Skin Intrinsic Fluorescence Is Associated With Hemoglobin A_{1c} and Hemoglobin Glycation Index but Not Mean Blood Glucose in Children With Type 1 Diabetes

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OBJECTIVE—To evaluate the relationship between skin advanced glycation end products (sAGEs) with mean blood glucose (MBG), hemoglobin A_{1c} (HbA_{1c}), and MBG-independent, between-patient differences in HbA_{1c} among children with type 1 diabetes.

RESEARCH DESIGN AND METHODS—Children aged 5 to 20 years with type 1 diabetes of at least 1 year duration participated. At a clinic visit, sAGE was estimated noninvasively by measurement of skin intrinsic fluorescence (SIF). SIF data were adjusted to correct for variation in skin pigmentation. MBG-independent, between-patient differences in HbA_{1c} were examined by statistically controlling HbA_{1c} for MBG or alternatively by use of a hemoglobin glycation index (HGI). Results were similar whether HbA_{1c}, MBG, and HGI were analyzed as single values from the time of the SIF examination visit or as the mean values from all available visits of the patient.

RESULTS—HbA_{1c} was correlated with MBG ($r = 0.5$; $P < 0.001$; $n = 110$). HbA_{1c} and HGI, but not MBG, were statistically associated with SIF after adjustment for age, duration of diabetes, race, sex, and BMI *z*-score. SIF increased with age and duration of diabetes and was higher in girls than boys.

CONCLUSIONS—sAGE levels estimated by SIF increase with age, duration of diabetes, and female sex. sAGE is correlated with MBG-independent biological variation in HbA_{1c}, but not with MBG itself. These results suggest that factors besides MBG that influence HbA_{1c} levels also contribute to accumulation of sAGE.

Diabetes Care 34:1816–1820, 2011

Chronically elevated blood glucose levels are associated with the development of serious microvascular complications in patients with type 1 diabetes (1). One mechanism for development of complications is through enhanced nonenzymatic glycation of important proteins facilitated by hyperglycemia (2,3). As conventionally understood, the formation of glycated proteins is dependent on glucose concentration. For example, over short periods of time glucose can reversibly attach to hemoglobin and other proteins to form a Schiff base. With more time, the Schiff base can chemically rearrange in a nearly irreversible process to form an Amadori product, glycated hemoglobin (HbA_{1c}) (4). Clinically, HbA_{1c} is highly correlated with mean blood glucose (MBG) levels from the preceding weeks and months (5), and both have been shown to be predictors for the development and progression of microvascular diabetes complications (1,6).

Other proteins besides hemoglobin are also susceptible to nonenzymatic glycation. Structural proteins have a longer life span than the 120 days typical of hemoglobin. Over weeks to months, the glycated moieties of long-lived proteins can form covalent complexes known as advanced glycation end products (AGEs). Formation of AGEs in tissues causes alteration in the normal structure and function of the involved proteins, which can elicit pathological changes (2,3). Tissue burden of AGEs has been assessed by direct chemical measurement of these substances from biopsy samples. The Epidemiology of Diabetes Interventions and Complications (EDIC) study found that AGE levels from biopsied skin were predictive for the progression of microvascular complications in patients with type 1 diabetes (7,8). Furthermore, these investigators found that HbA_{1c} levels were correlated with skin AGE burden (8). Thus concentrations of an early-stage, shorter-lived glycation product, HbA_{1c}, are associated with the amount of AGEs accumulated in tissue over time. Thus both HbA_{1c} and AGEs appear to be predictors for development and progression of microvascular diabetes complications (8).

The high correlation between HbA_{1c} and MBG has led to the more easily measurable HbA_{1c} becoming a widely used method to estimate glycemic control and guide therapy in patients with diabetes. In addition to MBG, HbA_{1c} levels have been shown to be influenced by MBG-independent factors, which are manifested by consistent between-individual differences in HbA_{1c} level regardless of the preceding background MBG (9,10). Previously, we developed a hemoglobin glycation index (HGI) to quantify such between-patient

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Received 9 January 2011 and accepted 5 May 2011.
DOI: 10.2337/dc11-0049
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc11-0049/-/DC1.

differences from readily available clinical data (9–11). We found that MBG-independent between-patient HbA_{1c} differences are predictive for development of diabetes complications (10). As HbA_{1c} and MBG are highly correlated, we hypothesized that tissue AGEs would be associated with both MBG from patient self-monitored capillary blood samples as well as corresponding HbA_{1c} levels. Furthermore, we hypothesized that MBG-independent differences in HbA_{1c} would also be associated with tissue accumulation of AGEs.

Recently, methods have been developed to noninvasively estimate skin AGEs in vivo by measurement of skin intrinsic fluorescence (SIF) (12–15). SIF is highly correlated with AGEs assayed directly from skin biopsy (15). The technology can rapidly and noninvasively estimate skin AGEs from large numbers of individuals and thus is particularly suitable for use in children. We used the SIF technology to test our hypotheses in a well-characterized group of children with type 1 diabetes.

RESEARCH DESIGN AND METHODS

Patient recruitment

Children with type 1 diabetes of at least 1 year duration, attending diabetes clinics at Children's Hospital New Orleans, were invited to participate. Informed consent was obtained from parents and consent/assent from the patient. Data regarding duration of diabetes, height, weight, date of birth, race, and sex were collected at the time of visit, and additional data regarding prior HbA_{1c} and MBG levels were extracted from patient medical records. The study was approved by the institutional review board at Louisiana State University Health Sciences Center and the Children's Hospital New Orleans.

Assessment of glycemic control

At the time of clinic visit, the patient's glucose meter was inspected for proper operation. If no problems were found, the MBG from the 30 days prior to the visit was calculated and recorded. A blood sample at the time of the clinic visit was obtained for HbA_{1c} and glucose (blood glucose at time of clinic visit [cBG], measured by Accu-Chek Inform, Model 2001201, Roche Diagnostics, Indianapolis, IN). HbA_{1c} was measured using a National Glycohemoglobin Standardization Program–certified assay. The HGI quantifies between-individual biological variation in HbA_{1c} not due to MBG. HGI at the time of the clinic visit was calculated as the difference between a patient's observed HbA_{1c} minus the HbA_{1c} level predicted from the patient's observed MBG based on the population regression of HbA_{1c} on MBG (predicted $HbA_{1c} = 0.021 \times MBG + 4.3$) (16). We have previously shown the intra- and interindividual consistency over time of HbA_{1c}, MBG, and HGI collected from clinic data (16).

Measurement of SIF

During one clinic visit, SIF levels were noninvasively measured from the volar surface of the left forearm from each subject using a SCOUT DS instrument (VeraLight, Inc.). The device sequentially excited the skin surface using different light-emitting diodes (LEDs) that had peak excitation wavelengths of 375, 405, and 420 nm. The LEDs excite varying degrees of fluorescence from different fluorophores such as AGEs. Fluorescence generated from each LED was detected over a 435–600-nm emission window. Skin reflectance was measured for each excitation LED, and a white light LED was used to measure skin reflectance over the emission region.

Spectral data collected by the instrument were transmitted directly to VeraLight, Inc. for further analysis to adjust for the impact of skin pigmentation, hemoglobin content, light scattering, and other dermal characteristics. Data were adjusted mathematically using two different k_x/k_m parameter sets on the measured reflectance at the excitation and emission wavelengths. For the first k_x/k_m set (designated as set u), k_x and k_m were 1.0 and 0.0, respectively. For the second k_x/k_m set (set c), k_x and k_m were 0.5 and 0.5, respectively, which is useful for populations with a wide range of skin melanin (17) (further technical detail in Supplementary Data). SIF data are reported in arbitrary relative fluorescence units as a function of excitation wavelength and which k_x/k_m adjustment ("u" or "c") used. VeraLight, Inc. investigators were blinded to the clinical and laboratory status of the patients.

Statistical analysis

Data were analyzed using HbA_{1c}, MBG, HGI, and cBG at the time of the visit when SIF was measured. In addition, the means of MBG, HbA_{1c}, and HGI from all available prior clinic visits for the patient were also evaluated. As results were similar whether using data for that clinic visit or the mean of data from all available clinic visits, we report here the mean data. Simple correlation analyses between variables were performed by Pearson method. The influence of biological variation in HbA_{1c} on SIF was evaluated in multivariate regression models using either HGI or by substituting HbA_{1c} adjusted for MBG in the model. For evaluation purposes, cBG was substituted in the models for MBG for single clinic visits. Models were further adjusted for chronological age, duration of diabetes, sex, race, and BMI *z*-score (z-BMI) using the GLM procedure in SAS software (SAS Institute, Cary, NC). Statistical significance was considered to be at $P < 0.05$.

RESULTS—Characteristics of the patient population studied at the time of SIF measurement in clinic are presented in Table 1. In the patient population, mean HbA_{1c} ($mHbA_{1c}$) was correlated with mean MBG (mMBG) ($r = 0.5$; $P < 0.001$) but not the visit cBG ($r = 0.07$; $P = 0.51$). mMBG and cBG were not correlated with each other, or with mHGI. $mHbA_{1c}$ was correlated with mean HGI (mHGI) ($r = 0.8$; $P < 0.001$). The SIF data for the three excitation wavelengths were highly intercorrelated within the same adjustment set whether "u" or "c". However, correlations between "u" and "c" data were considerably lower (Supplementary Table 3).

Results from the single visit data were similar to the results using mean values from multiple visits. Data are reported as the mean from multiple visits. Simple correlation analyses were performed between "u" and "c" adjustments of SIF with mMBG, $mHbA_{1c}$, mHGI, age, and duration of diabetes (Table 2). The average number of prior patient visits was 8.2 with a range of 1–21. mHGI and $mHbA_{1c}$ were correlated with all SIF data whether "u" or "c" was adjusted. mMBG, cBG, and z-BMI were not correlated with any of the SIF data. Age and duration of diabetes

Table 1—*Patient characteristics at time of clinic visit* (n = 110)

Age (years)	13.2 ± 3.8
Race	
White	82 (71%)
Black	33 (29%)
Sex (M/F)	60/55
Duration of diabetes (years)	5.9 ± 3.6
BMI (kg/m^2)	21.6 ± 4.5
z-BMI	0.6 ± 0.9
HbA_{1c} (%)	9.5 ± 2.0
MBG (mg/dL)	204.6 ± 39.6
cBG (mg/dL)	239.9 ± 93.3

Table 2—*Pearson correlation between "u" and "c" set SIF adjustments with mMBG, $mHbA_{1c}$, mHGI, age, and duration of diabetes*

	mMBG	$mHbA_{1c}$	mHGI	Age	Duration of diabetes
SIF375u	0.14	0.39	0.39	0.02	0.01
	0.14	<0.0001	<0.0001	0.82	0.89
SIF405u	0.12	0.42	0.44	0.22	0.14
	0.22	<0.0001	<0.0001	0.02	0.14
SIF420u	0.10	0.41	0.43	0.28	0.18
	0.30	<0.0001	<0.0001	0.002	0.05
SIF375c	0.15	0.38	0.34	0.45	0.39
	0.12	<0.0001	0.0003	<0.0001	<0.0001
SIF405c	0.05	0.26	0.26	0.53	0.39
	0.60	0.005	0.007	<0.0001	<0.0001
SIF420c	0.04	0.22	0.21	0.52	0.38
	0.69	0.02	0.02	<0.0001	<0.0001

The top number in each cell is the correlation coefficient (*r*), and the bottom number is the significance level (*P* value); $n = 110$.

were consistently correlated with all "c"-adjusted SIF data.

Multivariate regression analysis showed that $mHbA_{1c}$ adjusted for mMBG, or mHGI substituted in the model for $mHbA_{1c}$, was consistently associated with all SIF excitation wavelengths, both "u" and "c", after also controlling for presence of age, race, sex, z-BMI, and duration of diabetes (Table 3). mMBG and z-BMI were not statistically significant in relationship with any of the SIF measures. Figure 1 depicts the relationship between 405-nm excited SIF using set "c" adjustment versus mMBG, mHGI, or $mHbA_{1c}$.

Age, duration of diabetes, and sex were statistically significant covariates for the "c" SIF data at all three wavelengths. SIF levels increased with age and were higher in girls than boys at any given age (Fig. 2). Race was a significant covariate for all SIF excitation wavelengths with the "u" adjustment, but only for 375 nm in the "c"-adjusted SIF data, blacks being higher than whites.

CONCLUSIONS—To our knowledge this is the first in-depth study of the relationship of skin AGEs (sAGEs) with HbA_{1c}, MBG, HGI, and other characteristics in a biracial population of children with type 1 diabetes. We used a novel technology to estimate sAGEs by measuring SIF using a SCOUT DS device at three different excitation wavelengths and two mathematical adjustment sets. Prior reports using this device have focused on its ability to identify adults with abnormalities of glucose tolerance (13,14) and the relationship of SIF with coronary artery calcifications in type 1 diabetes (12).

Spectral information read by the SCOUT device from the skin is a combination of reflected light and fluorescence excited in the tissue by LEDs. Because of variability in skin pigmentation, skin thickness, hemoglobin, and other factors, the excitation and emission data returning to the device must be adjusted to minimize the distortion of the skin fluorescence by the aforementioned factors. How adjustment can affect interpretation of data can be seen by comparing "u" and "c" adjustments of the SIF data (Table 3). With the "u" adjustment, all data from the three excitation SIF wavelengths evaluated were consistently higher in black than in white patients, consistent with differences in skin pigmentations. Such a difference was only found at the 375-nm SIF excitation wavelength with the "c" adjustment. Thus the "c" adjustment eliminated a considerable amount of artifact due to skin pigmentation. sAGEs and auto-fluorescence have been shown to increase with aging and duration of diabetes in dermal biopsy samples from patients with diabetes (15,18,19). However, the "u" adjustment from our SIF data was insensitive to differences related to duration of diabetes and less

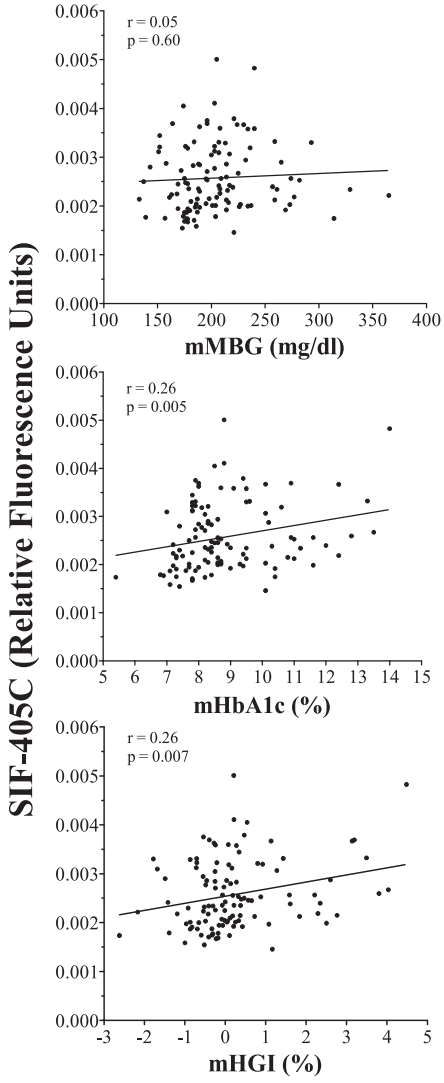

Figure 1—*The relationship between 405-nm excited SIF using set "c" adjustment with mMBG, mHGI, and $mHbA_{1c}$.*

Table 3—*Multivariate regression analysis between "u"- and "c"-adjusted SIF levels at each wavelength as dependent variables with patient age, duration of diabetes (DOD), sex, race, z-BMI, HbA_{1c} or HGI, and MBG*

SIF wavelength and adjustment	Age (years)	DOD (years)	Sex F > M	Race B > W	z-BMI	HbA_{1c} or HGI (%)	MBG (mg/dL)
375u				X		X	
375c	X	X	X	X		X	
405u	X			X		X	
405c	X	X	X			X	
420u	X			X		X	
420c	X	X	X			X	

Statistically significant relationships, $P = 0.05$ or less, for the covariates in the model are indicated by X.

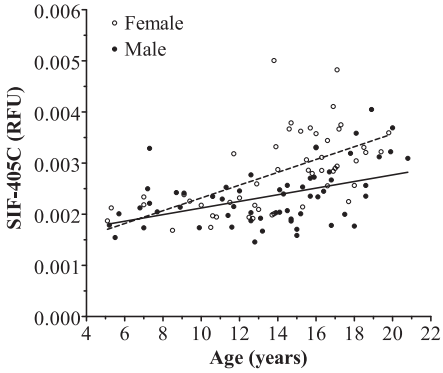

Figure 2—*The relationship of age and sex in SIF405c data. SIF levels increased with age and were higher in girls than boys.*

sensitive to age-related change. Our finding of a significant increase in "c"-adjusted SIF at all excitation wavelengths with patient age and duration of diabetes in children is in accordance with prior biopsy data, albeit from adults.

We found a difference in the "c"-adjusted SIF levels between girls and boys that was independent of age, MBG, or HbA_{1c} level. There was no sex difference discernable using the "u" adjustment, which agrees with reported findings in adults using a similar method (20), with the exception of smokers (21). Sex differences are known to occur in skin (22). Potentially the detected sex difference in SIF might represent a biochemical difference in accumulation of fluorescent AGEs between the sexes during childhood and adolescence. Alternatively this difference may represent non-AGE differences and be an artifact of the adjustment method or other factors.

It is conventionally understood that formation and accumulation of glycated proteins such as HbA_{1c} (4) and AGEs (2) is a concentration-dependent function of glucose concentration. The SIF data demonstrated a strong relationship with HbA_{1c} or HGI whether "u" or "c" adjusted. Interestingly there was no influence of MBG or cBG on SIF. Lack of relationship of SIF with randomly obtained cBG is not surprising. Previous investigators have studied skin autofluorescence during oral glucose tolerance testing and noted no changes related to acute fluctuations in blood glucose (23). This makes sense from the conventional understanding of how proteins are glycated. Initial nonenzymatic attachment of glucose to a protein such as hemoglobin occurs quickly and reversibly, but the formation of the stable Amadori product takes much longer, and progression to AGEs even more time. Thus a randomly obtained glucose level, while correlated with the labile Schiff base precursor to HbA_{1c}, is negligibly associated with stable HbA_{1c} levels (24).

The lack of association of SIF with MBG is more surprising. Both MBG and HbA_{1c} levels have been associated with the development of complications in type 1 diabetes (6). HbA_{1c} is strongly correlated with MBG and widely used to estimate MBG (25). However in simple Pearson correlation analysis, HbA_{1c} and HGI were correlated with the SIF levels whereas MBG was not. In the multivariate models, both HbA_{1c} and HGI were significantly related to SIF at that visit, but the visit MBG as a covariate was not. Considering the possibility that the 30-day average MBG from the clinic visit might have been insufficiently long to be involved with detectable AGE formation, we substituted the average of all available prior MBG measurements from patients into the model (along with HGI or HbA_{1c}). There was still no significant association between MBG and SIF. These findings suggest that MBG-independent, between-patient differences in HbA_{1c} are predictive of AGE burden in the skin and potentially influence the development of diabetes complications. Thus factors besides MBG that can influence nonenzymatic glycation (24) may be more influential in formation of skin AGEs and development of diabetes complications than just MBG exposure alone.

Acknowledgments—N.M. and J.M. are employees of VeraLight, Inc. No other potential conflicts of interest relevant to this article were reported.

D.L.F. enrolled patients, collected data, maintained the study database, and cowrote the manuscript. J.M.H. helped analyze data, prepared figures, reviewed and edited the manuscript, and contributed to discussion. S.L. performed major statistical analysis of the dataset and reviewed and edited the manuscript. N.M. and J.M. analyzed SIF data, reviewed and edited the manuscript, and contributed to discussion. C.L. enrolled patients and collected data. S.A.C. performed overall supervision of the study, helped analyze data, and cowrote the manuscript.

The authors thank Kelly Allerton, Louisiana State University Health Sciences Center, for assistance in editorial preparation of the manuscript.

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