Original Article

Non-invasive measurement of skin autofluorescence to evaluate diabetic complications

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Although the accumulation of advanced glycation end-products (AGEs) of the Maillard reaction in our body is reported to increase with aging and is enhanced by the pathogenesis of lifestyle-related diseases such as diabetes, routine measurement of AGEs is not applied to regular clinical diagnoses due to the lack of conventional and reliable techniques for AGEs analyses. In the present study, a non-invasive AGEs measuring device was developed and the association between skin AGEs and diabetic complications was evaluated. To clarify the association between the duration of hyperglycemia and accumulation of skin fluorophores, diabetes was induced in mice by streptozotocin. As a result, the fluorophore in the auricle of live mice was increased by the induction of diabetes. Subsequent studies revealed that the fingertip of the middle finger in the non-dominant hand is suitable for the measurement of the fluorescence intensity by the standard deviation value. Furthermore, the fluorescence intensity was increased by the presence of diabetic microvascular complications. This study provides the first evidence that the accumulation of fluorophore in the fingertip increases with an increasing number of microvascular complications, demonstrating that the presence of diabetic microvascular complications may be predicted by measuring the fluorophore concentration in the fingertip.

Key Words: **advanced glycation end-product (AGEs), *N*δ-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1), diabetic complications, autofluorescence, diagnosis**

The excessive intake of energy and lack of exercise increase the pathogenesis of lifestyle-related diseases, which becomes problematic regarding medical and social aspects. The number of diabetic patients has increased worldwide and was reported to be 10.1 million in 2012 in Japan. Diabetes causes complications such as retinopathy, nephropathy and neuropathy[1] and results in the loss of vision, kidney failure, dysautonomia and neuralgia.[2–4] However, because the fundamental treatment of lifestyle-related diseases such as atherosclerosis and diabetic complications is difficult, early treatment with suitable medicine is the most important approach to prevent the diseases. Therefore, the early diagnosis and accurate evaluation of the therapeutic effect are necessary to prevent the progress of the above diseases. Although hemoglobin A1c (HbA1c), an early stage product of the Maillard reaction between the β chain of hemoglobin with glucose, is measured clinically as an index of blood glucose control of the past 1–2 months, it is invalid for predicting the progression of diabetic complications. Recent studies demonstrate that advanced glycation end products (AGEs) of the Maillard reaction accumulate in our bodies with aging and are enhanced by the pathogenesis of diabetic complications, including nephropathy,[5–7] retinopathy[8–10] and atherosclerosis.[11–13] Therefore, because the measurement of AGEs is predicted to be an early marker for diabetic complications, the development of a rapid and reliable method to measure AGEs is also anticipated.

Meerwaldt *et al.*[14,15] estimated skin AGEs by measuring characteristic fluorescence wavelengths. However, this method is rarely used in hospitals in Asian countries because the presence of subdermal vessels and change in skin color may also affect the measurement of the fluorescence intensity in each subject. The original fluorescent AGE analyzer was developed for Caucasians in The Netherlands, and the usage of the apparatus for Asians is limited due to the lack of a correlation with diabetes. In the present study, we aimed to develop a non-invasive, rapid and highly reproducible detection system for skin fluorophores. Therefore, we determined the most relevant fluorescent wavelength and selected ideal regions less likely to be influenced by the change in skin color.

Materials and Methods

Chemicals. Streptozotocin (STZ) was purchased from Sigma-Aldrich Japan (Tokyo, Japan). Acetonitrile, H_2O and formic acid were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals were of the best grade available from commercial sources.

Fluorescence measurement of human serum. It is known that serum AGEs is increased by the pathogenesis of renal dysfunction.[7–9] Since skin fluorescence intensity is too weak to select the optimum wavelength for measuring biological AGEs, sera in patients with renal dysfunction and normal subjects were used. A total of 8 patients (61.9 ± 10.5 years, M/F: 3/5) who were admitted in Division of Nephrology and Rheumatology, Fukuoka University Hospital (Fukuoka, Japan) from April to November 2014. Two and four of them had a diagnosis of chronic kidney disease (CKD) stage 3 and 5, respectively. The remaining two patients were received hemodialysis. Blood samples were

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collected after an overnight fast, and the serum was separated from blood cells. A part of serum was aspirated and stored at –80°C until analyses were performed. The Ethic Committee of Fukuoka University and Tokai University (Protocol Number: 14035) approved this study protocol, and informed consent was obtained from all the patients. Fluorescence spectrum data of sera were recorded by fluorescence spectrophotometer using a FL-4500 (Hitachi, Tokyo, Japan).

Measurement of *N*δ-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1) in the serum by LC-MS/MS. MG-H1 contents in the serum was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described previously.[16] Briefly, low molecular weight fractions (<3,000) of serum (50 μl) were obtained by VIVASPIN 500 (Sartorius Stedim Biotech, Goettingen, Germany), and reduced by $NaBH_4$. Standard [$^2H_2$] MG-H1 (PolyPeptide Laboratories, Strasbourg, France) and [$^{13}C_6$] Lysine (Cambridge Isotope Laboratories, Inc., Tewksbury, MA) were added to the reduced fractions. The sample was passed over a Strata-X-C column (Phenomenex, Torrance, CA) and assayed by LC-MS/MS using a TSQ Vantage triple stage quadrupole mass spectrometer (Thermo Fisher Scientific, Waltham, MA). The retention time for MG-H1 and Lysine were approximately 12 and 13 min, respectively. MG-H1, Lysine, and the standard were detected by electrospray positive ionization-mass spectrometric multiple reaction monitoring.

Animal experiments. The experimental protocol was approved by the ethics review committee of Japan Women's University for animal experimentation. Five-week-old male ddY mice (weight ~20 g) were purchased from SLC (Shizuoka, Japan). Diabetes was induced by an abdominal injection of STZ (150 mg/kg body weight) in 200 μl of 0.05 M saline-citrate buffer (pH 4.5). Age-matched control mice were given abdominal injections of same buffer alone. These mice were housed in a pathogen-free barrier facility (12 h/12 h light/dark cycle) and were fed a normal rodent chow diet (Clea, Tokyo, Japan). The accumulation of AGEs in the skin was estimated by measuring the auricle fluorescence intensity. Similar to previous reports,[17] skin fluorescence intensity was recorded with a prototype (fiber-type) consisting of a light emitting diode (LED) light source, a spectral apparatus system by a 2048-pixel CCD linear image sensor and grating and a biantennary silica-based optical fiber (please see Supplemental Fig. 1*). A mouse skin surface area measuring approximately 0.38 mm in diameter was irradiated by an LED light source of 365 nm at the excitation peak. For the detection of fluorescence signals at the same location on the skin, a Y-shaped flexible optical fiber coated in polyurethane was used (please see Supplemental Fig. 1*). The quartz Y-shape fiber consists of two core fibers (0.6 mm in diameter), and one side of the fibers was connected to a spectrometer while the other side was connected to an LED light source. The mouse skin autofluorescence spectrum of the auricle showed a stable emission peak at 440–460 nm. All measurements were performed in a semi-dark, temperature-controlled room.

Measurement of skin melanin contents. A Mexameter® (Courage-Khazaka Electronic, Cologne, Germany) was used to measure the melanin index (MI) on 49 human volunteers (26 males, 23 females, Average: 46.6 ± 9.1 years of age). The MI was collected at 4 points, two points on the left side and two points on the right side, on the forearms of each volunteer. Fluorescence measurements were subsequently performed at the same positions used to measure the MIs.

Measurement of fingertip fluorescence by a clip-type sensor. A finger clip feature was adopted to measure the intensity of fluorescence from the middle finger of the non-dominant hand for clinical experiments. Standard deviation of average the fluorescence intensity of three fingers in the dominant and non-dominant hand were compared. Each finger has an axisymmetric arrangement for each hand, and we could resist body movement during the measurement. In addition, the clip pinched each finger at 5.5–6.0 N. The detection unit of fluorescence (please see Supplemental Fig. 1*) was utilized as previously described with a quartz Y-shape fiber consisting of multi-core fibers (received fiber: 0.6 mm in diameter, irradiated 13-fiber loop to encircle the received fiber, 0.19 mm in diameter).

Human clinical study. A total of 168 subjects (82 subjects with type 2 diabetes and 86 subjects without type 2 diabetes) who were admitted to Kumamoto University Hospital between 2013 and 2015 were recruited. Type 2 diabetes was diagnosed according to the World Health Organization criteria (Table 1).[18] Patients with type 1 diabetes were excluded, as were patients positive for glutamic acid decarboxylase antibody, those with a history of ketoacidosis, and patients dependent on insulin therapy. Patients with severe hepatic disease, malignancy, or acute/chronic inflammatory disease were also excluded. The patient characteristics are listed in Table 1. All subjects included in this study were Japanese. Each patient participated in a detailed interview of his/her personal disease. The study protocol was approved by the Human Ethics Review Committee of Kumamoto University (Protocol Number: 1737), and all subjects provided their written informed consent. Blood samples were collected from the diabetic participants in the early morning after an overnight fast. Fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), serum total cholesterol (TC), triglycerides (TG) and high-density lipoprotein (HDL) cholesterol were measured by standard methods.

Table 1. Characteristics of subject (*n* = 82)

Age (years)	64.9 ± 11.1
Sex (% female)	52.4
Duration of diabetes (years)	12.2 ± 10.9
BMI (kg/m^2)	25.5 ± 11.4
SBP (mmHg)	131.2 ± 16.0
DBP (mmHg)	75.1 ± 10.1
FPG (mmol/L)	7.02 ± 1.72
HbA1c (%)	7.42 ± 1.34
TC (mmol/L)	4.77 ± 0.95
TG (mmol/L)	1.54 ± 0.72
HDL cholesterol (mmol/L)	1.39 ± 0.34
LDL cholesterol (mmol/L)	2.67 ± 0.86
non-HDL cholesterol (mmol/L)	3.40 ± 0.96
hs-CRP (mg/L)	0.31 ± 1.13
ACR (mg/g)	171.6 ± 737.5
Hypertension (%)	64.6
Hyperlipidemia (%)	76.8
Diabetic microangiopathy (%)	
Retinopathy	19.5
Neuropathy	20.7
Nephropathy	41.5
Diabetes medication (%)	
Oral hypoglycemic agents	65.9
Insulin	8.5
Oral hypoglycemic agents + insulin	8.5
Statins (%)	56.1
ARBs and/or ACEIs (%)	53.7
CCBs (%)	48.8
Ant-platelet agents (%)	30.5

Data are means ± SD. SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; TC, total cholesterol; TG, triglyceride; hs-CRP, high sensitive C-reactive protein; ACR, urinary albumin-creatinin ratio; ARBs, angiotensin II receptor blockers; ACEIs, angiotensin converting enzymes; CCBs, calcium channel blockers.

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Low-density lipoprotein (LDL) cholesterol was determined using the Friedewald formula.[19] HbA1c (%) was estimated as National Glycohemoglobin Standardization Program (NGSP) equivalent values (%), calculated using the formula HbA1c (%) = 1.02 × HbA1c [Japan Diabetes Society (JDS)] (%) + 0.25%, in consideration of the relational expression of HbA1c (JDS) (%) measured by the previous Japanese standard substance and measurement methods and HbA1c (NGSP).[20] Diabetic retinopathy was assessed by ophthalmologists and graded according to the International Clinical Classification of Diabetic Retinopathy.[21] Diabetic nephropathy was defined as the presence of microalbuminuria provided by an albumin-to-creatinine ratio ≥30 mg/g.[22] Diabetic neuropathy diagnosed by the presence of two of the following three factors, as recommended in the simplified diagnostic criteria proposed by the Diabetic Neuropathy Study Group in Japan[23]: i) subjective symptoms in the bilateral lower limbs or feet; ii) loss of or decreased ankle jerk; and iii) decreased vibration perception, assessed using a C128 tuning fork and bilaterally measured at the medial malleoli. The presence of microvascular complications was defined as meeting the criteria of microalbuminuria, neuropathy and/or retinopathy. The following clinical data of the control group were collected: age, sex, BMI and blood pressure.

Statistical analysis. All statistical analyses were performed using the SPSS software program ver. 20 for Windows (SPSS Inc., Chicago, IL). All data are presented as the means ± SD or as actual numbers. The Mann-Whitney *U* test and chi-square test were used for categorical variables. An unpaired Student's *t* test was used for normally distributed variables. A p value <0.05 was considered to be statistically significant.

Results

Fluorescence measurement of human serum. To detect fluorophores in physiological samples, the maximum excitation and emission wavelengths of human serum were measured by a fluorometric detector. As shown in Fig. 1A, the sera from patients with renal disease gave a fluorescent spectrum with an excitation maximum at 330–350 nm and emission maximum at 420–450 nm. To clarify the maximum wavelength for excitation, fluorescent properties in the sera of normal subjects and of patients with renal dysfunction were measured. To acquire the optimum excitation wavelength, the detection position was fixed at 440 nm and surveyed the excitation wavelength (Fig. 1B). Both group showed fluorescent spectra with an excitation maximum at 340 nm and emission maximum at 440 nm (Fig. 1C). The maximum fluorescent intensities of nephropathy were higher than that of normal subjects (Fig. 1D).

Change in the auricle fluorescence intensity in diabetic mice. To demonstrate the change of fluorophore contents by diabetes, diabetes was induced in mice by STZ and the fluorescence intensity on the auricle was measured. The blood glucose level steeply increased after the induction of diabetes, but decreased after 3 months (Fig. 2A) most likely due to the progression of type 1 diabetes. Although the body weight of normal mice

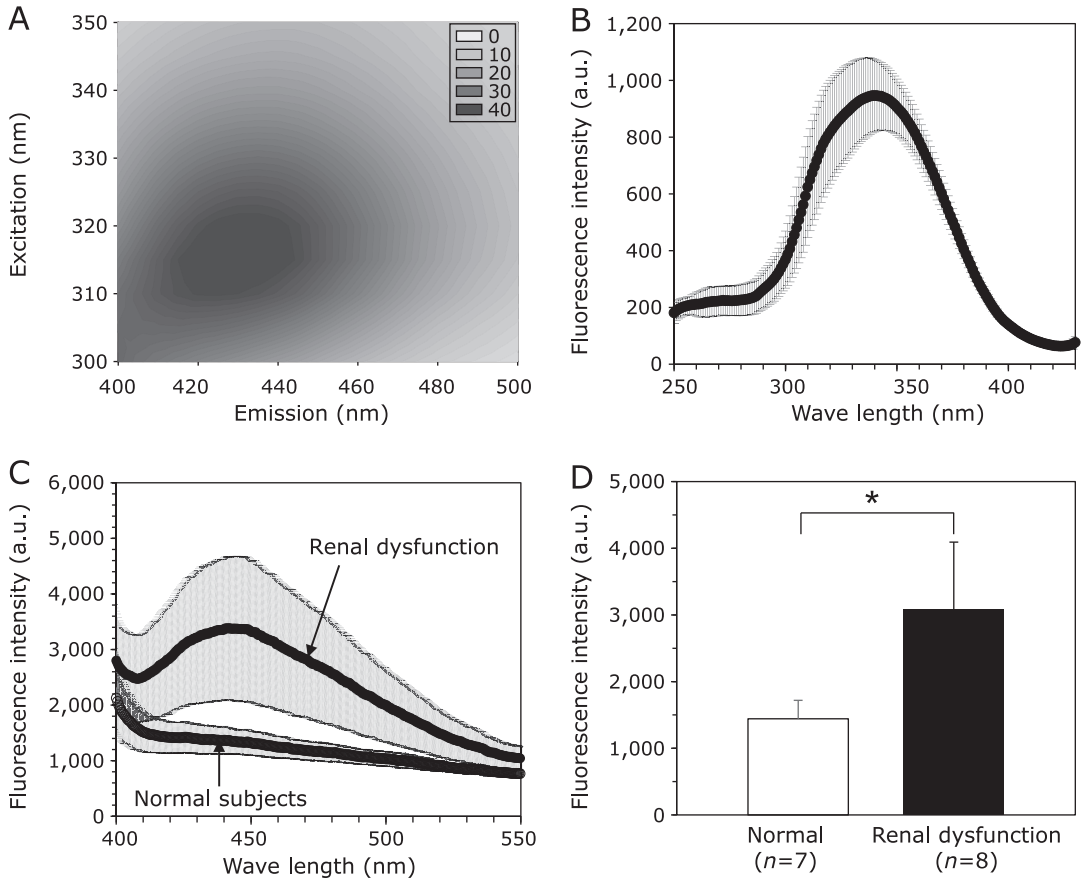

Fig. 1. Fluorescence measurement of human sera. Typical fluorescent wavelength scanning of sera from patients with renal dysfunction (A). The maximum wavelength for excitation fluorescence spectra in the sera of patients with renal dysfunction (n = 8, closed circle) were measured at a constant emission wavelength (440 nm) (B). The emission fluorescence spectra were recorded at a constant excitation wavelength (340 nm) in sera from normal subjects (n = 7, open circle) and in patients with renal dysfunction (n = 8, closed circle) (C). The florescent intensities by excitation at 340 nm and emission at 440 nm were compared (D). Data are presented as the mean ± SD. *p<0.01 vs normal subjects.

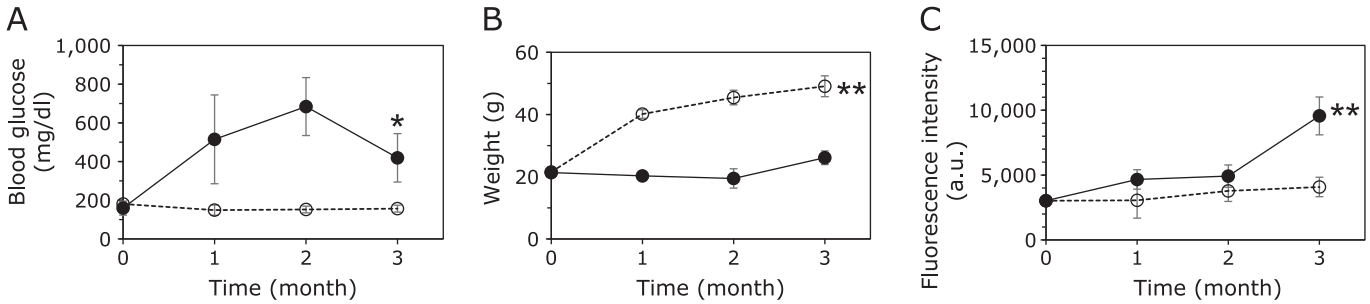

Fig. 2. Change in the blood glucose, body weight and auricle fluorescence intensities in diabetic mice. Diabetes was induced in mice by streptozotocin and changes in the blood glucose (A), body weight (B) and fluorescence intensities on the auricle (C) of normal mice (n = 8, open circle) and diabetic mice (n = 8, closed circle) were measured. Data are presented as the means ± SD. *p<0.05, **p<0.01 vs normal mice.

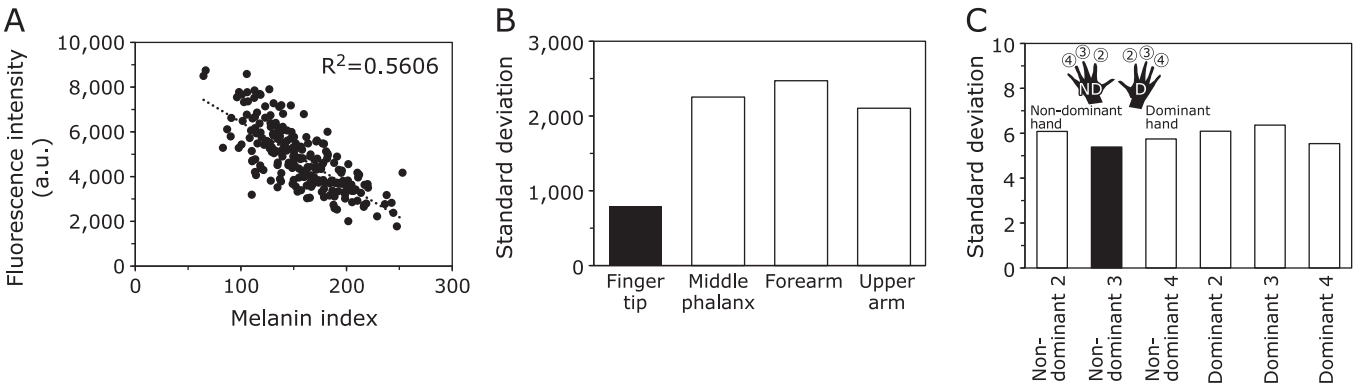

Fig. 3. Evaluation of optimal regions for measurement of fluorescence intensities. The melanin content of the forearm was measured by a Mexameter® as described in the materials and method and compared with the fluorescence intensities (n = 49) (A). The standard deviation of the fluorescence intensity in several regions such as the fingertip, middle phalanx, forearm and upper arm (n = 11) were evaluated (B). The standard deviation of the fingertip fluorescence intensity on the index finger, middle finger and annular finger of the dominant or non-dominant arm were also determined (n = 86) (C).

increased in a time-dependent manner that of diabetic mice slightly increased at 3 months (Fig. 2B). Furthermore, auricle fluorescence intensities of diabetic mice increased at 3 months, whereas that of normal mice did not change during the experimental period (Fig. 2B), indicating that the accumulation of fluorophores increased under diabetic conditions during the experimental period.

Evaluation of optimal regions for measurement of fluorescence intensity. Because colored pigments *in vivo* such as melanin and hemoglobin may block the transmission of light, the correlation between melanin contents and fluorescent intensity in the skin was measured. As shown in Fig. 3A, fluorescence intensities of the forearm decreased with increasing melanin index (MI), strongly demonstrating that the measurement of skin fluorescence intensities is significantly affected by melanin contents in control subjects. Next, the fluorescence intensities of several regions such as the fingertip, middle phalanx, forearm and upper arm were evaluated to clarify which regions show low accidental error. The characteristics of subjects without type 2 diabetes were as follow: Age: 53.9 ± 17.1, % female: 65.1%, BMI: 23.2 ± 4.5. The fluorescence intensity in several human regions as well as the mouse auricle was measured by a fiber-type sensor (Fig. 3B). As shown in Fig. 3B, although the fingertip showed the lowest fluorescence intensity, it also showed the lowest SD among the tested regions. These results demonstrated that the fingertip is one of the prominent regions to stably measure fluorescence intensity. The fluorescence intensity in the mouse auricle and human forearm were measured by the fiber-type sensor, whereas that in the human fingertip was measured by the clip-type sensor (please see Supplemental Fig. 1*) because the fingertip is one of the less melanin-containing regions in our body and the clip makes it possible to clamp the fingertip with the same pressure. Because the thickness of the skin fingertip is altered by its frequency of use, the hands were divided by the dominant vs non-dominant side, and the fluorescence intensities of the fingertips on the index finger, middle finger and annular finger was measured by the clip-type sensor. As a result, the middle finger of the non-dominant hand showed the lowest standard deviation among the fingers.

Relationship between fingertip fluorescence intensity and diabetic complications. The fluorescence intensity of the fingertip in patient with diabetes was measured and compared with their number of complications. Characteristics of patients with type 2 diabetes are listed in Table 1. As shown in Fig. 4A, the fluorescence intensity significantly increased with an increasing number of complications. In contrast, the levels of HbA1c did not change even with an increasing number of complications (Fig. 4B). Furthermore, the sera were obtained from the same subjects and MG-H1 levels in the sera were measured by LC-MS/MS. As a result, the MG-H1 level correlated with the fingertip fluorescence intensity (Fig. 4C).

Discussion

Immunochemical and instrumental studies have demonstrated that AGEs accumulate in age-related diseases such as atherosclerosis[24] and diabetic complications.[25] Furthermore, Ottum *et al.*[26]

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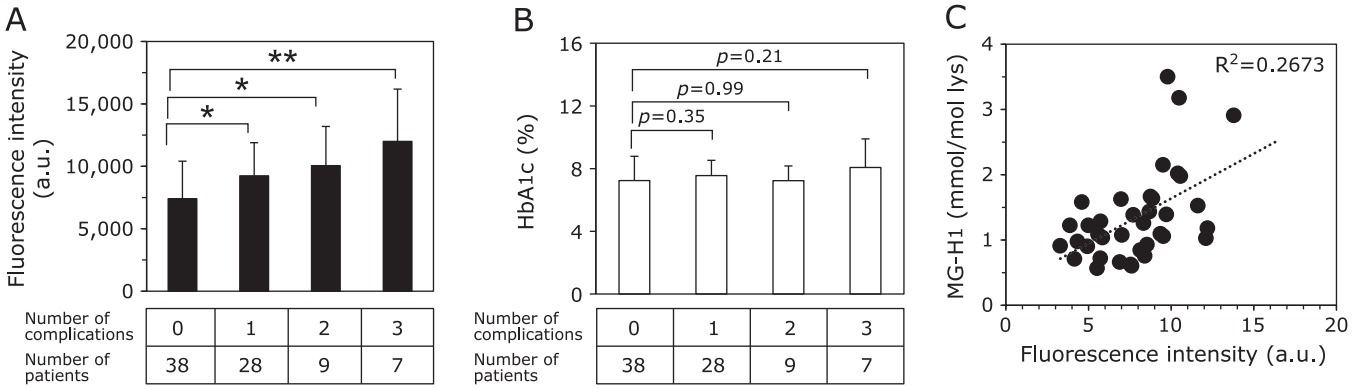

Fig. 4. Relationship among the fingertip fluorescence intensities, HbA1c, diabetic complications and serum MG-H1 levels. The fluorescence intensity of the fingertip (A) and HbA1c value (B) in patients with diabetes was measured and compared with their number of diabetic complications. Sera were obtained from the same subjects and the MG-H1 levels in the sera were measured by LC-MS/MS as described in the materials and methods (C).

reported that high AGEs diets overwhelm innate defenses of enzymes and receptor-mediated endocytosis and promote cell damage via receptor for AGEs, indicating that measurement of AGEs levels in our body are valuable for evaluation of diabetic microvascular complications. However, since multiple preparation steps and time-consuming processes are required to measure AGEs in physiological samples by instrumental analyses, the measurement of AGEs for clinical diagnosis has not been applied, unlike HbA1c.

Previous studies by Meerwaldt *et al.*[14,15] demonstrated that the accumulation of AGEs, which was measured by skin fluorescence, is increased in patients with diabetes compared to normal subjects. However, there are 3 critical issues to be solved for the measurement of skin AGEs by fluorescence. First, it is known that the measurement of the fluorescence intensity is suppressed by the presence of brown pigments, therefore, changes in the skin color due to sunburns and differences in race can affect the analysis. In fact, the fluorescence intensity in the forearm showed a highly inverse correlation with the melanin contents (Fig. 3A), strongly demonstrating that the measurement of the skin fluorescence on the fingertip is a more reliable site than that of the lower arm since low melanin contents are not as influenced by colored races and seasonal skin variation due to sun exposure. Second, because blood pigments, such as hemoglobin, also interfere with the measurement of fluorescence intensity, the presence or absence of veins in the skin cause accidental errors. Our preliminary study showed that the skin fluorescence intensity on veins was approximately 1.5-fold higher than that on skin without veins (data not shown). In contrast, because the capillary vein is dominant in the fingertip, the influence of the veins is negligible for the measurement of fluorescence. Third, to maintain constant measurement conditions, the fingertip was held by a clip with 5.5–6.0 N strength, and the analysis was performed within 5 s. This system allows us to reduce small vibrations at the time of measurement. Since the thickness of the skin fingertip changes by its frequency of use, the fingertip fluorescence intensity in the dominant vs non-dominant hand was determined. The fingertip fluorescence intensity was measured in the index finger, middle finger and annular finger because it is possible to place the palm on the device without unreasonable posture and measure the fluorescence intensities of those 3 fingertips. We revealed that standard deviation of fingertips in the non-dominant side was lower than that in dominant side, and standard deviation of fingertips of middle finger was lowest among three fingertips in the non-dominant side, suggesting that fingertip of the middle finger in the non-dominant hand is suitable to receive stable fluorescence intensity data as much as possible in this system. Taken together, in addition to determining the suitable wavelength to measure skin fluorescence, the non-invasive evaluation of diabetic complications was achieved by evaluating the fingertip, which has low melanin contents, few veins and readily holds the same amount of stress as the forearm.

We demonstrated that the level of HbA1c was not correlated with the number of complications (Fig. 4B). Since HbA1c is metabolized from blood content by a few months and is affected by treatment with anti-diabetic agents, it is seems that HbA1c is unsuitable marker for predicting diabetic complications. On the other hand, we also demonstrated that fluorescence intensity on fingertip increased in proportion to the number of complications (Fig. 4A). The clinical application of the devise clearly demonstrated that the fluorescence intensity increased with an increasing number of complications (Fig. 4A). Moreover, fluorescence intensity on fingertip was positively correlated with serum level of HG-M1 (Fig. 4C), suggesting that fluorescence intensity on fingertip represented cumulative dose of AGEs. Because AGEs are comparatively stable and cumulative substances, fluorescence intensity on fingertip may correlate positively with the number of complications rather than HbA1c. Taken together, our present study suggests that the measurement of fluorescence intensity on fingertip is useful for predicting diabetic microvascular complications.

In conclusion, this study provided the first evidence that the measurement of fluorescence intensity on the fingertip plays an important role in the early diagnosis and may prevent the pathogenesis of lifestyle-related diseases.

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Conflict of Interest

No potential conflicts of interest were disclosed.

References

1 Kitada M, Zhang Z, Mima A, King GL. Molecular mechanisms of diabetic vascular complications. *J Diabetes Investig* 2010; **1**: 77–89.
2 Gilbert MP. Screening and treatment by the primary care provider of common diabetes complications. *Med Clin North Am* 2015; **99**: 201–219.
3 Aslam N, Kedar A, Nagarajarao HS, *et al*. Serum catecholamines and dysautonomia in diabetic gastroparesis and liver cirrhosis. *Am J Med Sci* 2015; **350**: 81–86.
4 Forbes HJ, Thomas SL, Smeeth L, *et al*. A systematic review and meta-analysis of risk factors for postherpetic neuralgia. *Pain* 2015. [Epub ahead of print]
5 Beisswenger PJ, Moore LL, Brinck-Johnsen T, Curphey TJ. Increased collagen-linked pentosidine levels and advanced glycosylation end products in early diabetic nephropathy. *J Clin Invest* 1993; **92**: 212–217.
6 Chabroux S, Canoui-Poitrine F, Reffet S, *et al*. Advanced glycation end products assessed by skin autofluorescence in type 1 diabetics are associated with nephropathy, but not retinopathy. *Diabetes Metab* 2010; **36**: 152–157.
7 Bolton WK, Cattran DC, Williams ME, *et al*. Randomized trial of an inhibitor of formation of advanced glycation end products in diabetic nephropathy. *Am J Nephrol* 2004; **24**: 32–40.
8 Murata T, Nagai R, Ishibashi T, Inomuta H, Ikeda K, Horiuchi S. The relationship between accumulation of advanced glycation end products and expression of vascular endothelial growth factor in human diabetic retinas. *Diabetologia* 1997; **40**: 764–769.
9 Kang P, Tian C, Jia C. Association of RAGE gene polymorphisms with type 2 diabetes mellitus, diabetic retinopathy and diabetic nephropathy. *Gene* 2012; **500**: 1–9.
10 Miller AG, Zhu T, Wilkinson-Berka JL. The renin-angiotensin system and advanced glycation end-products in diabetic retinopathy: impacts and synergies. *Curr Clin Pharmacol* 2013; **8**: 285–296.
11 Kume S, Takeya M, Mori T, *et al*. Immunohistochemical and ultrastructural detection of advanced glycation end products in atherosclerotic lesions of human aorta with a novel specific monoclonal antibody. *Am J Pathol* 1995; **147**: 654–667.
12 Sakata N, Imanaga Y, Meng J, *et al*. Increased advanced glycation end products in atherosclerotic lesions of patients with end-stage renal disease. *Atherosclerosis* 1999; **142**: 67–77.
13 Rebholz CM, Astor BC, Grams ME, *et al*. Association of plasma levels of soluble receptor for advanced glycation end products and risk of kidney disease: the Atherosclerosis Risk in Communities study. *Nephrol Dial Transplant* 2015; **30**: 77–83.
14 Meerwaldt R, Links T, Graaff R, *et al*. Simple noninvasive measurement of skin autofluorescence. *Ann N Y Acad Sci* 2005; **1043**: 290–298.
15 Meerwaldt R, Lutgers HL, Links TP, *et al*. Skin autofluorescence is a strong predictor of cardiac mortality in diabetes. *Diabetes Care* 2007; **30**: 107–112.
16 Jo-Watanabe A, Ohse T, Nishimatsu H, *et al*. Glyoxalase I reduces glycative and oxidative stress and prevents age-related endothelial dysfunction through modulation of endothelial nitric oxide synthase phosphorylation. *Aging Cell* 2014; **13**: 519–528.
17 Ohno R, Moroishi N, Sugawa H, *et al*. Mangosteen pericarp extract inhibits the formation of pentosidine and ameliorates skin elasticity. *J Clin Biochem Nutr* 2015; **57**: 27–32.
18 World Health Organization, Department of Noncommunicable Disease Surveillance. *Definition, Diagnosis and Classification of Diabetes and its Complications: Report of a WHO Consultation. Part 1: Diagnosis and Classification of Diabetes*. Geneva: World Health Organiation, 1999; 1–59.
19 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.
20 Seino Y, Nanjo K, Tajima N, *et al*; The Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the committee on the classification and diagnostic criteria of diabetes mellitus. *J Diabetes Investig* 2010; **1**: 212–228.
21 Wilkinson CP, Ferris FL 3rd, Klein RE, *et al*; Global Diabetic Retinopathy Project Group. Proposed international clinical diabetic retinopathy and diabetic macular edema disease severity scales. *Ophthalmology* 2003; **110**: 1677–1682.
22 Katayama S, Moriya T, Tanaka S, *et al*; Japan Diabetes Complications Study Group. Low transition rate from normo- and low microalbuminuria to proteinuria in Japanese type 2 diabetic individuals: the Japan Diabetes Complications Study (JDCS). *Diabetologia* 2011; **54**: 1025–1031.
23 Yasuda H, Sanada M, Kitada K, *et al*. Rationale and usefulness of newly devised abbreviated diagnostic criteria and staging for diabetic polyneuropathy. *Diabetes Res Clin Pract* 2007; **77**: S178–S183.
24 Nagai R, Brock JW, Blatnik M, *et al*. Succination of protein thiols during adipocyte maturation: a biomarker of mitochondrial stress. *J Biol Chem* 2007; **282**: 34219–34228.
25 Khan MW, Qadrie ZL, Khan WA. Antibodies against gluco-oxidatively modified human serum albumin detected in diabetes-associated complications. *Int Arch Allergy Immunol* 2010; **153**: 207–214.
26 Ottum MS, Mistry AM. Advanced glycation end-products: modifiable environmental factors profoundly mediate insulin resistance. *J Clin Biochem Nutr* 2015; **57**: 1–12.

doi: 10.3164/jcbn.15-132