annually.

For the SIF substudy, all living subjects who participated during year 16 or 17 were eligible for inclusion unless they met exclusion criteria: history of extreme photosensitivity (n = 11), skin cancer (n = 10), no informed consent (n = 5), birthmarks, tattoos, skin rashes, or chemical hair removal (n = 5), or missing the study participation window (n = 73). As a result, 1,185 of 1,289 active EDIC participants were included (92%).

**SIF measurement**

Duplicate measurements of SIF were obtained from the skin on the underside of the left forearm using a SCOUT skin fluorescence spectrometer (VeraLight, Inc., Albuquerque, NM). SIF was excited with a light-emitting diode centered at 375 nm and was detected over the emission range of 435–655 nm. Skin reflectance was assessed with a white light-emitting diode over the 435–655 nm spectral region. The measured skin reflectance was used to compensate for absorbance due to melanin and hemoglobin as well as subject-specific light scattering using the intrinsic fluorescence correction\(^5\) formula expressed in Eq. 1:

\[
f_{sm} = \frac{F_{xm}}{R_{sm}R_{nm}}
\]

where the measured fluorescence, \(F_{xm}\), is divided by reflectance values from the excitation and white light-emitting diodes, \(R_x\) and \(R_{nm}\), respectively. The reflectance values are adjusted by the dimensionless exponents, \(k_x\) and \(k_m\). For these analyses, the 375-nm excited fluorescence was used with \(k_x\) set to 0.6 and \(k_m\) set to 0.2. The resulting intrinsic fluorescence, \(f_{sm}\), was integrated over the 435–655 nm spectral region and multiplied by 1,000 to represent SIF, reported in arbitrary units (AU). These values of \(k_x\) and \(k_m\) were previously determined to be relevant for the 375-nm excited fluorescence, which had the strongest association with diabetes-related complications in the Pittsburgh Epidemiology of Diabetes Complications cohort.\(^19\)

**Clinical outcomes**

Demographic data and health history were self-reported. A physical examination measured clinical status. Laboratory measurements were performed at the DCCT/EDIC Central Biochemistry Laboratory as previously described.\(^17\) HbA1c was measured every 3 months during the DCCT and yearly during the EDIC study.\(^2\,17\) Long-term stability of the HbA1c
assay has been described. AER was measured annually during the DCCT and in alternate years during the EDIC study using a timed 4-h urine collection and expressed per 24-h period. Serum lipids were measured using enzymatic methods from fasting samples obtained yearly during the DCCT and in alternate years during the EDIC study. Serum creatinine was measured annually within the DCCT/EDIC. Estimated glomerular filtration rate (eGFR) was calculated from serum creatinine specific for age, sex, and race using the Chronic Kidney Disease–Epidemiology Collaboration equation.

Time-weighted eGFR was computed by taking the mean of the eGFR values for each subject over the entire DCCT/EDIC time period up to the year the SIF measurement was performed. For all linear regression models a categorical variable of any eGFR < 60 mL/min/m² from DCCT enrollment to date of the SIF measurement was used because it was the renal variable most strongly correlated with SIF.

Clinic latitude was hypothesized to be a surrogate for potential differences in vitamin D levels due to sun exposure and was incorporated into the data analysis as a categorical variable, with EDIC clinics below latitude 37°N designated as southern clinics (n = 7) and those above as northern clinics (n = 21). Skin tone was considered a measure of the light reflected by the subject’s skin. Skin tone was measured by the SCOUT device across the 435–655 nm spectral region and was calculated by summing light reflectance in that range. Smoking status was determined by subject self-report and categorized as “never smoked” (≤ 100 cigarettes in a subject’s lifetime), “previous smoker” (quit ≥1 year ago), or “current smoker.” Subject age at the time of the SIF measurements was used for all analyses. Mean HbA1c for a given number of years was calculated by taking the mean of the HbA1c values of the given time period. Total mean HbA1c was calculated by summing (DCCT/EDIC eligibility HbA1c × duration of diabetes at study baseline), (DCCT mean HbA1c × years of follow-up in DCCT), and (EDIC mean HbA1c × years of follow-up in EDIC) and dividing by total duration of diabetes.

**Statistical analyses**

Demographic and clinical characteristics were compared using the Wilcoxon rank-sum test to evaluate treatment group differences for ordinal and numeric variables. The contingency χ² test was used for categorical variables. The first SIF measurement per subject was used for all analyses except the intrasubject, same-day variation in SIF, which was assessed using the method of the Hoorn study. The correlations between log(SIF) and EDIC phenotype data were computed by summing (DCCT/EDIC eligibility HbA1c × duration of diabetes at study baseline), (DCCT mean HbA1c × years of follow-up in DCCT), and (EDIC mean HbA1c × years of follow-up in EDIC) and dividing by total duration of diabetes.

**Results**

At the time of SIF measurement, the population had a mean age of 51.5 years and diabetes duration of 29.8 years (Table 1). The mean SIF values did not differ significantly by DCCT treatment cohort (P = 0.82) or by gender (P = 0.62). However, there was a significant difference in SIF between the primary prevention (22.2 ± 4.8 AU) and secondary intervention (23.2 ± 4.8 AU) cohorts of the DCCT (P < 0.0001), which is explained by the younger age of the primary prevention cohort (51.7 ± 7 vs. 52.7 ± 7 years, P < 0.01) and, at the start of DCCT, the shorter duration of diabetes (1–5 years vs. 1–15 years) and absence of renal compromise (AER < 40 mg/24-h period).

In the 1,185 EDIC subjects with SIF measurement, the intraday Hoorn coefficient of variation was 4.2%, and the between-measurement correlation was 0.963 (R² = 0.927), which explains 7.3% of the total variance in the SIF measurement. The range of SIF values in the EDIC cohort was 8.7–54.0 AU, with a mean value of 22.7 ± 4.8 AU. The distribution had a log normal characteristic, so SIF was natural logarithm-transformed for further statistical analyses, resulting in a mean value of the log(SIF) of 3.10 ± 0.21 AU.

The correlations (R²) of log(SIF) with increasing lengths of glycemic exposure as assessed by mean HbA1c are reported in Table 2. Each row represents a different interval of glycemic exposure, ranging from the most recent 5 years of EDIC to the subject’s glycemic exposure from entry into DCCT to the most recent year of EDIC (29.8 ± 4.9 years). For all time intervals, the intensive therapy group showed a lower correlation with current SIF than did the conventional therapy group. The correlation of log(SIF) with glycemic exposure increased with increasing length of glycemic exposure from an R² of 0.035 for the most recent 5 years of EDIC to 0.063 for all of the EDIC study. As shown in Table 2, the trend of increasing correlation with time holds true for all time periods in the conventional therapy group, but in the intensive therapy group, the addition of the DCCT period to the EDIC period disrupts the trend, resulting in lower correlation.

To determine the clinical factors associated with log(SIF), univariate and multivariate associations were examined for factors that could be influential, including time-weighted mean HbA1c, age, gender, body mass index, DCCT treatment group, DCCT cohort assignment, duration of diabetes, use of statins at any time, time-weighted mean high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and diastolic blood pressure. Through multivariate analysis (see Table 1), we found the following clinical/technical factors significantly associated with log(SIF): glycemic exposure, age, any eGFR < 60 mL/min/m², smoking status, skin tone, and clinic latitude. These variables were included in the multivariate models for log(SIF). Because there was not a significant interaction due to DCCT treatment group or primary versus secondary cohort,
Table 1. Characteristics of the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications Skin Intrinsic Fluorescence (SIF) Population at the Time of SIF Assessment Overall and by Original DCCT Treatment Group

<table>
<thead>
<tr>
<th>Former treatment group</th>
<th>Intensive</th>
<th>Conventional</th>
<th>P value</th>
<th>Overall</th>
<th>Univariate R² with log(SIF)</th>
<th>P value</th>
<th>Multivariate R² with log(SIF)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number of patients</td>
<td>612</td>
<td>573</td>
<td>—</td>
<td>1,185</td>
<td></td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>SIF (arbitrary units)</td>
<td>22.6±4.7</td>
<td>22.7±4.9</td>
<td>0.8207</td>
<td>22.7±4.8</td>
<td></td>
<td>0.005</td>
<td>0.0237</td>
<td>0.0001</td>
</tr>
<tr>
<td>Demographic characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>317 (52)</td>
<td>308 (54)</td>
<td>0.5006</td>
<td>625 (53)</td>
<td>&lt;0.001</td>
<td>0.8482</td>
<td></td>
<td>0.003</td>
</tr>
<tr>
<td>Age (years)</td>
<td>51.9±6.9</td>
<td>51.0±6.9</td>
<td>0.0203</td>
<td>51.5±6.9</td>
<td>0.136</td>
<td>&lt;0.0001</td>
<td>0.110</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>30.0±4.9</td>
<td>29.5±4.9</td>
<td>0.0990</td>
<td>29.8±4.9</td>
<td>0.015</td>
<td>&lt;0.0001</td>
<td>0.0615</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>29.4±9.3</td>
<td>28.3±4.9</td>
<td>0.0635</td>
<td>28.9±7.5</td>
<td>&lt;0.001</td>
<td>0.6615</td>
<td>&lt;0.0001</td>
<td>0.2181</td>
</tr>
<tr>
<td>Cohort assignment, primary</td>
<td>298 (49)</td>
<td>294 (51)</td>
<td>0.3681</td>
<td>292 (50)</td>
<td>0.014</td>
<td>&lt;0.0001</td>
<td>0.6187</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Skin tone, arbitrary units</td>
<td>260.4±46.8</td>
<td>255.6±48.8</td>
<td>0.0513</td>
<td>258.1±47.8</td>
<td>0.015</td>
<td>&lt;0.0001</td>
<td>0.036</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Clinic latitude &gt;37° N</td>
<td>444 (73)</td>
<td>427 (75)</td>
<td>0.4423</td>
<td>871 (74)</td>
<td>0.017</td>
<td>&lt;0.0001</td>
<td>0.015</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Smokingb</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>372 (61)</td>
<td>352 (61)</td>
<td>0.7436</td>
<td>724 (61)</td>
<td></td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>Ever smoker</td>
<td>154 (25)</td>
<td>149 (26)</td>
<td>0.065</td>
<td>303 (26)</td>
<td></td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>Current smoker</td>
<td>86 (14)</td>
<td>72 (13)</td>
<td>0.4953</td>
<td>158 (13)</td>
<td></td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>ESRA (mg/dL)</td>
<td>6 (1)</td>
<td>8 (1)</td>
<td>0.5080</td>
<td>14 (1)</td>
<td>0.013</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>Time-weighted serum creatinine (mg/dL)</td>
<td>0.8±0.2</td>
<td>0.8±0.2</td>
<td>0.4565</td>
<td>0.8±0.2</td>
<td>0.019</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>Time-weighted log(AER)</td>
<td>2.5±0.7</td>
<td>2.7±0.9</td>
<td>0.0022</td>
<td>2.6±0.8</td>
<td>0.026</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>Any eGFR &lt;60 mL/min/m² to date</td>
<td>40 (7)</td>
<td>44 (8)</td>
<td>0.4436</td>
<td>84 (7)</td>
<td>0.056</td>
<td>&lt;0.0001</td>
<td>0.017</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Ever use of statins (yes vs. no)</td>
<td>360 (59)</td>
<td>329 (57)</td>
<td>0.6238</td>
<td>689 (58)</td>
<td>0.005</td>
<td>0.0130</td>
<td>0.001</td>
<td>0.1578</td>
</tr>
<tr>
<td>Time-weighted HDLC (mg/dL)</td>
<td>55.3±13.4</td>
<td>107.9±21.0</td>
<td>0.7300</td>
<td>55.3±12.8</td>
<td>&lt;0.001</td>
<td>0.9841</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>Time-weighted TDLC (mg/dL)</td>
<td>109.7±19.9</td>
<td>55.3±12.1</td>
<td>0.1789</td>
<td>108.9±20.5</td>
<td>0.013</td>
<td>&lt;0.0001</td>
<td>0.001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Time-weighted triglycerides (mg/dL)</td>
<td>86.6±42.2</td>
<td>83.1±42.1</td>
<td>0.0389</td>
<td>84.9±42.2</td>
<td>0.008</td>
<td>0.0018</td>
<td>0.3981</td>
<td></td>
</tr>
<tr>
<td>Time-weighted BP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diastolic</td>
<td>74.4±5.2</td>
<td>74.1±5.1</td>
<td>0.3991</td>
<td>74.3±5.2</td>
<td>&lt;0.001</td>
<td>0.8704</td>
<td>&lt;0.001</td>
<td>0.3540</td>
</tr>
<tr>
<td>Systolic</td>
<td>118.8±8.1</td>
<td>118.5±8.4</td>
<td>0.6217</td>
<td>118.7±8.3</td>
<td>0.025</td>
<td>&lt;0.0001</td>
<td>0.5828</td>
<td></td>
</tr>
<tr>
<td>Use of antihypertensive medications ever</td>
<td>340 (56)</td>
<td>337 (59)</td>
<td>0.2575</td>
<td>677 (57)</td>
<td>0.014</td>
<td>&lt;0.0001</td>
<td>0.1907</td>
<td></td>
</tr>
<tr>
<td>Most recent HbA1c (%)</td>
<td>7.9±1.2</td>
<td>7.9±1.2</td>
<td>0.3392</td>
<td>7.9±1.2</td>
<td>0.022</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>EDIC mean HbA1c (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last 5 years</td>
<td>7.9±1.1</td>
<td>7.8±1.1</td>
<td>0.1637</td>
<td>7.9±1.1</td>
<td>0.035</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>Last 10 years</td>
<td>7.9±1.1</td>
<td>7.8±1.1</td>
<td>0.1293</td>
<td>7.9±1.1</td>
<td>0.046</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>All years</td>
<td>8.0±1.1</td>
<td>7.9±1.0</td>
<td>0.7549</td>
<td>8.0±1.0</td>
<td>0.063</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>EDIC+DCCT mean HbA1c (%)</td>
<td>7.8±0.9</td>
<td>8.2±0.9</td>
<td>&lt;0.0001</td>
<td>8.0±1.0</td>
<td>0.058</td>
<td>&lt;0.0001</td>
<td>Not used</td>
<td>—</td>
</tr>
<tr>
<td>EDIC+DCCT+baseline mean HbA1c (%)</td>
<td>8.2±0.9</td>
<td>8.4±0.9</td>
<td>&lt;0.0001</td>
<td>8.2±0.9</td>
<td>0.070</td>
<td>&lt;0.0001</td>
<td>0.043</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Data are n (%) or mean±SD values. P values are for treatment group differences comparing former intensive versus conventional therapy based on the contingency χ² test for qualitative variables and the Wilcoxon rank-sum test for quantitative variables.

aData are squared semipartial R² correlations from a linear regression model regressing log(SIF) on all potential explanatory variables, resulting in a R² of 0.345 for the full model. Variables that were redundant or weaker than other similar variables are marked as not used and were not included in the model. Specifically, Epidemiology of Diabetes Interventions and Complications (EDIC) + DCCT baseline mean hemoglobin A1c (HbA1c) trumped all other mean HbA1c variables, triglycerides trumped high-density lipoprotein cholesterol (HDLC) and low-density lipoprotein cholesterol (LDLC), and any estimated glomerular filtration rate (eGFR) <60 mL/min/m² trumped continuous eGFR, serum creatinine, and end-stage renal disease (ESRD).

bCurrent smoker defined as currently smoking or smoking within the last year; ever smoker defined as smoking more than a year ago.

cTotal mean HbA1c is calculated by summing (DCCT/EDIC eligibility HbA1c × duration of diabetes at study baseline), (DCCT mean HbA1c × years of follow-up on DCCT), and (EDIC mean HbA1c × years of follow-up in EDIC) and dividing by total duration of diabetes.
Table 2. Log(Skin Intrinsic Fluorescence) Correlation with Increasing Lengths of Glycemic Exposure as Assessed by Time-Weighted Mean Hemoglobin A1c, Stratified by Original Diabetes Control and Complications Trial Treatment Group

<table>
<thead>
<tr>
<th></th>
<th>EDIC mean HbA1c</th>
<th>EDIC + DCCT mean HbA1c</th>
<th>EDIC + DCCT + baseline mean HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intensive</td>
<td>Conventional</td>
<td>Overall</td>
</tr>
<tr>
<td></td>
<td>group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last 5 years</td>
<td>0.027</td>
<td>0.046</td>
<td>0.035</td>
</tr>
<tr>
<td>Last 10 years</td>
<td>0.036</td>
<td>0.059</td>
<td>0.046</td>
</tr>
<tr>
<td>All years</td>
<td>0.052</td>
<td>0.078</td>
<td>0.063</td>
</tr>
</tbody>
</table>
| Data are squared semipartial $R^2$ correlations from five separate linear regression models regressing log(skin intrinsic fluorescence [SIF]) on various glycemic exposure intervals. $^a$Total mean hemoglobin A1c (HbA1c) is calculated by summing (Diabetes Control and Complications Trial [DCCT]/Epidemiology of Diabetes Intervention and Control [EDIC] eligibility HbA1c $\times$ duration of diabetes at study baseline), (DCCT mean HbA1c $\times$ years of follow-up on DCCT), and (EDIC mean HbA1c $\times$ years of follow-up in EDIC) and dividing by total duration of diabetes. $^b$Current smoker defined as currently smoking or smoking within the last year; ever smoker defined as smoking more than a year ago.

data are squared semipartial $R^2$ correlations from five separate linear regression models regressing log(skin intrinsic fluorescence [SIF]) on various glycemic exposure intervals. $^a$Total mean hemoglobin A1c (HbA1c) is calculated by summing (Diabetes Control and Complications Trial [DCCT]/Epidemiology of Diabetes Intervention and Control [EDIC] eligibility HbA1c $\times$ duration of diabetes at study baseline), (DCCT mean HbA1c $\times$ years of follow-up on DCCT), and (EDIC mean HbA1c $\times$ years of follow-up in EDIC) and dividing by total duration of diabetes. $^b$Current smoker defined as currently smoking or smoking within the last year; ever smoker defined as smoking more than a year ago.

data are squared semipartial $R^2$ correlations from five separate linear regression models regressing log(skin intrinsic fluorescence [SIF]) on various glycemic exposure intervals. $^a$Total mean hemoglobin A1c (HbA1c) is calculated by summing (Diabetes Control and Complications Trial [DCCT]/Epidemiology of Diabetes Intervention and Control [EDIC] eligibility HbA1c $\times$ duration of diabetes at study baseline), (DCCT mean HbA1c $\times$ years of follow-up on DCCT), and (EDIC mean HbA1c $\times$ years of follow-up in EDIC) and dividing by total duration of diabetes. $^b$Current smoker defined as currently smoking or smoking within the last year; ever smoker defined as smoking more than a year ago.

Table 3. Association of Log(Skin Intrinsic Fluorescence) with Varying Glycemic Exposure Intervals

<table>
<thead>
<tr>
<th></th>
<th>$\beta$ ($R^2$) at glycemic exposure interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EDIC</td>
</tr>
<tr>
<td>Mean HbA1c (%)</td>
<td>0.028 (0.023)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.011 (0.137)</td>
</tr>
<tr>
<td>Any eGFR &lt; 60 mL/min/m²</td>
<td>0.140 (0.030)</td>
</tr>
<tr>
<td>to date (yes vs. no)</td>
<td>0.070 (0.058)</td>
</tr>
<tr>
<td>Smoking (current, former, never $^b$)</td>
<td>0.0008 (0.034)</td>
</tr>
<tr>
<td>Skin tone (arbitrary units)</td>
<td>0.0008 (0.034)</td>
</tr>
<tr>
<td>Clinic latitude $&gt;37^b$ (south vs. north)</td>
<td>0.055 (0.014)</td>
</tr>
<tr>
<td>Total $R^2$ (all variables)</td>
<td>0.308</td>
</tr>
</tbody>
</table>

Data are $\beta$ coefficients and squared semipartial $R^2$ correlations from five separate linear regression models regressing log(skin intrinsic fluorescence) on various glycemic exposure intervals. Each model was simultaneously adjusted for age, any estimated glomerular filtration rate (eGFR) $< 60$ mL/min/m², smoking status, skin tone, and clinic latitude. $^a$Total mean hemoglobin A1c (HbA1c) is calculated by summing (Diabetes Control and Complications Trial [DCCT]/Epidemiology of Diabetes Intervention and Control [EDIC] eligibility HbA1c $\times$ duration of diabetes at study baseline), (DCCT mean HbA1c $\times$ years of follow-up on DCCT), and (EDIC mean HbA1c $\times$ years of follow-up in EDIC) and dividing by total duration of diabetes. $^b$Current smoker defined as currently smoking or smoking within the last year; ever smoker defined as smoking more than a year ago.
FIG. 1. (A) Unadjusted and (B) adjusted log(skin intrinsic fluorescence [SIF]) versus 25-year mean hemoglobin A1c (HbA1c). Adjustment was done simultaneously for age, any estimated glomerular filtration rate < 60 mL/min/m², smoking status, skin tone, and clinic latitude.
defense may be important. As shown in our own and other studies, among people without diabetes, the interindividual rate of accumulation of specific AGE products in skin can vary by a factor of 2. Also, in both people without diabetes and those with T1DM, we found similar interindividual variation in the rate of accumulation of oxidized methionine residues in skin collagen. Taken together, these findings suggest a hypothesis that, in the absence of diabetes, the large variation in the rate of AGE accumulation is possibly linked to individual specific oxidative stress. Unfortunately, there is no convenient measure for “oxidative stress,” so we were unable to assess its contribution to SIF.

We did find similarities with DCCT skin biopsy data collected in 1992. The article of Monnier et al.\(^5\) reports correlations with mean HbA1c and biopsy for the six different AGE assays after adjustment for age and duration of diabetes that range from 0.205 (\(R^2 = 0.042\)) for acid-soluble collagen to 0.440 (\(R^2 = 0.194\)) for furosine. The adjusted SIF measurement had a correlation of 0.46 (0.414–0.503) to 25-year mean HbA1c (Fig. 1B), which is of the same magnitude as the furosine correlation, although with the exception of age, the SIF adjustments were different than those used for furosine. It is interesting that SIF was correlated with the furosine assay (\(R = 0.25\)) in the 175 subjects who had both skin biopsy in 1992 and the SIF measurement 17–19 years later. Finally, although the 1992 biopsy data showed significant differences in AGE products between the intensive and conventional therapy groups, there was no difference in SIF between these groups, possibly due to study-wide adoption of intensive therapy and the passage of time.

Loss to follow-up during the EDIC study was higher in subjects who developed end-stage renal disease or who had a coronary artery calcification score of >200 Agatston units, potentially giving rise to a survivor bias, which may have eliminated differences in SIF between DCCT treatment groups for these outcomes (Table 1). However, compared with the few (\(n = 104\)) active DCCT/EDIC participants without an SIF measure, those examined showed no differences in age (\(P = 0.97\)) and duration of diabetes (\(P = 0.64\)). Nonparticipants had a higher mean total (EDIC + DCCT + baseline) HbA1c (8.4 ± 1.0% vs. 8.2 ± 0.9%; \(P = 0.02\)) (data not shown).

In subjects without diabetes, chronological age correlates more strongly with SIF (\(R^2 = 0.261\))\(^{28,29}\) than in the current study (\(R^2 = 0.147\)). We hypothesize that the higher and more variable glycomic exposure in subjects with T1DM as well as increased likelihood of compromised renal function contributed to the weakened correlation of chronological age with SIF in the EDIC SCOUT substudy cohort. Several studies have shown that renal failure leads to increased levels of skin AGEs independent of glycemic control, and the combination of diabetes plus renal failure can lead to high levels of skin AGEs and SIF.\(^{15,30,31}\)

We hypothesized the variance explained by skin tone was due to imperfections in the intrinsic correction of the measured fluorescence. To test this hypothesis, we changed the intrinsic correction coefficient, \(k_0\), from a value of 0.6 to 0.8 to compute new SIF values and then rebuilt the multivariate models. For the new SIF variable, the model \(R^2\) increased from 0.33 to 0.46, and the variance explained by skin tone increased from 0.034 to 0.161, whereas the variance explained by the other model variables decreased. This analysis confirmed that the primary function of the skin tone variable was to compensate for imperfections in the intrinsic correction.

The clinic latitude variable was used to describe subjects who attended DCCT clinics above 37° N latitude. As shown in the multivariate analysis, clinic latitude did contribute to a small but statistically significant portion of the variance in SIF. However, we cannot definitively say whether this was due to differences in vitamin D, sun exposure, genetics, or lifestyle.

Approximately 13% of the study cohort were current smokers, and another 26% were former smokers. As smoking is known to enhance AGE formation and is a source of oxidative stress,\(^{28}\) the finding that smoking is a contributor to the variance in SIF is consistent with the literature.

Imperfections of SIF that could contribute to the unexplained variance include the superposition of epidermal fluorescence from NADH and FAD\(^{28}\) on dermal AGE fluorescence and interday, within-subject measurement variance. As NADH and FAD are not as stable as dermal AGEs and their levels fluctuate in response to changes in metabolism and oxidative stress,\(^{34}\) their superposition on the dermal AGE signal could reduce the reflection of glycemic exposure in SIF because the SIF signal only takes into account the intensity of the fluorescence and not the spectral shapes.

Finally, we tested to see if instrument bias was a source of error by adding clinic number to the multivariate model because there was a one-to-one mapping between instruments and clinics. This test showed that instrument bias was not a significant contributor to the overall variance in SIF.

Strengths of this study include the excellent characterization of 92% of all living EDIC subjects. Potential weaknesses include the absence of measures of smoking by pack-years and the absence of measures of long-term oxidative stress. In addition, there are gaps in the HbA1c history due to annual measurement of HbA1c during the EDIC study instead of quarterly during the DCCT. Finally, use of clinic latitude as an indicator of vitamin D levels or sun exposure is an approximation.

**Conclusions**

In the DCCT/EDIC cohort, clinical factors significantly associated with SIF include age, smoking status, time-weighted mean HbA1c, skin tone, clinic latitude >37° N, and any eGFR <60 mL/min/m². The unadjusted SIF is weakly but significantly correlated (\(R = 0.26\)) with glycemic exposure, and the strength of the correlation increases with increasing length of glycemic exposure with the exception that the HbA1c during the DCCT did not contribute to current SIF values for the intensive treatment group. When SIF is predicted by a multivariate model that uses the aforementioned clinical factors, the correlation with glycomic exposure becomes modest (\(R = 0.46\)).

These findings suggest that SIF may be associated with diabetes-related complications because it reflects age, long-term glycomic exposure, smoking, and chronic kidney disease, which are known correlates and risk factors.

**Acknowledgments**

The authors would like to thank all members of the DCCT/EDIC Research Group.\(^{25}\) The authors would also like to thank the contributors of free or discounted supplies and/or equipment: Abbott, Animas, Aventis, BD, Bayer, Can-AM, Eli
Lilly, Lifescan, Medtronic Minimed, Omron, and Roche. In addition, the authors would like to acknowledge support from contracts with the Division of Diabetes, Endocrinology and Metabolic Diseases of the National Institute of Diabetes and Digestive and Kidney Diseases, the National Eye Institute, the National Institute of Neurological Disorders and Stroke, the General Clinical Research Centers Program and the Clinical and Translational Science Awards Program, National Center for Research Resources, and VeraLight and by Genentech through a Cooperative Research and Development Agreement with the National Institute of Diabetes and Digestive and Kidney Diseases. P.A.C. and J.M. researched the data, wrote significant portions of the manuscript, reviewed, edited the manuscript, and contributed to the discussion. B.H.B. researched the data, wrote a portion of the manuscript, reviewed/edited the manuscript, and contributed to the discussion. T.O., T.J.L., C.C., and R.A.G.-K. each wrote a portion of the manuscript, reviewed/edited the manuscript, and contributed to the discussion. J.W. and K.A. researched the data. A.B. reviewed/edited the manuscript and contributed to the discussion. J.W. and K.A. researched the data. A.B. reviewed/edited the manuscript and contributed to the discussion. S.V. reviewed/edited the manuscript.

Author Disclosure Statement

P.A.C., B.H.B., C.C., R.A.G.-K., K.A., and S.V. have no relevant conflicts of interest to disclose. J.M. and J.W. are employees and stock option holders of VeraLight, Inc. A.B. is an independent insulin pump trainer who receives payment from Medtronic, Animas, Spirit, and Omnipod pumps. T.O. has received grant support from VeraLight, Inc. and serves as a consultant to Gilead and Aegerion. T.J.L. has received grant support from VeraLight, Inc. in the past.

References


Address correspondence to:
Patricia A. Cleary, MS
George Washington University
6110 Executive Boulevard, Suite 750
Rockville, MD 20852
E-mail: cleary@bsc.gwu.edu
This article has been cited by: