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ORIGINAL ARTICLE

Skin Autofluorescence and Complications of Diabetes: Does Ethnic Background or Skin Color Matter?

Mohamed Ahdi, MD,[1] Victor E.A. Gerdes, MD, PhD,[1,2] Reindert Graaff, MSc, PhD,[3] Saskia Kuipers, MD, PhD,[4] Andries J. Smit, MD, PhD,[3] and Eelco W. Meesters, MD[1]

Abstract

Aims: Skin autofluorescence (AF) has been associated with complications of diabetes. We evaluated the influence of skin color and ethnicity on the association between skin AF and the presence of diabetes-related complications.

Materials and Methods: In a multiethnic type 2 diabetes cohort we investigated all patients with available skin AF measurements. The associations between skin AF and hemoglobin A1c (HbA1c) and the presence of complications of diabetes were estimated, stratified for ethnicity and quartiles of ultraviolet reflectance percentage (R%).

Results: In total, 810 patients (438 native Dutch, 372 non-Dutch) were included. Because of too low an R%, 32% of black Africans and 19% of Hindustanis were excluded. Non-Dutch patients had lower AF values compared with Dutch patients (median AF = 2.69 [interquartile range (IQR), 2.26–3.09] vs. 3.06 [IQR, 2.65–3.50] arbitrary units; $P < 0.001$), but the R% was also lower (non-Dutch, median R% = 12% [IQR, 9–15%]; Dutch, median R% = 18% [IQR, 14–23%]; $P = 0.027$). In the multivariate analysis, skin AF was only a determinant for complications in patients with R% 25th percentile (macrovascular, odds ratio [OR] = 1.71 [95% confidence interval (CI), 1.05–2.77] vs. 1.15 [95% CI, 0.55–2.40] in the lowest quartile of R%; microvascular, OR = 1.81 [95% CI, 1.20–2.75] vs. OR = 0.87 [95% CI, 0.50–1.51]). A similar pattern was observed for nephropathy, neuropathy, and retinopathy separately. In non-Dutch patients AF was not a significant determinant for diabetes complication risk, whereas HbA1c was for nephropathy, retinopathy, and neuropathy.

Conclusions: Skin AF measurement is a valuable tool for the assessment of micro- and macrovascular complication risk in patients with light skin color types. Even after exclusion of patients with too low a reflectance, the current performance of the AGE Reader™ (DiagnOptics Technologies BV, Groningen, The Netherlands) was insufficient in darker-skinned patients.

Introduction

Skin autofluorescence (AF) is a measure of tissue accumulation of advanced glycation end products (AGEs). The noninvasive assessment of AGEs using skin AF with the AGE Reader™ (DiagnOptics Technologies BV, Groningen, The Netherlands) has been validated against specific AGE levels in skin biopsy specimens of patients with diabetes or end-stage renal disease and healthy individuals.[1,2]

In patients with type 2 diabetes, skin AF was not only associated with the presence and degree of both micro- and macrovascular complications, but was also a predictor of their development.[3,4] Lutgers et al.[5] demonstrated an additional value for the AGE Reader on top of the traditional risk factors in determining cardiovascular risk and mortality and all-cause mortality. In the same cohort study of 973 primary care diabetes patients with a mean hemoglobin A1c (HbA1c) level of 53 (±14) mmol/mol, skin AF measurement led to the reclassification of 27% of patients with a low-intermediate United Kingdom Prospective Diabetes Study risk score to high risk, and vice versa.

In most studies performed so far, only patients of white and Asian descent were included[6–8] because of the AGE Reader's limitation to measure accurately in patients with very dark

[1]Department of Internal Medicine, Slotervaart Hospital, Amsterdam, The Netherlands.
Departments of [2]Vascular Medicine and [4]Internal Medicine, Academic Medical Centre, Amsterdam, The Netherlands.
[3]Department of Internal Medicine, University Medical Centre Groningen, Groningen, The Netherlands.

(Fitzpatrick class V–VI) skin types.[9] The reflectance of ultraviolet (UV) light, which is necessary for AF measurement, can be hampered by a dark skin color. Moreover, the fluorescence emitted by the AGE is also absorbed to a larger extent in the case of a darker skin color (mainly by melanin). Therefore, without correction, measurements in patients with darker skin colors (UV reflectance of <10%) typically result in lower AF values than in those with fair skin colors.[10]

In a previous study we provided a method for calculating skin AF independent of skin color at lower levels of reflectance (as low as 6%). This algorithm was validated based on the spectral characteristics of melanin and hemoglobin, as the strongest contributing absorbers for the lower skin AF values. These spectral characteristics were derived from reflectance and emission spectra in the UV and visible range.[11] This algorithm was developed and subsequently validated using two subsets of skin AF data previously obtained in healthy subjects from Afro-Caribbean, Asian, or African descent living in The Netherlands and of southern Chinese people in China. However, so far, no validation studies for this algorithm in other ethnic cohorts have been published.

In the present study we applied the algorithm for skin color-independent assessment of skin AF in a secondary care multiethnic type 2 diabetes cohort. We determined the proportion of patients with too low a reflectance and investigated the association among AF, HbA1c, and complications of diabetes in the remaining data, stratified by ethnicity and UV reflectance.

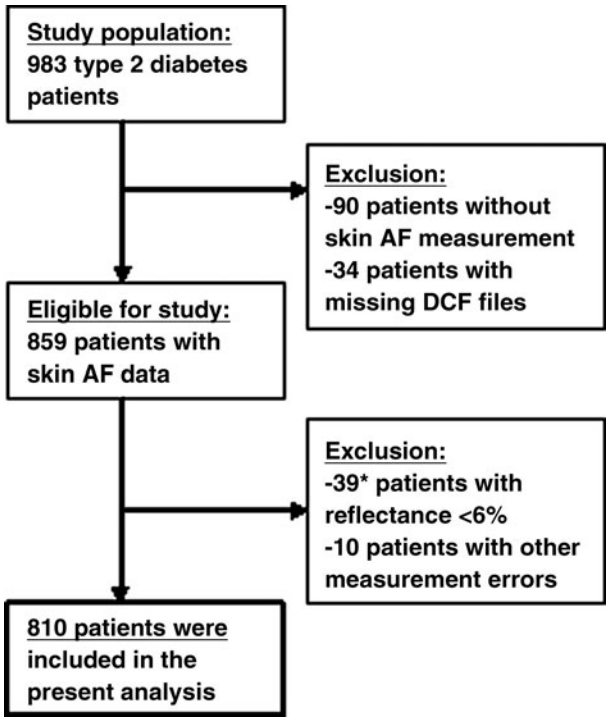

FIG. 1. Study flow chart. *Because of too low a reflectance, the proportion of excluded patients per ethnic group was as follows: black Africans, 18 of 56 (32%); Hindustani, 11 of 59 (19%); other ethnicity, five of 98 (5%); Turks, one of 81 (1%); Moroccans, two of 185 (1%); and Dutch, two of 504 (0.5%). AF, autofluorescence; DCF, Diagnoptics compact files.

Materials and Methods

Description of patients

For the present analysis, we selected all patients with type 2 diabetes with a valid skin AF measurement from a multiethnic diabetes cohort. The AF measurement was performed during the annual comprehensive diabetes evaluation at the outpatient clinic of Slotervaart Hospital (Amsterdam, The Netherlands), between May 2009 and December 2010. An overview of the selection procedure is shown in Figure 1. All study patients provided written informed consent, and the local medical ethics committee of Slotervaart Hospital approved the study.

Clinical definitions

Ethnicity was defined according to the country of birth of either the patient or his or her parents. The following ethnic groups were considered: native Dutch (whites), Turks, Moroccans, Hindustani (Surinamese or Indian origin), black African (Surinamese, Netherlands Antillean, or West African descent), and a group of persons of other ethnicities.

Complications of diabetes were defined as follows: nephropathy as the presence of (un)treated microalbuminuria (≥30 mg/24 h) or macroalbuminuria (≥300 mg/24 h). Retinopathy was considered present in the case of background retinopathy, (pre-)proliferative retinopathy, and/or planned or previous laser coagulation therapy. Neuropathy was defined as the presence of peripheral polyneuropathy (clinically diagnosed by a neurologist or by electromyography) or obviously disturbed sensibility during foot examination. Macrovascular complications were defined as the presence of a history of myocardial infarction, (unstable) angina pectoris, cerebrovascular accident or transient ischemic attack, or proven peripheral vascular disease.

Data collection and (laboratory) measurements

Clinical data (age, sex, ethnicity, current smoking, body mass index, systolic and diastolic blood pressure, ''known'' diabetes duration, complications, and medication) and laboratory results (serum creatinine, blood urea nitrogen, fasting lipids [total cholesterol, low-density lipoprotein-cholesterol, high-density lipoprotein (HDL)-cholesterol, and triglycerides] and glucose, HbA1c, high-sensitivity C-reactive protein [hs-CRP], and microalbuminuria) were obtained approximately 2 weeks before the skin AF measurement.

Blood pressure was measured using an automated Vital Signs Monitor, 300 series device (Welch Allyn Protocol Inc., Beaverton, OR). Five consecutive readings were taken at 3-min intervals, and the mean systolic and diastolic blood pressures of the fourth and fifth measurements were recorded. Blood samples were obtained by standard phlebotomy after a 10-h overnight fast. The routine analysis of these samples for HbA1c was part of the assessment of glycemic control in our patients and was performed using a Menarini (Adams™ HA-8160; Arkray Inc., Kyoto, Japan) automated high-performance liquid chromatography analyzer. Levels of serum total and HDL-cholesterol and triglycerides were determined using standard laboratory procedures within 4 h after sampling with an automated analyzer (Synchron® LX20; Beckman Coulter Inc., Fullerton, CA). The low-density lipoprotein-cholesterol level was calculated using the formula of Friedewald et al.[12]

The non–HDL-cholesterol level was calculated as total cholesterol minus HDL-cholesterol. The hs-CRP level was determined with a near-infrared particle immunoassay rate methodology (Beckman, Brea, CA). Analytical sensitivity, defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence, was 0.2 mg/mL. Microalbuminuria was assessed using rate nephelometry, with a threshold of 1.9 mg/dL, by the Immage Immunochemistry System (Beckman, Brea). Renal function was assessed by calculating the estimated glomerular filtration rate (eGFR), using the MDRD formula.[13]

Skin AF

The skin AF results were obtained with the AGE Reader as previously described in detail.[1,3] In brief, the AGE Reader illuminates a skin surface of approximately 4 cm^2 with an excitation light source with peak intensity at approximately 370 nm. Emission light and reflected excitation light from the skin were measured with a spectrometer in the 300–600 nm range, using a glass fiber. AF was calculated by dividing the average emitted light intensity per nm in the range between 420 and 600 nm by the average reflected excitation light intensity per nm in the range between 300 and 420 nm and was expressed as arbitrary units (AU), multiplied by 100. Furthermore, for the skin color correction, properties of the reflectance spectrum that were obtained with a white light-emitting diode are used by the AGE Reader in subjects with skin reflectance of $<12\%$. Skin AF was measured at three positions at the volar side of the arm (approximately 10 cm below the elbow fold).

Prior to any statistical analysis, the spectra of all measured data were determined with AGE Reader software (version 2.3.0), which uses the previously validated algorithm that accounts for skin color with an UV reflectance percentage (R%) of $>6\%$.[11] For triple measurements with an SD of <12.5% of mean AF, the mean was used in the analysis, and for measurements with an SD of >12.5% of mean AF, the median value was used, in order to eliminate errors that sometimes occur during one of the triple measurements.

Statistical analysis

Categorical data were presented as absolute numbers with percentages, and because of a non-normal distribution of skin AF (Kolmogorov–Smirnov test, $P<0.05$), continuous variables were presented as medians with interquartile range (IQR). Spearman rank correlation coefficient (r_s) values were calculated to study the relation between skin AF and risk factors of diabetes-related complications. Odds ratios (ORs) and their 95% confidence intervals (CIs) for the risk of complications of diabetes (nephropathy, retinopathy, neuropathy, any microvascular complications, and macrovascular disease) associated with skin AF and HbA1c were estimated using binary logistic regression. Individuals without complications of diabetes were used as a reference category. The calculated ORs for each complication of diabetes represent the increased risk per unit of skin AF (AU) or HbA1c (%), respectively. The analysis was stratified by ethnicity in order to evaluate the clinical applicability of AGE Reader assessment in patients of non-Dutch origin. To investigate the influence of skin color type on AF measurement, the analysis was also stratified by UV reflectance (R% $\leq 25^{th}$ percentile vs. R% $>25^{th}$ percentile). Statistical analyses were performed using SPSS version 18.0 software package (SPSS Inc., Chicago, IL).

Results

From a total of 983 patients with type 2 diabetes, 810 had valid skin AF data and were included in the present analysis. The main reasons to exclude the 173 patients were no skin AF measurement performed (52%), R% below 6% (23%), missing Diagnoptics compact files data, which contain the raw measurements (20%), and other measurement errors (6%). Thirty-two percent of the black Africans and 19% of the Hindustanis had too low a UV reflectance, compared with 0.5% of the patients of Dutch origin.

There were no significant differences in demographics, clinical variables, and complications between patients with and without available skin AF data, except for ethnicity. In the cohort, the median age was 63 (range, 25–91) years, and the median HbA1c was 54 (IQR, 46–63) mmol/mol. About half of the cohort was of non-native Dutch origin: Turkish ($n=74$), Moroccan ($n=149$), Hindustani ($n=41$), black African ($n=28$), and other ($n=80$). The characteristics, distribution of complications, and their associated risk factors for the overall cohort and for the Dutch and non-Dutch populations separately are presented in Table 1. Non-Dutch patients were significantly younger, had a lower systolic blood pressure, lower levels of HDL-cholesterol and triglycerides, and better renal parameters, and less frequently smoked ($P\leq 0.001$). In addition, they had significantly higher HbA1c levels and more retinopathy but less neuropathy and macrovascular disease ($P<0.001$). Further analysis of the individual ethnic minorities in the non-Dutch group revealed no significant differences in risk factors and complications of diabetes.

Skin AF and UV reflectance

The median skin AF value of this cohort was 2.86 (IQR, 2.46–3.35) AU, and the median UV reflectance was 15% (IQR, 11–20%). A positive significant correlation was found between skin AF and R% ($r_s=0.299$, $P<0.001$). Despite the algorithm in the current software version that accounts for the selective absorption of skin compounds, non-Dutch patients had lower AF values compared with native Dutch (median AF, 2.69 [IQR, 2.26–3.09] vs. 3.06 [2.65–3.50] AU), but the R% was also lower (indicating a darker skin color) among non-Dutch patients (R% 12% [IQR, 9–15%] vs. 18% [IQR, 14–23%]). Figure 2 shows the AF values and R% by ethnic group. Within the individual ethnic groups, a statistically significant correlation between skin AF and R% was only found for individuals from Turkish, Moroccan, and ''other ethnic'' origins ($r_s=0.235$, 0.219, and 0.381, respectively).

Skin AF, risk factors, and complications

In the overall cohort, skin AF was positively correlated with age ($r_s=0.460$, $P<0.001$), systolic blood pressure ($r_s=0.153$, $P<0.001$), microalbuminuria ($r_s=0.118$, $P=0.001$), creatinine ($r_s=0.251$, $P<0.001$), blood urea nitrogen ($r_s=0.073$, $P=0.0380$), current or previous smoking ($r_s=0.077$, $P=0.029$), and hs-CRP ($r_s=0.079$, $P=0.025$). A significant negative correlation was found with eGFR ($r_s=-0.283$, $P<0.001$) and diastolic blood pressure ($r_s=-0.072$, $P=0.044$), and there was a trend for an association between

Table 1. Complications of Diabetes and Associated Risk Factors, for All Patients and According to Ethnicity

	All patients (n = *810*)	*Dutch* (n = *438*)	*Non-Dutch* (n = *372*)	P *value*[a]
Risk factors				
Age (years)	63 (25–91)	66 (37–91)	59 (25–84)	0.000
Diabetes duration (years)	11 (0–75)	12 (0–53)	11 (0–75)	0.132
Male gender [*n* (%)]	424 (52)	242 (55)	182 (49)	0.072
BMI (kg/m^2)	30.5 (27.5–34.5)	30.5 (27.2–34.4)	30.5 (27.7–34.8)	0.773
Fasting glucose (mmol/L)	7.9 (6.6–9.5)	7.8 (6.7–9.4)	7.9 (6.6–9.5)	0.877
HbA1c				
%	7.1 (6.4–7.9)	6.9 (6.3–7.6)	7.3 (6.7–8.3)	0.000
mmol/mol	54 (46–63)	52 (45–58)	56 (49–67)	0.000
Smoking [*n* (%)]				
Current	158 (20)	104 (24)	54 (15)	0.001
Previous	274 (34)	182 (42)	92 (25)	0.000
Blood pressure (mm Hg)				
Systolic	127 (116–138)	128 (119–140)	125 (115–136)	0.002
Diastolic	74 (68–80)	74 (68–81)	74 (68–80)	0.675
Serum creatinine (μmol/L)	82 (70–100)	89 (74–106)	78 (65–91)	0.000
Blood urea nitrogen (mmol/L)	5.4 (4.3–6.9)	5.8 (4.6–7.5)	4.8 (4.0–6.3)	0.000
eGFR (mL/min/1.73 m^2)	88 (69–107)	80 (63–98)	95 (76–118)	0.000
Microalbuminuria (mg/24 h)	15 (6–45)	17 (7–42)	13 (6–56)	0.217
hs-CRP (mg/L)	2.40 (1.07–5.11)	2.30 (1.12–4.86)	2.73 (1.05–5.31)	0.417
Total cholesterol (mmol/L)	4.08 (3.52–4.77)	4.13 (3.56–4.84)	4.01 (3.48–4.72)	0.109
HDL-cholesterol (mmol/L)	1.02 (0.85–1.25)	1.04 (0.88–1.28)	0.99 (0.83–1.22)	0.005
Triglycerides (mmol/L)	1.45 (1.06–2.13)	1.62 (1.14–2.31)	1.31 (0.94–1.85)	0.000
LDL-cholesterol (mmol/L)	2.25 (1.82–2.77)	2.20 (1.79–2.75)	2.34 (1.83–2.79)	0.153
Non–HDL-cholesterol (mmol/L)	3.00 (2.48–3.59)	2.98 (2.48–3.61)	3.02 (2.48–3.52)	0.589
Complications [*n* (%)]				
Nephropathy	407 (50)	220 (50)	187 (50)	0.967
Retinopathy	211 (26)	87 (20)	124 (33)	0.000
Neuropathy	190 (23.5)	122 (28)	68 (18)	0.001
Microvascular (any)	548 (68)	299 (68)	249 (67)	0.757
Macrovascular disease	248 (31)	165 (38)	83 (22)	0.000
Medication [*n* (%)]				
Metformin	716 (88)	380 (87)	336 (90)	0.135
Insulin	557 (69)	289 (66)	268 (72)	0.064
ACEi/ARBs	566 (70)	314 (72)	252 (68)	0.222
β-Blockers	319 (39)	199 (45)	120 (32)	0.000
Calcium antagonists	201 (25)	113 (26)	88 (24)	0.482
Diuretics	273 (34)	170 (39)	103 (28)	0.001
Statins	546 (67)	281 (64)	265 (71)	0.032

Data are number (percentage) or median (interquartile range). Age and diabetes duration are given as median (range).
[a]*P* value for Dutch versus non-Dutch patients.
ACEi, angiotensin converting enzyme inhibitor; ARBs, angiotensin receptor blockers; BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; hs-CRP, high-sensitivity C-reactive protein; LDL, low-density lipoprotein.

the AF value and both diabetes duration ($r_s = 0.062$, $P = 0.079$) and low-density lipoprotein-cholesterol ($r_s = -0.067$, $P = 0.060$). No associations were found between skin AF and gender, HbA1c, and non-HDL cholesterol.

Complication-associated risk for skin AF and HbA1c

Table 2 shows the risk of complications of diabetes associated with skin AF and HbA1c stratified by R%. In the patients with R% below the 25th percentile (i.e., 11.12%), the AF value was not a determinant of the risk of complications of diabetes, in both the univariate and multivariate logistic regression analysis, whereas HbA1c was for nephropathy, retinopathy, neuropathy, and any microvascular complication. In patients with lighter skin color types, R% >25th percentile (i.e., 11.13%), the associated risk per unit of skin AF was higher for all complications, including macrovascular complications, than the risk per unit of HbA1c.

As shown in Table 3, the analysis was also stratified according to ethnic background. In the overall cohort, significant associations between skin AF and reported complications of diabetes were found, with ORs, adjusted for the confounders of age, sex, diabetes duration, current smoking, body mass index, systolic blood pressure, eGFR, and (fasting) non-HDL cholesterol, of 1.46 (95% CI, 1.06–2.00) for any microvascular complication and 1.64 (95% CI, 1.12–2.39) for macrovascular disease. A separate analysis for native Dutch patients compared with the non-Dutch population revealed more pronounced and significant associations for nephropathy and any microvascular and macrovascular complications in the Dutch

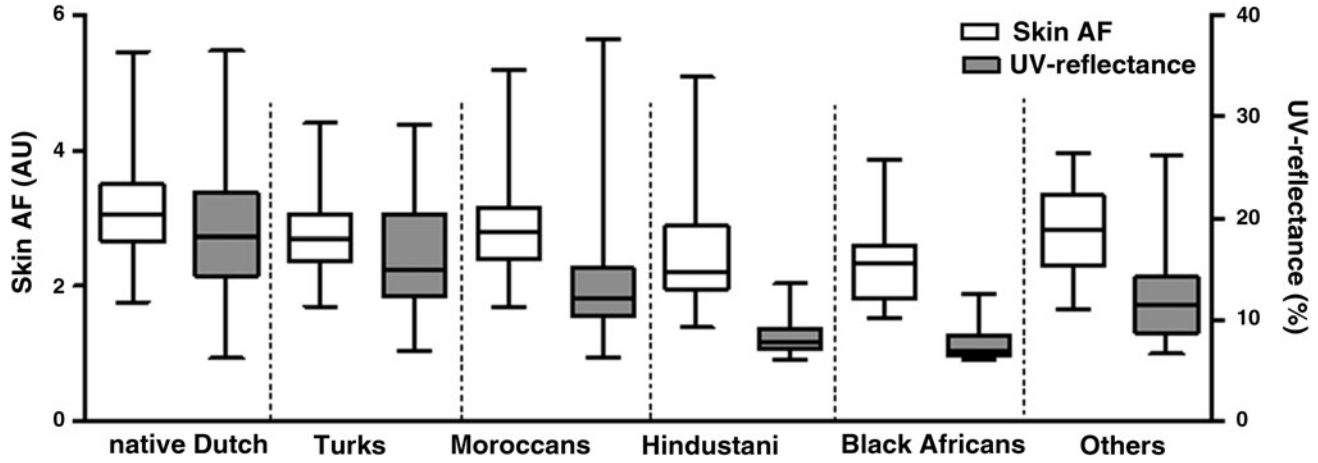

FIG. 2. Skin autofluorescence (AF) (in arbitrary units [AU]) and ultraviolet (UV) reflectance (percentage) by ethnic group: native Dutch, 3.06 (interquartile range, 2.65–3.50) and 18% (14–23%); Turks, 2.70 (2.37–3.06) and 15% (12–20%); Moroccans, 2.78 (2.40–3.16) and 12% (10–15%); Hindustani, 2.21 (1.95–2.89) and 8% (7–9%); black Africans, 2.34 (1.81–2.59) and 7% (6.5–8.5%); and other ethnic origins, 2.84 (2.32–3.33) and 12% (9–14%), respectively. Data are median (interquartile range) values.

group ($P = 0.053$, $P = 0.066$, and $P = 0.027$, respectively), but not for retinopathy and neuropathy.

It is interesting that skin AF per unit was a stronger determinant for the risk of macrovascular disease than HbA1c by percentage (adjusted OR = 1.81 vs. 1.14 in Dutch and 1.42 vs. 1.11 in non-Dutch). This was also true for the individual microvascular complications in the Dutch group, except for retinopathy. In non-Dutch patients, however, AF was not a stronger determinant than HbA1c for microvascular complications. Further stratification for the different ethnic minority groups resulted in loss of statistical power, with large CIs of the calculated ORs (data not shown).

Discussion

In the present observational cohort study among 810 patients with type 2 diabetes, of which 49% of non-native Dutch origin, we demonstrate that skin AF is a stronger determinant for complications of diabetes than HbA1c in patients with fair skin colors but not in patients with darker skin color types. Despite the application of an algorithm that accounts for the selective absorption of skin compounds, the performance of the AGE Reader remains poor in patients with very dark skin types, in particular, Hindustani and black Africans.

Our finding of a stronger association for skin AF with complications of diabetes than for HbA1c, as observed in patients with R% above approximately 11%, has been described before.[1,3,14] It has been hypothesized that the relative high turnover rate of HbA1c (approximately 8 weeks) represents only a ''short-term memory,'' whereas skin AF provides a more ''long-term memory'' for glycemic stress on the end organs.[15] In accordance with Gerrits et al.,[4] we found a strong adjusted predictive value of skin AF for all diabetes-

Table 2. Odds Ratios for Diabetes-Related Complications, Associated with Skin Autofluorescence and Hemoglobin A1c, and the Influence of Low Reflectance Percentage

Analysis, parameter	*None* (n = *187*)	*Nephropathy* (n = *407*)	*Retinopathy* (n = *211*)	*Neuropathy* (n = *190*)	*Microvascular* (n = *548*)	*Macrovascular* (n = *248*)
Univariate						
Skin AF						
R% ≤11.12	1	1.14 (0.69–1.89)	1.30 (0.73–2.33)	1.72 (0.90–3.27)	1.19 (0.74–1.93)	1.52 (0.82–2.81)
R% ≥11.13	1	2.51 (1.74–3.63)	2.04 (1.36–3.06)	2.93 (1.88–4.58)	2.37 (1.66–3.39)	3.02 (2.02–4.52)
HbA1c						
R% ≤11.12	1	1.44 (1.10–1.88)	1.84 (1.27–2.66)	1.42 (1.00–2.00)	1.41 (1.09–1.82)	1.13 (0.77–1.65)
R% ≥11.13	1	1.15 (0.95–1.38)	1.46 (1.18–1.82)	1.19 (0.98–1.46)	1.15 (0.96–1.38)	0.91 (0.74–1.13)
Multivariate						
Skin AF						
R% ≤11.12	1	0.88 (0.48–1.61)	0.80 (0.40–1.62)	1.17 (0.54–2.53)	0.87 (0.50–1.51)	1.15 (0.55–2.40)
R% ≥11.13	1	1.92 (1.23–2.98)	1.90 (1.15–3.13)	1.81 (1.10–3.00)	1.81 (1.20–2.75)	1.71 (1.05–2.77)
HbA1c						
R% ≤11.12	1	1.59 (1.13–2.25)	3.38 (1.85–6.15)	1.63 (1.04–2.57)	1.68 (1.22–2.33)	1.38 (0.81–2.33)
R% ≥11.13	1	1.24 (1.00–1.53)	1.70 (1.32–2.20)	1.35 (1.08–1.70)	1.27 (1.04–1.55)	1.05 (0.82–1.35)

Data are presented as odds ratios with 95% confidence intervals per unit of skin autofluorescence (AF) (in arbitrary units) and per unit of hemoglobin A1c (HbA1c) (in %), according to ultraviolet reflectance percentage (R%). In the multivariate analysis, odds ratios were adjusted for age, sex, diabetes duration, current smoking, body mass index, systolic blood pressure, estimated glomerular filtration rate (by MDRD equation), and fasting non–high-density lipoprotein-cholesterol.

Table 3. Odds Ratios for Diabetes-Related Complications, Associated with Skin Autofluorescence and Hemoglobin A1c, Stratified by Ethnicity

Analysis, parameter	*None (n = 187)*	*Nephropathy (n = 407)*	*Retinopathy (n = 211)*	*Neuropathy (n = 190)*	*Microvascular (n = 548)*	*Macrovascular (n = 248)*
Univariate						
Skin AF (overall)	1	2.05 (1.54–2.71)	1.86 (1.35–2.58)	2.70 (1.89–3.88)	1.99 (1.52–2.62)	2.72 (1.96–3.76)
Dutch	1	2.76 (1.79–4.25)	2.41 (1.45–4.02)	2.56 (1.58–4.15)	2.52 (1.67–3.81)	3.12 (1.97–4.92)
Non-Dutch	1	1.53 (1.01–2.32)	1.78 (1.13–2.78)	2.46 (1.40–4.34)	1.55 (1.03–2.33)	1.79 (1.08–2.97)
HbA1c (overall)	1	1.22 (1.05–1.43)	1.55 (1.29–1.87)	1.24 (1.04–1.47)	1.22 (1.05–1.41)	0.95 (0.79–1.14)
Dutch	1	1.23 (0.97–1.58)	1.57 (1.16–2.13)	1.22 (0.93–1.60)	1.18 (0.93–1.50)	0.97 (0.73–1.29)
Non-Dutch	1	1.28 (1.05–1.57)	1.51 (1.19–1.92)	1.45 (1.14–1.86)	1.33 (1.09–1.62)	1.09 (0.84–1.41)
Multivariate						
Skin AF (overall)	1	1.52 (1.09–2.13)	1.45 (0.99–2.13)	1.71 (1.15–2.56)	1.46 (1.06–2.00)	1.64 (1.12–2.39)
Dutch	1	1.70 (0.99–2.91)	1.56 (0.82–2.95)	1.61 (0.92–2.82)	1.57 (0.97–2.55)	1.81 (1.07–3.06)
Non-Dutch	1	1.17 (0.72–1.91)	1.33 (0.79–2.23)	1.44 (0.76–2.71)	1.18 (0.74–1.88)	1.42 (0.78–2.60)
HbA1c (overall)	1	1.34 (1.12–1.60)	1.91 (1.52–2.39)	1.43 (1.17–1.74)	1.37 (1.16–1.61)	1.10 (0.88–1.37)
Dutch	1	1.53 (1.13–2.07)	2.20 (1.44–3.37)	1.51 (1.07–2.12)	1.40 (1.06–1.86)	1.14 (0.83–1.58)
Non-Dutch	1	1.26 (1.00–1.60)	1.78 (1.33–2.37)	1.56 (1.17–2.08)	1.38 (1.11–1.72)	1.11 (0.80–1.55)

Data are presented as odds ratios with 95% confidence interval, per unit of skin autofluorescence (AF) (in arbitrary units) and per unit of hemoglobin A1c (HbA1c) (in %), according to ethnicity. In the multivariate analysis, odds ratios were adjusted for age, sex, diabetes duration, current smoking, body mass index, systolic blood pressure, estimated glomerular filtration rate (by MDRD equation), and fasting non–high-density lipoprotein-cholesterol.

related microvascular complications except for retinopathy. Given the heterogeneity of this cohort and an in part unreliable measurement of skin AF (as discussed below), we can at this point only speculate on the possible explanations.

Based on the comparable distribution of cardiovascular risk factors in this cohort, it is not expected that patients from non-native Dutch origin have a substantial lower amount of AGEs in comparison with native Dutch patients. Yue et al.[8] compared the AF values of healthy Chinese individuals with the expected values of healthy whites of similar age and found no significant difference in skin AF between the groups, as long as the reflectance was 10% or higher. Our finding of significantly lower skin AF values in Hindustani and black African patients is therefore most likely related to the also lower R% compared with native Dutch patients.

Failure of the algorithm used seems an obvious explanation for the loss of association between skin AF and complications of diabetes in the lowest R% quartile. As mentioned before, the adaptations in the algorithm for skin color correction were developed in healthy persons only and were not or insufficiently validated in persons with diabetes without and with complications. Assuming similar behavior of AGE formation and breakdown among different ethnic groups, as suggested above, our finding of an association between skin AF and R%, especially among ethnic groups with lighter skin color types, also implies that there is opportunity for further improvement of the algorithm.

Another explanation is failure of the measurement technique. It may be that the absorption of (especially the UV) light in the upper and lower epidermis by melanin is so effective that a reliable measurement is simply not possible. However, the adequate performance of the 2.3 algorithm in tracking age-dependent changes in skin AGE levels suggests that this, to some extent, is not true and probably much depends on the R% (the lower, the poorer) and perhaps also on ethnic differences not only related to skin color.

Is the observed lack of association between skin AF and diabetes-related complications in the non-Dutch population possibly ''true''? There is, to our knowledge, no evidence for a different pathogenetic role of AGEs in the development of complications between ethnic groups. Theoretically, the skin might be an exception: AGE formation and accumulation in the dermis take place (like elsewhere in tissues with slow turnover) under the combined influence of glycemic and systemic oxidative stress, but may also be enhanced locally in the skin by UV radiation-induced oxidative stress, actinic elastosis, and other UV-enhanced factors, like photosensitizers and extracellular matrix components (such as fibronectin and fibrillin). The upper dermis is the part of the skin that is probably the most important in determining the AGE Readers' skin AF signal. Melanocytes in the lower epidermis, and to a smaller extent also in the upper dermis, form dendrites between the keratinocytes in the dermis, in which the melanosomes protect, for example, the nuclei of the keratinocytes. Perhaps they similarly protect collagen, elastin fibers, and fibroblasts (-cytes) in the upper dermis against UV but also against oxidative/glycemic stress.[16–18] So, a more effective protection against UV radiation-induced skin damage and AGE formation in the skin of people with substantial amounts of melanin might, to some extent, also protect them against glycemic or oxidative stress. Skin biopsy studies in persons with Fitzpatrick skin phototypes V and IV are needed to confirm this hypothesis.

Some aspects of the current study require further comment. The skin AF measurements were performed as part of the annual comprehensive diabetes evaluation, for 5 days during 1 week. Because the device is equipped with a temperature calibration correction and because no changes over time of the day were found, it is unlikely that factors related to time of the day such as heating of the lamp in the device have influenced the performance of the AGE Reader. However, further analysis of the time-dependent behavior of our measurements revealed a gradual decrease over the whole inclusion period of the AF value divided by the predicted value of AF as a function of age for healthy subjects.[19] This decrease could not be explained by changes in the composition

of the study population during the inclusion period or the population characteristics, including age, sex, HbA1c, and smoking history (data not shown). This might suggest that AGE Reader–related properties might change over time, even when the lamp is intermittently replaced and even though the skin AF as a ratio of emission and excitation light intrinsically corrects for changes in excitation light intensity. Finally, the exclusion of study subjects because of too low an R% resulted in loss of statistical power, and therefore no reliable estimates for the complication-associated risk of HbA1c could be calculated for the Hindustani and black African population.

In conclusion, skin AF is a valuable tool for the risk assessment of diabetes-related complications in patients with a UV reflectance of approximately 11% or higher. However, with the existing algorithm for skin color-independent assessment of skin AF, the noninvasive measurement of skin AF falls short in discriminating those with and without complications, in diabetes patients with darker skin color types. Future improvement of the skin color correction is therefore required before widespread use of the AGE Reader in patients of non-white origin or with a low UV reflectance.

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Author Disclosure Statement

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M.A. and V.E.A.G. designed the study protocol, wrote the manuscript, collected data, and performed statistical analyses. S.K. performed statistical analyses, contributed to the discussion, and reviewed/edited the manuscript. R.G. researched data, contributed to the discussion, and reviewed/edited the manuscript. A.J.S. and E.M. contributed to the discussion and reviewed/edited the manuscript. M.A. and V.E.A.G. had full access to all data in the study and take responsibility for the integrity of data and the accuracy of data analysis.

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Address correspondence to:
Mohamed Ahdi, MD
Department of Internal Medicine
Room 9B-v010
Slotervaart Hospital
Louwesweg 6
1066 EC Amsterdam, The Netherlands

E-mail: Mohamed.Ahdi@slz.nl