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Skin Advanced Glycation End Product Accumulation Is Poorly Reflected by Glycemic Control in Type 2 Diabetic Patients (ZODIAC-9)

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Abstract

Background:

Glycemic memory can be reflected by tissue accumulation of advanced glycation end products (AGEs). In type 1 diabetes mellitus (T1DM) patients, hemoglobin A1c (HbA1c) levels over various time periods poorly predicted the accumulation of different AGEs in skin biopsies. Our aim was to investigate whether HbA1c assessments can predict the change in skin AGEs during time in type 2 diabetes mellitus (T2DM).

Methods:

We included 452 T2DM patients participating in a shared-care setting, who are screened annually for HbA1c and diabetic complications. Baseline and follow-up levels of skin AGEs were assessed with a validated noninvasive autofluorescence (AF) method, which is based on the fluorescence characteristics of certain AGEs.

Results:

Our study population had a mean age of 65 years and 54% were female. After a mean follow-up duration of 3.3 years, linear regression analyses showed weak relationships among different assessments of HbA1c (baseline, maximum, mean, and variance of HbA1c) and skin AF at follow-up. Baseline skin AF and age were predictors of skin AF at follow-up, but diabetes duration, smoking, and creatinine were of less or no predictive value for skin AF at follow-up.

Conclusions:

In our T2DM population, integrated HbA1c assessments over years poorly predict the change in skin AGE level measured by skin AF. These findings agree with results in patients with T1DM. This suggests either the need for longer exposure to glucose disturbances to change tissue AGEs or other mechanisms, such as oxidative stress, leading to AGE accumulation.

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Abbreviations: (ACR) albumin-to-creatinine ratio, (AF) autofluorescence, (AGE) advanced glycation end product, (AFR) autofluorescence reader, (AU) arbitrary units, (DCCT) Diabetes Control and Complications Trial, (EDIC) Epidemiology of Diabetes Interventions and Complications, (HbA1c) hemoglobin A1c, (T1DM) type 1 diabetes mellitus, (T2DM) type 2 diabetes mellitus, (ZODIAC) Zwolle Outpatient Diabetes project Integrating Available Care

Keywords: advanced glycosylation end products, fluorescence, hemoglobin A1c, type 2 diabetes mellitus, ZODIAC-9

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Introduction

Short-term glycemic memory can be reflected by hemoglobin A1c (HbA1c), which represents the degree of glycemic control over the last 6 to 8 weeks. Another more long-term glycemic index encompasses the level of tissue accumulation of advanced glycation end products (AGEs).[1] These stable end products of glycation of proteins are formed nonenzymatically in the Maillard reaction from Amadori products such as HbA1c. AGEs can also be formed by reactive carbonyl compounds (e.g., glyoxal, methylglyoxal, arabinose, glycoaldehyde) in conditions of enhanced oxidative stress in general. AGEs form cross-links in and accumulate on long-lived proteins such as skin collagen, which has an estimated lifetime of 15–20 years. Quantization of these collagen-bound AGEs could provide information about cumulative glycemic and oxidative stress over several years. Part of this metabolic process is a consequence of poor metabolic control over a considerable period, probably years.

Important evidence regarding the relationship between poor metabolic control and the development or progression of diabetic complications has been found earlier in T1DM in the Diabetes Control and Complications Trial (DCCT) and in T2DM in the United Kingdom Prospective Diabetes Study during the 1990s.[2,3] Relevance of the glycated collagen products compared to HbA1c, both as markers of diabetic complications, has been investigated in a DCCT–Epidemiology of Diabetes Interventions and Complications (EDIC) substudy.[1] Long-term intensive treatment compared to conventional treatment of type 1 diabetes patients resulted in lower skin collagen glycation, glycoxidation, and cross-links. The accumulation of collagen AGEs, as measured in skin biopsies, explained an unexpectedly high percentage of variance in the incidence of diabetic complications, also after adjustment for HbA1c levels. Monnier *et al.*[2] also found that the AGE accumulation in skin biopsies of type 1 diabetic patients was poorly predicted by HbA1c levels over several different time periods.

Advanced glycation end product accumulation can be quantified by tissue measurements, invasively by skin biopsies, but also noninvasively with an autofluorescence reader (AFR) or currently AGE reader. This newly developed device measures certain tissue AGEs, with the concept of reflecting metabolic control over several years, and has already been established as a risk marker for micro- and macrovascular complications and mortality in T2DM.[4,5] Controlling glycemic and metabolic status is an important issue in preventing long-term diabetic complications, and it is not clearly defined which marker for metabolic control over a certain period is the best predictor in relation to the development of chronic diabetic complications. We hypothesized that the accumulation of AGEs in type 2 diabetes patients can be predicted by the course of HbA1c during a certain period prior to the skin autofluorescence (AF) measurement. The aim of our study was to investigate to what extent skin AF, reflecting tissue AGE accumulation, can be predicted by different integrated assessments of HbA1c in type 2 diabetic patients.

Methods

Study Population

Subjects were recruited from a large type 2 diabetes cohort participating in the Zwolle Outpatient Diabetes project Integrating Available Care (ZODIAC) study.[6] Baseline skin AF measurements were performed in a cohort of 973 patients between May 2001 and May 2002.[7] At the end of follow-up, from June 2004 until October 2005, a second skin AF was assessed randomly in 452 patients from the initial population of 973 patients who were still visiting the diabetes outpatient clinic. Because of the limitation of the applied AFR to measure accurately in dark skin types, patients with a Fitzpatrick class V–VI skin type were excluded from the beginning. All participating patients visited the outpatient clinic annually and all of them had given informed consent. Approval by the local ethical committee had been obtained.

Autofluorescence Reader

Assessment of tissue AGEs by the AFR device, a prototype of the current AGE reader (DiagnOptics Technologies BV, Groningen, The Netherlands), is based on a technique that enlists the fluorescence properties of certain AGEs, as mentioned before, and expresses the level of skin AF. This noninvasive measurement is obtained by positioning the ventral side of the lower arm on a window in a box containing an excitation ultraviolet A light source with a peak wavelength around a 370-nm excitation light, integrated over the 300- to 420-nm range. Reflected light from the skin and emission light in the 420- to 600-nm range are measured with an integrated spectrometer. Dividing the average light intensity of the emission spectrum by the average light intensity of the excitation spectrum expresses the skin AF level in arbitrary units (AU). To calculate skin reflection, white

reference measurements (with a white Teflon block, assuming 100% reflectance) were performed before every measurement. Over all AF measurements, the mean age-corrected AF per measuring month, per examiner, and per AFR system did not differ significantly. The AFR has been described more in detail elsewhere.[8]

Clinical Data and Laboratory Assessments

Clinical data were obtained on the date of the first skin AF measurement; these data were derived from the Diabetic Electronic Monitoring System containing data of all patients in the shared-care project.[6] Laboratory assessments were measured according to the standard hospital procedures of the Isala Clinics, Zwolle, The Netherlands. HbA1c was measured with a Primus CLC-385 using boronate affinity chromatography and high-performance liquid chromatography (reference value 4.0–6.0%). The presence of microvascular disease was defined as the occurrence of at least one of the following diabetic complications: retinopathy, neuropathy, and/or nephropathy. We used the albumin-to-creatinine ratio (ACR) in our definition of nephropathy. The relationship between skin AF and ACR was shown in the original study population, and the predictive value of skin AF for the development of nephropathy has been studied as well.[4,7] Macrovascular disease was defined as the presence of at least one aspect of the following cardiovascular complications: coronary heart, cerebrovascular, and/or peripheral vascular disease. All complications are described in detail elsewhere.[7]

Skin AF assessments were performed at baseline and at the end of follow-up.

Statistical Analysis

Comparing means of skin AF between patients with and without a diabetic complication was performed by analysis of variance.

Linear regression was used to determine the relationship between skin AF at follow-up and various HbA1c measures. The different integrated assessments of HbA1c used in the analyses were as follow: the variance of HbA1c, mean HbA1c, maximum HbA1c, and HbA1c at baseline. Mean and variance of HbA1c were calculated over the annually assessed HbA1c measurements between baseline and the end of follow-up. Maximum HbA1c is the highest HbA1c level of the annually assessed HbA1c measurements between baseline and the end of follow-up.

Adjustment for baseline skin AF was performed in all the analyses. In multivariate regression, in addition to baseline skin AF, adjustments for age, diabetes duration, creatinine, and smoking were made (all variables were assessed at baseline). These variables are all independently related to skin AF and could all affect the rate of formation and accumulation of AGEs during years.[7]

Results

Baseline characteristics are shown in **Table 1**. Mean age was 65 years with 255 (54%) female patients. The mean follow-up duration was 3.3 ± 0.4 years. At baseline, patients were well controlled with a mean HbA1c of 6.8 ± 1.2%, and mean HbA1c at follow-up was 7.0 ± 1.0%. Patients with either a microvascular or a macrovascular complication had higher levels of skin AF baseline **(Table 2)**.

The total amount of annually collected samples of HbA1c per patient was minimal 3 and maximal 5; the mean of collected samples of HbA1c was 4.2.

Table 1.
Baseline Characteristics[a]

Patient characteristic	*N* = 452
Age (years)	65 (11)
Gender (F/M)	244/208 (54%/46%)
Smoking (%)	19
Body mass index (kg/m^2)	29.5 (4.9)
Systolic blood pressure (mm Hg)	145 (20)
Diastolic blood pressure (mm Hg)	81 (10)
Diabetes duration (years)	3.6 (1.5–7.5)[b]
HbA1c (%)	6.8 (1.2)
Creatinine (µmol/liter)	94 (17)
Total cholesterol (mg/dl)	197 (37)
Microvascular disease (%)	46
Macrovascular disease (%)	35
Skin AF at t0 (AU)	2.71 (0.69)
Skin AF at t follow-up (AU)	2.79 (0.77)

[a] Values are expressed as mean (SD).
[b] Median and interquartile range. Reference values of the laboratory: 4.0–6.0% HbA1c, 70–110 µmol/liter creatinine, 135–193 mg/dl total cholesterol.

Table 2.
Mean Skin AF ± Standard Deviation (Arbitrary Units) of Patients with or without a Micro/Macrovascular Complication

Complication	Yes (*n*)	No (*n*)	*p* value
Microvascular	2.81 ± 0.70 (208)	2.63 ± 0.67 (244)	0.005
Macrovascular	2.91 ± 0.74 (156)	2.61 ± 0.63 (296)	< 0.001

The relationship between skin AF at follow-up (adjusted for the also presented skin AF at baseline) and the various assessments of HbA1c is shown in **Table 3**. Skin AF at follow-up showed weak but significant relationships with all different integrated assessments of HbA1c: regression coefficients < 0.1; $p \leq 0.025$ [with addition of baseline skin AF: overall adjusted R^2 ~0.45; $p < 0.001$]. **Table 4** shows the linear regression analyses of skin AF at follow-up versus different integrated assessments of HbA1c with adjustment for baseline skin AF, age, diabetes duration, creatinine, and smoking. In all analyses, adjusted R^2 of skin AF at follow-up was 0.48 ($p < 0.001$), although the regression coefficients of all the different integrated HbA1c assessments were low (maximum 0.069) and, therefore, added little prognostic value to skin AF level at follow-up. The skin AF level at baseline proved to be the best predictor for skin AF at follow-up (regression coefficient = 0.65 for mean HbA1c, maximum HbA1c, HbA1c at baseline, and 0.66 for the variance of HbA1c; $p < 0.001$). Age and creatinine were of less predictive value than skin AF at baseline. In these models, diabetes duration and smoking were not of any prognostic value for skin AF at follow-up.

Table 3.
Linear Regression Models of Skin AF at Follow-Up (Adjusted for Skin AF at Time 0) versus Different Integrated Assessments of HbA1c

HbA1c assessment Baseline skin AF	Regression coefficient	95% confidence interval	*p* value
Variance of HbA1c	0.067	0.020–0.114	0.005
Baseline skin AF	0.745	0.669–0.822	<0.001
Mean HbA1c	0.075	0.018–0.133	0.010
Baseline skin AF	0.728	0.650–0.806	<0.001
HbA1c max	0.053	0.014–0.091	0.007
Baseline skin AF	0.735	0.658–0.812	<0.001
HbA1c at baseline	0.052	0.007–0.098	0.025
Baseline skin AF	0.734	0.656–0.811	<0.001

Table 4.
Multivariate Linear Regression Models of Skin AF at Follow-Up (Adjusted for Baseline Skin AF, Age, Diabetes Duration, Creatinine, and Smoking) versus Different Integrated Assessments of HbA1c

HbA1c assessment Covariates	Regression coefficient	95% confidence interval	*p* value
Variance of HbA1c	0.069	0.024–0.115	0.003
Skin AF time 0	0.658	0.577–0.739	<0.001
Age	0.012	0.007–0.018	<0.001
Diabetes duration	0.003	−0.006–0.012	0.512
Creatinine	0.004	0.000–0.007	0.027
Smoking	0.064	−0.070–0.198	0.350
Mean HbA1c	0.069	0.011–0.127	0.021
Skin AF time 0	0.647	0.565–0.729	<0.001
Age	0.012	0.007–0.018	<0.001
Diabetes duration	0.000	−0.009–0.010	0.959
Creatinine	0.003	0.000–0.006	0.040
Smoking	0.067	−0.067–0.201	0.327
HbA1c max	0.052	0.014–0.090	0.007
Skin AF time 0	0.650	0.569–0.732	<0.001
Age	0.013	0.007–0.018	<0.001
Diabetes duration	0.001	−0.008–0.010	0.851
Creatinine	0.003	0.000–0.006	0.037
Smoking	0.064	−0.070–0.198	0.346
HbA1c at baseline	0.052	0.007–0.097	0.022
Skin AF time 0	0.649	0.567–0.731	<0.001
Age	0.012	0.007–0.018	<0.001
Diabetes duration	0.001	−0.008–0.010	0.848
Creatinine	0.004	0.001–0.007	0.023
Smoking	0.066	−0.069–0.200	0.339

Discussion

This study showed that skin AF, reflecting tissue AGE accumulation and proposed as an exponent of glycemic long-term memory, is poorly predicted by the degree of short-term glycemic control in a type 2 diabetes population. The variance, mean, maximum, and baseline measurements of HbA1c during a 3.3-year period prior to the skin AF measurement at the end of the follow-up period were all of little value for predicting the change in AGE accumulation. These findings confirm the observations by Monnier and colleagues[2] in the DCCT–EDIC cohort in T1DM. In the DCCT–EDIC substudy analyses of skin collagen variables against HbA1c levels at various time points regarding the date of biopsy were adjusted for age and diabetes duration only.[1] In our cohort, age and skin AF at baseline were the most pronounced factors that influenced skin AF at follow-up, which is not surprising. A stable or small increase of the accumulation of AGEs was seen during the relatively short follow-up period. This also confirms the slow process of formation and accumulation of AGEs in human tissue, but could also be because of the on average well-controlled study population (mean HbA1c of 6.8%). The mean increase in skin AF of 0.08 AU over 3.3 years is also in line with the similarly small age decade-related differences in skin AF levels in our previous cross-sectional study in the same cohort.[7] Furthermore, patients with a decrease in skin AF level do have a lower HbA1c level at baseline

compared to patients who have a stable or increased skin AF level. If the attribution of hyperglycemic stress to the formation of AGEs is important, one could postulate that a decrease in the AGE level is accompanied with a lower HbA1c level. The acceleration of formation of AGEs could be more pronounced at higher HbA1c levels.

Renal function, as reflected by serum creatinine, affects the capacity for AGE removal and, therefore, plays an independent role in the accumulation of tissue AGEs. In the present study, it was indeed confirmed that serum creatinine contributes to the change in skin AF at follow-up. Cross-sectional data showed that smoking resulted in increased skin AF levels in both T2DM and controls, but in the present longitudinal analyses, smoking at baseline did not correlate to skin AF levels at follow-up.[7] This could be because of the short follow-up period and/or the strict diabetes treatment regimen of patients participating in the shared care project, which included stimulation of patients to stop smoking. The percentage of smokers was low in our cohort. Because tobacco smoke is a source of precursors of AGEs and free radicals, which both enhance AGE formation and accumulation, cessation of smoking could result in stabilization or probably decrease the rate of AGE accumulation.[9,10]

In our study, unfortunately, no data were available on the effects of short-term glycemic variability nor on the degree of oxidative stress. Hyperglycemia is a well-known endogenous source of oxidative stress.[11,12] Monnier and associates[13] confirmed that glucose fluctuations during postprandial periods exhibited a more specific triggering effect on oxidative stress than chronic sustained hyperglycemia in type 2 diabetic patients. However, no relationship was seen between high glucose variability and elevated levels of oxidative stress in patients with type 1 diabetes, although these patients had higher urinary levels of oxidative stress than healthy controls.[14] Markers of oxidative stress will be measured in future studies to determine the degree of oxidative stress and to study its relationship with skin AF, reflecting the accumulation of AGEs, and glycemic variability.

The relatively small numbers of total collected samples of HbA1c per patient (due to the short follow-up period) and the overall good glycemic control in our study population are two other limitations of our study; the latter could be considered as a limitation in assessing the rate of AGE accumulation. We cannot exclude that extending the range of glycemic control could have resulted in a stronger contribution of the HbA1c parameters to skin AF. Perhaps a stronger relationship would also have been found in poorly controlled subjects studied over a longer time period.

This study confirmed that the level of skin AF is partly determined by glycemic control defined as different assessments of HbA1c. Information about skin AGE levels could be of more important value than HbA1c levels for the development of diabetic complications, which could make the noninvasive AFR a practical adjuvant in clinical practice. Follow-up studies have already shown the usefulness of skin AF as a new marker in predicting diabetic complications, which turned out to be a stronger predictor than HbA1c. This was found for microvascular complications as well as for macrovascular morbidity and mortality.[4,5] The mainstay of diabetes management is the prevention of chronic complications, especially cardiovascular disease. The presence of a considerable number of patients with micro- and macrovascular disease in our well-controlled study population at baseline supports the consensus that HbA1c parameters, reflecting at best medium-term glycemic control, cannot be considered as a substitute for the use of skin AF, reflecting the accumulation of AGEs with its vascular-damaging effects.

Conclusions

In conclusion, the present type 2 diabetes study showed glycemic control, measured by different assessments of HbA1c, as a small contributor to AGE accumulation expressed as skin AF. This poor relationship between HbA1c and skin AF in this group of type 2 diabetes mellitus patients is in agreement with the results of the DCCT in T1DM patients. Skin AGE levels could reflect the gravity of vascular damage even better than glycemic control by HbA1c. The finding also suggests other mechanisms for increased AGE accumulation, such as oxidative stress.

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Disclosure:

A. J. Smit is one of the founders of DiagnOptics B.V., The Netherlands, manufacturer of the AGE-Reader, which is based on the prototype used in this article.

References:

1. Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, Sivitz W, Monnier VM; DCCT Skin Collagen Ancillary Study Group. Glycation and carboxymethyllysine levels in skin collagen predict the risk of future 10-year progression of diabetic retinopathy and nephropathy in the diabetes control and complications trial and epidemiology of diabetes interventions and compliations participants with type 1 diabetes. Diabetes. 2005;54(11):3103-11.
2. Monnier VM, Bautista O, Kenny D, Sell DR, Fogarty J, Dahms W, Cleary PA, Lachin J, Genuth S. Skin collagen glycation, glycoxidation, and crosslinking are lower in subjects with long-term intensive versus conventional therapy of type 1 diabetes: relevance of glycated collagen products versus HbA1c as markers of diabetic complications. DCCT Skin Collagen Ancillary Study Group. Diabetes Control and Complications Trial. Diabetes. 1999;48(4):870-80.
3. Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, Hadden D, Turner RC, Holman RR. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ. 2000;321(7258):405-12.
4. Gerrits EG, Lutgers HL, Kleefstra N, Graaff R, Groenier KH, Smit AJ, et al. Skin autofluorescence: a tool to identify type 2 diabetic patients at risk for developing microvascular complications. Diabetes Care. 2008;31(3):517-21.
5. Meerwaldt R, Lutgers HL, Links TP, Graaff R, Baynes JW, Gans RO, Smit AJ. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. Diabetes Care. 2007;30(1):107-12.
6. Ubink-Veltmaat LJ, Bilo HJ, Groenier KH, Houweling ST, Rischen RO, Meyboom-de Jong B. Prevalence, incidence and mortality of type 2 diabetes mellitus revisited: a prospective population-based study in The Netherlands (ZODIAC-1). Eur J Epidemiol. 2003;18(8):793- 800.
7. Lutgers HL, Graaff R, Links TP, Ubink-Veltmaat LJ, Bilo HJ, Gans RO, Smit AJ. Skin autofluorescence as a noninvasive marker of vascular damage in patients with type 2 diabetes mellitus. Diabetes Care. 2006;29(12):2654-9.
8. Mulder DJ, Water TV, Lutgers HL, Graaff R, Gans RO, Zijlstra F, Smit AJ. Skin autofluorescence, a novel marker for glycemic and oxidative stress-derived advanced glycation endproducts: an overview of current clinical studies, evidence, and limitations. Diabetes Technol Ther. 2006;8(5):523-35.
9. Cerami C, Founds H, Nicholl I, Mitsuhashi T, Giordano D, Vanpatten S, Lee A, Al-Abed Y, Vlassara H, Bucala R, Cerami A.Tobacco smoke is a source of toxic reactive glycation products. Proc Natl Acad Sci U S A. 1997;94(25):13915-20.
10. Church DF, Pryor WA. Free-radical chemistry of cigarette smoke and its toxicological implications. Environ Health Perspect. 1985;64:111-26.
11. Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care. 1996;19(3):257-67.
12. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes. 2005;54(6):1615-25.
13. Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA. 2006;295(14):1681-7.
14. Wentholt IM, Kulik W, Michels RP, Hoekstra JB, DeVries JH. Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. Diabetologia. 2008;51(1):183-90.