

Increased Accumulation of Skin Advanced Glycation End Products Is Associated with Microvascular Complications in Type 1 Diabetes

Aleksandra Araszkiewicz, M.D., Ph.D.,¹ Dariusz Naskret, M.D., Ph.D.,¹ Pawel Niedzwiecki, M.D.,¹ Pawel Samborski, M.D.,² Bogna Wierusz-Wysocka, M.D., Ph.D.,¹ and Dorota Zozulinska-Ziolkiewicz, M.D., Ph.D.¹

Abstract

Background: Skin autofluorescence (AF) measured with an AF reader device is a noninvasive tool to measure the tissue accumulation of advanced glycation end products (AGEs). The aim of the study was to assess the association between AF and microvascular complications in type 1 diabetes mellitus (DM1).

Methods: The study population consisted of 140 DM1 patients, 28 years old (interquartile range [IQR], 23–35), 76 of whom were women, with disease duration of 13 years (IQR, 8–19). We used the AGE Reader (DiagnOptics, Groningen, The Netherlands) to measure the AF phenomenon, which occurs because of fluorescent properties of AGEs. The patients were divided according to the presence or absence of diabetes-associated microvascular complications: retinopathy, nephropathy, and neuropathy and any microangiopathy.

Results: The median AF was 2.0 (IQR, 1.7–2.4). In the univariate logistic regression AF was significantly associated with retinopathy (odds ratio [OR] 2.47, 95% confidence interval [CI] 1.26–4.84, $P = 0.008$), nephropathy (OR 3.15, 95% CI 1.34–7.39, $P = 0.008$), neuropathy (OR 3.17, 95% CI 1.46–6.90, $P = 0.003$), and any microvascular complication (OR 2.94, 95% CI 1.46–5.92, $P = 0.002$). Multivariate logistic regression showed that skin AF was independently associated only with diabetic neuropathy (OR 2.98, 95% CI 0.99–8.90, $P = 0.05$).

Conclusions: The tissue accumulation of AGE is significantly associated with microvascular complications in DM1.

Introduction

DESPITE ADVANCES IN THE TREATMENT of diabetes, chronic complications remain a significant clinical problem. It has been shown that good glycemic control is essential in prevention of vascular complications. The Diabetes Control and Complications Trial¹ and its follow-up Epidemiology of Diabetes Interventions and Complications² studies demonstrated that the improvement of glycemic control reduced the risk of development and progression of microangiopathy as well as macroangiopathy. Glycated hemoglobin (HbA1C) still remains the standard procedure of evaluating long-term metabolic control of diabetes. However, it illustrates only the average glucose levels over the past 3 months, without showing the fluctuations of glycemia. Sustained hyperglycemia leads to the glycation of many proteins and formation of advanced glycation end products (AGEs). Increasing evidence points to a major role of AGEs in the functional and morphologic changes that characterize complications of dia-

betes.^{3,4} AGE measurements are significantly higher in type 1 diabetes mellitus (DM1), as well as in type 2 diabetes mellitus, patients than in the healthy population.^{5,6} AGEs can be assessed with the use of several methods. Plasma levels of AGEs can be measured from blood samples. However, the analysis of AGEs in blood proteins or plasma does not necessarily reflect their tissue concentration.⁷ Tissue accumulation can be calculated with biochemical and immunochemical assays, but the use of these methods is limited.⁸ Some AGEs have fluorescent properties, which enables measurement of their level in tissue biopsy specimens. Recently, a new, noninvasive method based on the autofluorescence (AF) phenomenon has been developed.⁹ However, further investigations are needed to evaluate the correlation of AF with metabolic control of diabetes and the development of chronic complications.

The aim of this study was to assess the association between AF measured noninvasively with an AF reader device and microvascular complications in DM1.

Departments of ¹Internal Medicine and Diabetology and ²Internal Diseases, Metabolic Disorders and Dietetics, Poznan University of Medical Sciences, Poznan, Poland.

Patients and Methods

Study population

Our study involved 140 DM1 patients hospitalized in the Department of Internal Medicine and Diabetology in Poznan, Poland, with a minimum disease duration of 5 years and a median age of 28 years (interquartile range [IQR], 23–35), of whom 76 were women. The patients were admitted to the hospital for education, adjustment of appropriate insulin dose, and late diabetes complications assessment. A complete medical history with detailed information on diabetes was taken. Patients underwent physical examination, including evaluation of body weight, height, and blood pressure. The entire study population consisted of Caucasians. All the subjects were informed about the aim of the study and gave their consent. The study was approved by the Ethical Committee of Poznan University of Medical Sciences. The clinical characteristics of the study population is presented in Table 1.

Skin AF

Skin AF was measured by an AF reader device (AGE Reader, type 214D00102, DiagnOptics, Groningen, The Netherlands). The AF reader device is a noninvasive device to assess the accumulation of AGEs with fluorescent properties in tissues. The tool has an excitation light source and optic spectrometer. First, it lights up the skin with ultraviolet light of 300–420 nm spectrum. Subsequently the light emitted by the skin is assessed with a spectrometer in a specific range (between 300 and 600 nm). This measurement is divided by the range of light emitted by the light source, thus giving the AF score.⁹

For each patient AF was measured three times in series, and the score was the arithmetical mean from those assessments. All the evaluations were performed in room temperature. AF was measured on the ventral side of the forearm, about 5 cm distally to antecubital space. The skin in all subjects examined was free of tattoos or skin lesions.

Laboratory analyses

Blood samples were collected in a fasting state, defined as no caloric intake for at least 8 h. Serum concentrations of high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, and creatinine were measured using standard methods. The estimated glomerular filtration rate was calculated using the Modification of Diet in Renal Disease study equation. HbA1C was measured using high-performance liquid chromatography. The serum concentration of C-reactive protein was measured by a high-sensitivity method with a lower limit of detection of 0.03 mg/L.

Assessment of microvascular complications

We divided the patients according to the presence or absence of diabetes-associated microvascular complications: retinopathy, nephropathy and neuropathy and any microangiopathy, defined as the presence or absence of at least one of the above complications.

Diabetic retinopathy was diagnosed using direct ophthalmoscopy through dilated pupils followed in all the patients by fundus photography. Fundus examinations were performed using an indirect Volk lens. Subsequently, using a 45°

TABLE 1. CLINICAL CHARACTERISTICS OF THE STUDY GROUP

Characteristic	Value
<i>n</i>	140
Age (years)	28 (IQR, 23–35)
Sex (women/men)	76/64
Duration of diabetes (years)	13 (IQR, 8–19)
Smoking (%)	22.9
Insulin dose (units/kg/day)	0.65 ± 0.19
History of hypertension (% positive)	22.1
ACE-I treatment (%)	30.0
BMI (kg/m ²)	23.7 (IQR, 21.38–26.19)
SBP (mm Hg)	120 (IQR, 110–120)
DBP (mm Hg)	70 (IQR, 70–80)
HbA1C (%)	8.6 (IQR, 7.3–9.5)
CRP (mg/L)	0.9 (IQR, 0.3–2.3)
TG (mmol/L)	1.08 (IQR, 0.77–1.40)
LDL (mmol/L)	2.71 ± 0.76
HDL (mmol/L)	1.63 ± 0.41
Creatinine (μmol/L)	74.2 (IQR, 63.6–83.1)
GFR (MDRD) (mL/min)	100.8 (IQR, 89.7–115.6)
Skin autofluorescence	2.0 (IQR, 1.7–2.4)
Retinopathy incidence (%)	38.6
Neuropathy incidence (%)	21.4
Nephropathy incidence (%)	12.9
Any microangiopathy (%)	45.7

Data are median (interquartile range [IQR]), mean ± SD, or percentage of patients.

ACE-I, angiotensin converting enzyme inhibitors; BMI, body mass index; CRP, C-reactive protein; DBP, diastolic blood pressure; GFR, glomerular filtration rate estimated using the Modification of Diet in Renal Disease (MDRD) study equation; HbA1C, glycated hemoglobin; HDL, high-density lipoproteins; LDL, low-density lipoprotein; SBP, systolic blood pressure; TG, triglycerides.

digital camera (VISUCAM, Zeiss, Oberkochen, Germany), two fundus photographs were taken of each eye: one centered on the fovea and one centered on the optic disc. Evaluation of the results of both ophthalmoscopy and fundus photographs was performed for the entire group by the same ophthalmologist with experience in diabetic retinopathy. Diabetic retinopathy was graded according to the classification of the American Academy of Ophthalmology as no retinopathy or mild nonproliferative, moderate nonproliferative, severe nonproliferative, and proliferative retinopathy.¹⁰

Diabetic nephropathy was detected at the stage of albuminuria. Assessment of albuminuria was performed by measurement of urinary albumin excretion over a 12-h period. Albuminuria was defined as a urinary albumin excretion rate between 30 and 300 mg/24 h in two of three samples collected over a 3-month period after exclusion of secondary causes of microproteinuria (urinary tract infection, heart failure, acute febrile illness, hematuria, or excessive physical activity). Diabetic nephropathy was defined as the presence of albuminuria in connection with diabetes of over 10 years in duration or with diagnosed diabetic retinopathy.¹¹

Neuropathy assessment was performed using pressure sensation (10-g monofilament perception), vibration perception (128-Hz tuning fork), and ankle reflex tests. Diabetic neuropathy was diagnosed in patients with two or more of the following four components: the presence of symptoms of

TABLE 2. VARIABLES RELATED TO THE PRESENCE OF MICROVASCULAR COMPLICATIONS IN UNIVARIATE LOGISTIC REGRESSION

Variable	Retinopathy			Nephropathy			Neuropathy			Any microangiopathy		
	P	OR (95% CI)		P	OR (95% CI)		P	OR (95% CI)		P	OR (95% CI)	
Age	<0.00001	1.08 (1.03–1.13)		0.44	1.01 (0.97–1.06)		<0.00001	1.17 (1.10–1.25)		<0.00001	1.12 (1.06–1.17)	
Sex	0.32	1.41 (0.70–2.80)		0.69	1.21 (0.44–3.30)		0.25	1.61 (0.69–3.71)		0.55	1.22 (0.62–2.40)	
Duration of diabetes	<0.00001	1.23 (1.14–1.33)		0.0004	1.11 (1.05–1.18)		<0.00001	1.12 (1.05–1.19)		<0.00001	1.28 (1.17–1.39)	
Smoking	0.55	1.27 (0.56–2.84)		0.006	4.30 (1.52–12.15)		0.34	0.60 (0.20–1.75)		0.88	1.06 (0.47–2.36)	
Insulin dose	0.15	3.75 (0.60–23.16)		0.26	4.16 (0.32–52.92)		0.45	2.31 (0.25–21.16)		0.12	4.03 (0.67–24.21)	
History of hypertension	0.006	3.21 (1.39–7.38)		0.004	4.54 (1.60–12.88)		0.004	3.82 (1.54–9.48)		0.0008	4.76 (1.93–11.73)	
ACE-I treatment	<0.00005	5.25 (2.39–11.54)		<0.00005	17.59 (4.68–66.04)		0.0007	4.67 (1.94–11.23)		<0.00001	6.59 (2.86–15.18)	
BMI	0.19	1.06 (0.96–1.16)		0.32	0.93 (0.80–1.07)		0.11	1.09 (0.97–1.22)		0.10	1.07 (0.98–1.18)	
SBP	0.20	0.98 (0.96–1.00)		0.33	1.01 (0.98–1.05)		0.33	1.01 (0.98–1.04)		0.53	0.99 (0.96–1.01)	
DBP	0.98	0.99 (0.96–1.03)		0.53	1.01 (0.96–1.07)		0.96	1.00 (0.95–1.04)		0.83	0.99 (0.96–1.03)	
HbA1C	0.21	1.13 (0.93–1.38)		0.39	1.12 (0.85–1.49)		0.95	1.00 (0.78–1.28)		0.32	1.10 (0.90–1.34)	
CRP	0.19	1.13 (0.93–1.36)		0.77	0.95 (0.69–1.31)		0.72	1.03 (0.83–1.29)		0.28	1.10 (0.91–1.33)	
TG	0.95	0.99 (0.99–1.00)		0.21	1.00 (0.99–1.01)		0.92	0.99 (0.99–1.00)		0.84	0.99 (0.99–1.00)	
LDL	0.16	1.00 (0.99–1.02)		0.77	1.00 (0.98–1.02)		0.94	1.00 (0.98–1.01)		0.04	1.01 (1.00–1.02)	
HDL	0.80	0.99 (0.97–1.01)		0.89	1.00 (0.97–1.03)		0.56	1.00 (0.98–1.03)		0.64	1.00 (0.98–1.02)	
Creatinine	0.015	14.47 (1.68–124.11)					0.01	31.91 (2.21–459.58)		0.004	27.56 (2.81–270.20)	
AF	0.008	2.47 (1.26–4.84)		0.008	3.15 (1.34–7.39)		0.003	3.17 (1.46–6.90)		0.002	2.94 (1.46–5.92)	
Retinopathy	0.0001	6.35 (2.51–16.07)		0.0003	17.02 (3.67–78.77)		0.0001	6.35 (2.51–16.07)				
Neuropathy	0.0003	17.02 (3.67–78.78)		0.17	2.15 (0.70–6.58)							
Nephropathy							0.17	2.15 (0.70–6.58)				

ACE-I, angiotensin converting enzyme inhibitors; AF, skin autofluorescence; BMI, body mass index; CI, confidence interval; CRP, C-reactive protein; DBP, diastolic blood pressure; HbA1C, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio; SBP, systolic blood pressure; TG, triglycerides.

Significant values ($P < 0.05$) are shown in bold type.

TABLE 3. VARIABLES RELATED TO THE PRESENCE OF MICROVASCULAR COMPLICATIONS IN MULTIVARIATE LOGISTIC REGRESSION ADJUSTED FOR AGE WITH DIFFERENT INDEPENDENT VARIABLES

Variable	Retinopathy		Nephropathy		Neuropathy		Any microangiopathy	
	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
Male sex	0.97	1.01 (0.35–2.88)	0.80	1.23 (0.23–6.49)	0.02	4.32 (1.15–16.18)	0.78	0.86 (0.30–2.42)
Duration of diabetes	<0.00001	1.26 (1.15–1.39)	0.001	1.16 (1.05–1.27)	0.02	1.08 (1.01–1.16)	<0.00001	1.27 (1.15–1.40)
Smoking	0.59	1.36 (0.42–4.33)	0.005	9.65 (2.00–46.36)	0.30	0.50 (0.13–1.87)	0.94	1.04 (0.32–3.32)
History of hypertension	0.61	1.34 (0.41–4.37)	0.02	6.87 (1.24–37.88)	0.75	1.21 (0.35–4.16)	0.30	1.88 (0.55–6.37)
BMI	0.70	1.02 (0.89–1.17)	0.017	0.76 (0.61–0.95)	0.23	1.09 (0.93–1.28)	0.43	1.05 (0.92–1.21)
HbA1C	0.20	1.23 (0.89–1.71)	0.35	1.26 (0.77–2.06)	0.74	1.06 (0.72–1.57)	0.23	1.21 (0.87–1.67)
LDL	0.58	1.00 (0.98–1.02)	0.62	0.99 (0.97–1.01)	0.38	0.99 (0.97–1.01)	0.33	1.00 (0.99–1.02)
HDL	0.60	0.99 (0.96–1.02)	0.82	1.00 (0.95–1.05)	0.08	1.03 (0.99–1.07)	0.38	1.01 (0.98–1.04)
AF	0.90	1.05 (0.39–2.08)	0.69	0.76 (0.19–2.97)	0.045	2.98 (0.99–8.90)	0.78	1.14 (0.43–3.01)

AF, skin autofluorescence; BMI, body mass index; CI, confidence interval; HbA1C, glycated hemoglobin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; OR, odds ratio.

Significant values ($P < 0.05$) are shown in bold type.

neuropathy, the absence of ankle tendon reflexes, and/or abnormal scores for pressure and/or vibration perception.

Statistical analysis

Statistical analysis was performed using Statistica PL version 8.0 (StatSoft Inc., Tulsa, OK). The results of continuous variables are shown as mean \pm SD values for normally distributed data, as median values and IQR when the data were skewed, or as number and percentage of patients for categorical data. The Kolmogorov–Smirnov test with a Lilliefors correction was used to test for normality. In analyses a value of $P < 0.05$ was considered statistically significant. In the statistical analysis univariate logistic regression was used for determination of factors connected with diabetic microangiopathy, and multivariate logistic regression was used to determine independent relationship of variables with the presence of diabetic microangiopathy. Multivariate linear-regression analyses included sex, body mass index, diabetes duration, HbA1C, smoking, hypertension, skin AF, low-density lipoprotein cholesterol level, and high-density lipoprotein cholesterol level.

Results

In the study group with a median duration of diabetes of 13 years, we found 45.7% with any microvascular complication, 38.6% with retinopathy, 12.9% with nephropathy, and 21.4% with neuropathy. The median skin AF measured by the AGE Reader was 2.0 (IQR, 1.7–2.4). Patients with microangiopathy had higher median skin AF compared with subjects without microangiopathy (2.3 [IQR, 1.9–2.7] vs. 1.9 [IQR, 1.6–2.2]; $P = 0.0003$). In the subgroup with neuropathy we found higher skin AF than in subjects without any microangiopathy (2.4 [IQR, 1.9–2.8] vs. 1.9 [IQR, 1.6–2.2]; $P = 0.0002$). In the univariate logistic regression we have shown that in DM1 AF was significantly associated with retinopathy (odds ratio [OR] 2.47, 95% confidence interval [CI] 1.26–4.84, $P = 0.008$), nephropathy (OR 3.15, 95% CI 1.34–7.39, $P = 0.008$), or neuropathy (OR 3.17, 95% CI 1.46–6.90, $P = 0.003$) and with any microvascular complication (OR 2.94, 95% CI 1.46–5.92,

$P = 0.002$) (Table 2). It is interesting that the presence of any late complications was not associated with metabolic control of diabetes. Multivariate logistic regression showed that skin AF was independently associated only with diabetic neuropathy (OR 2.98, 95% CI 0.99–8.90, $P = 0.045$). Neuropathy was also associated with male sex (OR 4.32, 95% CI 1.15–16.18, $P = 0.02$) and duration of diabetes (OR 1.08, 95% CI 1.01–1.16, $P = 0.02$). Duration of diabetes was the only significant independent variable related to the presence of retinopathy and any microangiopathy. In multivariate logistic regression the presence of nephropathy was associated with disease duration, smoking, history of hypertension, and body mass index (Table 3).

Discussion

We have shown that skin AF measured by the AGE Reader was significantly associated with retinopathy, nephropathy, and neuropathy and with any microangiopathy in type 1 diabetes. In multivariate logistic regression analysis AF was independently associated only with diabetic neuropathy. The role of nonenzymatic glycation of proteins and formation of AGEs in the pathogenesis of late complications of diabetes has been well described.⁴ The AGE Reader used in our study to assess AGE accumulation in the skin is a simple, noninvasive device, constructed within the last few years. Meerwaldt et al.⁹ proved that skin AF measured with an AF reader device strongly correlated with tissue accumulation of AGEs in healthy people and in diabetes. The major benefit of assessing AF could be the possibility of identifying the risk of the development of late complications of diabetes. According to Lutgers et al.,⁶ AF correlates positively with the severity of type 2 diabetes complications. Meerwaldt et al.¹² reported association of AF with clinical manifestation of diabetic neuropathy. Recently, the relationship between coronary heart disease and AGE accumulation was described.¹³ However, these studies were conducted mostly on type 2 diabetes patients, and the information about the relationship between skin AF assessed noninvasively and microangiopathy in DM1 patients is very limited.¹⁴ We have shown the strong relationship between AF result and all microvascular complica-

tions in young DM1 subjects. Our results might confirm the usefulness of assessing AGE accumulation in DM1 to evaluate the patients' risk of vascular complications. However, future prospective studies are needed to examine the usefulness of AF determination as a risk factor of diabetic microangiopathy.

The correlation of AGE accumulation with long-term metabolic control of diabetes has been described previously.¹³ However, Genuth et al.¹⁵ showed on Diabetes Control and Complications Trial patients the predictive association of skin collagen glycation with progression of neuropathy and retinopathy independently from frequently measured HbA1C. The authors suggested that this association is not just a reflection of chronically elevated plasma glucose levels. There is a growing body of evidence that receptor for AGE interaction-mediated oxidative stress generation plays an important role in the pathogenesis of diabetic retinopathy.¹⁶ Through the interaction with the receptor for AGE, AGE activates secondary messenger pathways such as protein kinase C and nuclear factor κ B, increasing transcription of pro-inflammatory cytokines.¹⁷ As skin AF reflects the cumulative effect of chronic hyperglycemia and oxidative stress, it might be more informative for the risk of complications than HbA1C. We have shown that the presence of microangiopathy was not associated with the parameters of metabolic control, whereas it was strongly correlated with skin AF. Similarly, Meerwaldt et al.¹² showed that AF was associated with neuropathy independently of age, hyperglycemia, and albuminuria. However, we had just one-point HbA1C values, and this fact may cause the lack of association between microangiopathy and metabolic control of diabetes revealed in our study.

We have to be aware of some limitations of this method in the assessment of AGE accumulation.^{9,12,13} First, not all AGEs have fluorescent properties, and other tissue components that fluorescence in the same range of wavelength might be confounders. However, the method has been validated against specific AGE levels in skin biopsy specimens in healthy subjects, as well as in patients with diabetes and in subjects with end-stage renal disease.^{9,18} Second, skin AF is strongly associated with age.^{5,6,19} In our research, the patients' age range was relatively narrow. Moreover, we have chosen regression analyses that eliminate the effect of age on the results. Finally, the study was designed to be cross-sectional; therefore the interpretation of the results should be done with caution. The probable prognostic usefulness of this simple noninvasive method of the assessing the risk of microangiopathy should be proved in prospective observations.

Conclusion

The tissue accumulation of AGE is significantly associated with microvascular complications in DM1.

Author Disclosure Statement

No competing financial interests exist.

References

1. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Research Group. *N Engl J Med* 1993;329:977–986.
2. Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. *Diabetes Care* 1999;22:99–111.
3. Brownlee M: The pathobiology of diabetic complications: a unifying mechanism. *Diabetes* 2005;54:1615–1625.
4. Brownlee M: Negative consequences of glycation. *Metabolism* 2000;49:9–13.
5. Monnier VM, Vishwanath V, Frank KE, Elmetts CA, Dauchot P, Kohn RR: Relation between complications of type I diabetes mellitus and collagen-linked fluorescence. *N Engl J Med* 1986;314:403–408.
6. 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. *Diabetes Care* 2006;29:2654–2659.
7. Dorrian CA, Cathcart S, Clausen J, Shapiro D, Dominiczak MH: Factors in human serum interfere with the measurement of advanced glycation endproducts. *Cell Mol Biol* 1998;44:1069–1079.
8. Meerwaldt R, Links T, Zeebregts C, Tio R, Hillebrands JL, Smit A: The clinical relevance of assessing advanced glycation endproducts accumulation in diabetes. *Cardiovasc Diabetol* 2008;7:29.
9. Meerwaldt R, Graaff R, Oomen PH, Links TP, Jager JJ, Alderson NL, Thorpe SR, Baynes JW, Gans RO, Smit AJ. Simple non-invasive assessment of advanced glycation endproduct accumulation. *Diabetologia* 2004;47:1324–1330.
10. Wilkinson CP, Ferris FL 3rd, Klein RE, Lee PP, Agardh CD, Davis M, Dills D, Kampik A, Pararajasegaram R, Verdager JT; Global Diabetic Retinopathy Project Group: Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology* 2003;110:1677–1682.
11. KDOQI clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. *Am J Kidney Dis* 2007;49(2 Suppl 2):S12–S154.
12. Meerwaldt R, Links TP, Graaff R, Hoogenberg K, Lefrandt JD, Baynes JW, Gans RO, Smit AJ: Increased accumulation of skin advanced glycation end-products precedes and correlates with clinical manifestation of diabetic neuropathy. *Diabetologia* 2005;48:1637–1644.
13. 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;30:107–112.
14. Chabroux S, Canoui-Poitaine F, Reffet S, Mills-Joncour G, Morelon E, Colin C, Thivolet C: Advanced glycation end products assessed by skin autofluorescence in type 1 diabetes are associated with nephropathy, but not retinopathy. *Diabetes Metab* 2010;36:152–157.
15. Genuth S, Sun W, Cleary P, Sell DR, Dahms W, Malone J, Sivity W, Monnier VM: 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 Complications participants with type 1 diabetes. *Diabetes* 2005;54:3103–3111.
16. Yamagishi S, Ueda S, Matsui T, Nakamura K, Okuda S: Role of advanced glycation end products (AGEs) and oxidative stress in diabetic retinopathy. *Curr Pharm Des* 2008;14:962–968.
17. Goh SY, Cooper ME: The role of advanced glycation end products in progression and complications of diabetes. *J Clin Endocrinol Metab* 2008;93:1143–1152.

18. Gerrits EG, Lutgers HL, Kleefstra N, Graaff R, Groenier KH, Smit AJ, Gans RO, Bilo HJ: Skin autofluorescence. A tool to identify type 2 diabetic patients at risk for developing microvascular complications. *Diabetes Care* 2008;31: 517–521.
19. Monami M, Lamanna C, Gori F, Bartalucci F, Marchionni N, Mannucci E: Skin autofluorescence in type 2 diabetes: beyond blood glucose. *Diabetes Res Clin Pract* 2008;79:56–60.

Address correspondence to:
Aleksandra Araszkiewicz, M.D., Ph.D.
Department of Internal Medicine and Diabetology
Poznan University of Medical Sciences
Mickiewicza 2
60-834 Poznan, Poland
E-mail: olaaraszkiewicz@interia.pl