Introduction

Prevention of the onset and progression of complications in diabetes is one of the major goals in the treatment of diabetes mellitus. Ample evidence suggests that the onset and progression of microvascular complications are associated with chronic hyperglycemia, and they can be prevented by the correction of fasting hyperglycemia and hemoglobin A1c (HbA1c) levels [1–5]. While microvascular complications can be effectively prevented by monitoring HbA1c and fasting blood glucose levels, the reduction of the risk of macrovascular complications cannot be achieved through strict treatment based solely on HbA1c levels. Prevention of such complications requires full attention to hypoglycemia and postprandial hyperglycemia [5–8]. In relation to this, we previously reported a strong association between vascular endothelial dysfunction and fluctuation in blood glucose levels [9], and we assert that it is important to apply treatment protocols that can minimize glycemic excursions to prevent the onset and progression of macrovascular complications.

Glycemic excursions can be detected by continuous glucose monitoring (CGM) using parameters such as
the mean amplitude of glycemic excursions (MAGE) and standard deviation (SD). MAGE, in particular, has been shown to correlate with oxidative stress [10], coronary artery lesions [11], plaque instability [12], and vascular endothelial function [9]; hence, it is considered an important marker of macrovascular complications of diabetes. CGM, however, is not applicable to all patients, necessitating an assessment of fluctuation in blood glucose levels using glycemic parameters that can be measured in daily clinical practice. The objective of this study was to determine whether HbA1c and glycoalbumin (GA) levels, which are commonly used and easily measurable glycemic indices in daily practice, correlate with the parameters of glycemic excursion as measured by CGM.

Materials and Methods

Subjects

This cross-sectional study included patients with type 2 diabetes who were admitted to the University of Occupational and Environmental Health Hospital or affiliated hospitals between April 2014 and February 2016 for educational purposes and underwent CGM for at least 48 h within the first seven days of hospitalization with no change in treatment from admission. We excluded patients on any oral steroids, those in whom treatment was changed between the day of admission and the day of CGM placement or during the period of three months preceding enrollment in the study, those with active inflammatory disease, severe infection, pre- or postoperative status, serious trauma, anemia, hypoalbuminemia, impaired renal or liver function, thyroid dysfunction, malignancy, and those admitted with an acute condition on an emergency basis. This study was approved by the ethics committee of the University of Occupational and Environmental Health Hospital (H27–186). All patients provided written informed consent before enrolling in the study.

Study design

We measured the GA and HbA1c levels in all the study participants. For CGM, we used the CGMS System Gold (Medtronic Inc., Dublin, Ireland) or iPro2 (Medtronic Inc.). All the patients received optimal meals (25 kcal/kg of the ideal body weight; 60% carbohydrate, 15–20% protein, and 20–25% fat) during CGM.

CGM system

CGM data collected on the second day of CGM placement were used to calculate MAGE, SD, average blood glucose level (Average), percentage of time with blood glucose level ≥180 mg/dl (Time at >180), and percentage of time with blood glucose level <70 mg/dl (Time at <70). MAGE, which was proposed by Serv-Mace et al. [13], was calculated by identifying the arithmetic mean of the differences between consecutive peaks and nadirs using the Glycemic Variability Analyzer Program 1.1 (MATLAB® 2010b environment, MathWorks®, USA) [14]. CGM specifically measures glucose concentration in the interstitial fluid, which correlates well with venous blood glucose levels [15].

Laboratory tests

The HbA1c level (%) was measured by high-performance liquid chromatography with the Tosoh HLC-723 G8 system (Tosoh Co., Kyoto, Japan) and expressed using the National Glycohemoglobin Standardization Program values, which were calculated by adding 0.4% to the Japan Diabetes Society values [16]. GA level (%) was measured by the bromocresol purple method with the Lucica® GA-L kit (Asahi Kasei Pharma Co., Tokyo, Japan). All blood samples were collected in the early morning fasting state and subjected to analysis.

Data analysis and statistics

Values are expressed as mean ± SD. The distribution of data of each variable was tested by the Shapiro-Wilk test. The data of the GA and HbA1c levels and the GA/HbA1c ratio did not show normal distribution, so we analyzed the correlations of these variables with others by the Spearman’s test. Comparisons of the GA and HbA1c levels and GA/HbA1c ratio among the different MAGE subgroups was performed by the Mann-Whitney U test. A stepwise multiple regression analysis was also performed with MAGE as the dependent variable, and age, body mass index (BMI), duration of diabetes, urinary C-peptide, GA, and HbA1c as the independent variables. All analyses were performed with the SPSS Statistical Software 22.0 (SPSS Inc., Chicago, IL). Differences were considered significant at P<0.05.
Results

Baseline characteristics
The baseline characteristics of the patients are summarized in Table 1. The study population consisted of 66 patients (males = 44; females = 22). The mean age of the subjects was 59 ± 11 years, mean duration of diabetes 9.0 ± 9.8 years, mean body weight 71.8 ± 15.9 kg, and mean BMI 27 ± 5.1 kg/m². The mean GA was 23 ± 7.5%, mean HbA1c 9.2 ± 2.3%, mean GA/HbA1c ratio 2.5 ± 0.4, and mean fasting blood glucose level was 156.9 ± 46.5 mg/dl. An analysis of the CGM data of all the patients showed a mean MAGE of 101 ± 29 mg/dl and a mean SD of 39 ± 15 mg/dl. The mean average was also high, at 172 ± 51 mg/dl. The mean Time at >180 was 52 ± 13%, whereas the mean Time at <70 was as low as 2.7 ± 8.3%. On admission, 45 (68%) patients were not using any medications, while 16 (24%) patients were using only oral glucose-lowering agents. Four (6.1%) patients were on insulin therapy and 1 (1.5%) patient was on Glucagon-like peptide-1 receptor agonist therapy.

Association between glycemic indices and glycemic parameters obtained from CGM system
Table S1 shows the associations of GA, HbA1c, and GA/HbA1c ratio with MAGE, SD, Average, Time at >180, and Time at <70, measured by the CGM. MAGE correlated significantly with GA (r = 0.375, P = 0.002) and HbA1c (r = 0.367, P = 0.002), but not with the GA/HbA1c ratio (Figure 1). SD correlated significantly with GA (r = 0.418, P < 0.001), HbA1c (r = 0.377, P = 0.002), and the GA/HbA1c ratio (r = 0.291, P = 0.018) (Figure 1). The GA/HbA1c ratio did not correlate significantly with MAGE (P = 0.051), but did correlate significantly with SD (P = 0.018). The Average and Time at >180 correlated significantly with HbA1c, GA, and the GA/HbA1c ratio, while Time at <70 did not correlate with any of the glycemic indices.

Table 2 shows the results of multivariate analysis. The analysis identified GA as the most reflective of MAGE (adjusted multiple R² = 0.128, standardized coefficient β = 0.358, t = 3.068, and P = 0.003).

Service et al reported that the MAGE value of patients with stable diabetes was ≤75 mg/dl [17]. Accordingly, we divided the patients into two groups: one with MAGE ≤75 mg/dl (n = 10) and the other with MAGE >75 mg/dl (n = 56). When GA, HbA1c, and GA/HbA1c

### Table 1. Patient characteristics

| Parameter                        | mean ± SD, n, or n (%) |
|----------------------------------|------------------------|
| number of subjects (males/females) | 66 (44/22)            |
| Age (years)                      | 59 ± 11                |
| Body mass index (kg/m²)          | 27 ± 5.1               |
| Duration of diabetes (years)     | 9.0 ± 9.8              |
| Urinary C-peptide (µg/day)       | 85 ± 57                |
| Anti-hyperglycemic therapy       |                        |
| Diet only                        | 45 (68)                |
| Oral anti-diabetic agents        | 16 (24)                |
| Insulin only                     | 4 (6.1)                |
| Glucagon-like peptide-1 receptor agonist | 1 (1.5)            |

#### Glycemic indices

| GA (%)               | 23 ± 7.5               |
| HbA1c (%)            | 9.2 ± 2.3              |
| GA/HbA1c             | 2.5 ± 0.4              |

#### Glycemic parameters obtained from CGMS

| Parameter                  | mean ± SD |
|----------------------------|-----------|
| MAGE (mg/dl)              | 101 ± 29  |
| SD (mg/dl)                | 39 ± 15   |
| Average (mg/dl)           | 172 ± 51  |
| Time at >180 mg/dl (%)    | 52 ± 13   |
| Time at <70 mg/dl (%)     | 2.7 ± 8.3 |

Data are mean ± SD, n, or n (%). GA: glycoalbumin, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursion, SD: standard deviation.

### Table S1. Correlation coefficients between glycemic indices and glycemic parameters obtained from the continuous glucose monitoring system.

|                | GA                | HbA1c              | GA/HbA1c       |
|----------------|-------------------|--------------------|----------------|
|                | r                 | P                  | r              | P               |
| MAGE           | 0.375             | 0.002              | 0.367          | 0.002           | 0.241           | 0.051           |
| SD             | 0.418             | <0.001             | 0.377          | 0.002           | 0.291           | 0.018           |
| Average        | 0.688             | 0.001              | 0.663          | 0.001           | 0.367           | 0.002           |
| Time at >180   | 0.681             | <0.001             | 0.574          | <0.001          | 0.479           | <0.001          |
| Time at <70    | −0.218            | 0.790              | −0.287         | 0.519           | 0.03            | 0.810           |

Data are results of Spearman’s rank correlation analysis. *Measured using the continuous glucose monitoring system. GA: glycoalbumin, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursion, SD: standard deviation.
ratio were compared between the two groups, we observed significant differences in the GA levels and GA/HbA1c ratio ($P=0.028$ and $0.024$, respectively), but not in the HbA1c levels (Figure 2).

In order to identify the optimal cutoff value of the GA level that could allow the identification of patients with stable diabetes, i.e., those with MAGE $\leq 75$ mg/dl, we generated a receiver operating characteristic (ROC) curve and calculated the area under the curve (AUC) with a 95% confidence interval (CI). The ROC analysis estimated a GA level of 18.1% as the optimal cutoff value for identifying patients with small fluctuations in blood glucose levels (AUC = 0.725; 95% CI = 0.547–0.903; sensitivity = 82.8% and specificity = 50.0%) (Figure S1).

![Figure 1. Correlation of GA and HbA1c with MAGE and SD.](image)

Spearman’s correlation coefficient is shown in each panel. GA correlated significantly with MAGE (A) ($r = 0.375$, $p = 0.002$) and SD (B) ($r = 0.418$, $p < 0.001$). Also, HbA1c correlated significantly with MAGE (C) ($r = 0.367$, $p = 0.002$) and SD (D) ($r = 0.377$, $p = 0.002$). GA: glycoalbumin, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursion, SD: standard deviation.

| Table 2. Results of the multiple linear regression analysis |
|-----------------|-----------------|-----------------|-----------------|
|                 | Unstandardized  | Standardized    | $t$ value       | $P$ value       |
| B               | SE              | coefficients    | coefficients    |
| Intercept       | 69.164          | 10.873          | 6.361           | $<0.001$        |
| GA              | 1.367           | 0.446           | 0.358           | 3.068           | 0.003          |
| Adjusted multiple $R^2$ | 0.128          |                |                |

MAGE was the dependent variable and age, body mass index, duration of diabetes, urinary C-peptide, GA, and HbA1c, oral anti-diabetic agents, insulin only, Glucagon-like peptide-1 receptor agonist, Time at <70 were the independent variables. GA: glycoalbumin, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursion, SD: standard deviation.
Our study demonstrated that GA level is the most reflective of fluctuations in blood glucose levels in patients with type 2 diabetes. Our results showed no correlation between hypoglycemia and GA or HbA1c level. To our knowledge, this is the first report to evaluate the correlation between MAGE and GA in type 2 diabetes mellitus. Moreover, the GA level correlated with MAGE and was identified as the most reflective of MAGE in multivariate analysis.

With regard to the association between GA and HbA1c levels with the parameters of glycemic excursions measured by CGM, studies involving patients with type 1 diabetes mellitus have reported that GA levels and the GA/HbA1c ratio correlated with both SD and MAGE [18–20]. However, while previous studies demonstrated correlations between SD and GA levels [21] and between SD and MAGE [22] in patients with diabetes, including type 2 diabetes mellitus, none of these studies examined the direct relationship between MAGE and GA levels in type 2 diabetes mellitus. It has been reported that the annual variabilities in HbA1c and GA are greater in patients with type 1 diabetes than those with type 2 diabetes mellitus [23]. Therefore, it has been considered that the analysis of the associations of HbA1c and GA levels at any point

**Figure 2.** Box-and-whisker plots of GA and HbA1c levels and GA/HbA1c ratio for the MAGE ≤75 mg/dl and MAGE >75 mg/dl groups. Data shown are the results of the Mann-Whitney U-test. *P<0.05. There were significant differences between the two groups for GA (A) and GA/HbA1c (C) (P=0.028 and 0.024, respectively). There was no significant difference in HbA1c (B) between the two groups (P=0.107). In these plots, lines within the boxes represent median values; the upper and lower lines of the boxes represent the 25th and 75th percentiles, respectively; and the upper and lower bars outside the boxes represent the 90th and 10th percentiles, respectively. GA: glycoalbumin, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursion.

**Figure S1.** Receiver operating characteristic (ROC) curve analysis using GA levels for identifying patients with stable diabetes.
in time with CGM parameters is more accurate in patients with type 2 than type 1 diabetes mellitus. In our study, the observed correlation between GA level and MAGE may be explained by the fact that MAGE primarily reflects postprandial blood glucose levels, given the demonstrated correlation between 2-h postprandial blood glucose level with both GA and HbA1c, with a stronger correlation with the GA level in patients with type 2 diabetes mellitus [24]. In addition, our study showed no correlation between Time at <70 and the GA and HbA1c levels and GA/HbA1c ratio.

When the patients were divided into two groups based on the MAGE cutoff for stable diabetes (i.e., ≤ or >75 mg/dl), the GA level and GA/HbA1c ratio were significantly higher in patients with MAGE >75 mg/dl than in those with MAGE ≤75 mg/dl, whereas no significant difference in HbA1c level was found between the two groups. These findings suggest that GA level and GA/HbA1c ratio are more useful as indices for identifying patients with stable diabetes and minimal glycemic excursions. The cutoff value of GA for identifying patients with a MAGE of ≤75 mg/dl was 18.1%. To reduce glycemic excursions, appropriate glycemic control measures should be selected to achieve a GA level of 18.1% without hypoglycemic episodes. In relation to this, a previous study reported an association of presence of carotid plaque (a marker of arteriosclerosis) with GA [25]. The association of coronary artery disease, one form of arteriosclerosis, with GA has also been reported, where patients with type 2 diabetes mellitus with lesions in three coronary branches had significantly higher GA levels than those with lesions in two or fewer branches [26]. In addition, a GA level of ≥19% was reported as an independent predictor of coronary artery disease, and a GA level of ≥21% was found to be associated with an increased risk of three-branch coronary lesions [26]. For these reasons, glycemic control aims at achieving a GA level of 18.1%. The cutoff value identified in this study might ultimately be used for the prevention of arteriosclerosis.

Time at <70 correlated with neither GA, HbA1c, nor the GA/HbA1c ratio, suggesting that hypoglycemia cannot be reliably detected by the GA or HbA1c levels. Rather, it should be evaluated in a comprehensive manner based on symptoms, self-measured blood glucose levels, venous blood glucose levels, patient demographics, and the type of treatment administered.

This study has some limitations. The sample size of 66 inpatients is small. In particular, there were only 10 subjects with stable diabetes (MAGE ≤75 mg/dl). Subjects who were inpatients with type 2 diabetes within the first seven days of hospitalization with no change in treatment since admission included many patients with unstable diabetes in need of treatment and a few patients with stable diabetes. Therefore, the obtained results require further confirmation by an assessment of a large number of patients. In fact, the mean GA/HbA1c ratio in the current population was 2.5, which was smaller than that measured in a group of outpatients (2.8–2.9), as reported previously [20, 27]. In addition, since the subjects in this study were inpatients, their dietary intake and physical activity levels were considered to be different from those of outpatients. Although the evaluation of CGM was performed without changing the treatment at the beginning of hospitalization, it is possible that the blood glucose profile at admission might have been different from that of outpatients. Further studies are needed to examine the correlations between glycemic parameters obtained from outpatient CGM and outpatient laboratory data.

**Acknowledgments**

The authors thank Ms. N. Sakaguchi for her excellent technical assistance.

**Conflicts of Interest**

The authors declare no conflict of interest.

**References**

1. Stratton IM, Adler AI, Neil HAW *et al* (2000): Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. Br Med J 321(7258): 405–412

2. Turner R (1998): Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). Lancet 352 (9131): 837–853
3. Gubitosi-Klug RA (2014): The diabetes control and complications trial/epidemiology of diabetes intervention and complications study at 30 years: summary and future directions. Diabetes Care 37 (1): 44–49
4. Dyck PJ, Davies JL, Clark VM et al (2006): Modeling chronic glycemic exposure variables as correlates and predictors of microvascular complications of diabetes. Diabetes Care 29 (10): 2282–2288
5. Patel A, MacMahon S, Chalmers J et al (2008): Intensive blood glucose control and vascular outcomes in patients with type 2 diabetes. New Engl J Med 358 (24): 2560–2572
6. Gerstein HC, Miller ME, Byington RP et al (2008): Effects of intensive glucose control lowering in type 2 diabetes. New Engl J Med 358 (24): 2545–2559
7. Desouza C, Salazar H, Cheong B, Murgo J & Fonseca V (2003): Association of hypoglycemia and cardiac ischemia: a study based on continuous monitoring. Diabetes Care 26 (5): 1485–1489
8. Tominaga M, Eguchi H, Manaka H et al (1999): Impaired glucose tolerance is a risk factor for cardiovascular disease, but not impaired fasting glucose: the Fuku- nagata Diabetes Study. Diabetes Care 22 (6): 920–924
9. Torimoto K, Okada Y, Mori H & Tanaka Y (2013): Relationship between fluctuations in glucose levels measured by continuous glucose monitoring and vascular endothelial dysfunction in type 2 diabetes mellitus. Cardiovasc Diabetol 12: 1
10. Monnier L, Mas E, Ginet C et al (2006): Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. J Am Med Assoc 295(14): 1681–1687
11. Su G, Mi S, Tao H et al (2011): Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. Cardiovasc Diabetol 10: 19
12. Kuroda M, Shinke T, Sakaguchi K et al (2015): Effect of daily glucose fluctuation on coronary plaque vulnerability in patients pre-treated with lipid-lowering therapy: a prospective observational study. JACC Cardiovasc Interv 8(6): 800–811
13. Service FJ, Molnar GD, Rosevear JW et al (1970): Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes 19(9): 644–655
14. Marics G, Lendvai Z, Lódi C et al (2015): Evaluation of an open access software for calculating glucose variability parameters of a continuous glucose monitoring system applied at pediatric intensive care unit. Biomed Eng Online 14: 37
15. Boyne MS, Silver DM, Kaplan J & Saudek CD (2003): Timing of changes in interstitial and venous blood glucose measured with a continuous subcutaneous glucose sensor. Diabetes 52(11): 2790–2794
16. Seino Y, Nanjo K, Tajim N et al (2010): Report of the committee on the classification and diagnostic criteria of diabetes mellitus. J Diabetes Invest 1(5): 212–228
17. Service FJ & Nelson RL (1980): Characteristics of glycemic stability. Diabetes Care 3(1): 58–62
18. Matsumoto H, Murase-Mishiba Y, Yamamoto N et al (2012): Glycated albumin to glycated hemoglobin ratio is a sensitive indicator of blood glucose variability in patients with fulminant type 1 diabetes. Intern Med 51(11): 1315–1321
19. Tsutsumi C, Imagawa A, Onishi M et al (2013): Glycated albumin as a useful clinical biomarker for glycemic variability in type 1 diabetes assessed by continuous glucose monitoring. Diabetol Int 4(3): 156–159
20. Ogawa A, Hayashi A, Kishihara E et al (2012): New indices for predicting glycaemic variability. PLoS ONE 7(9) e46517
21. Suwa T, Ohta A, Matsui T et al (2010): Relationship between clinical markers of glycemia and glucose excursion evaluated by Continuous Glucose Monitoring (CGM). Endocr J 57(2): 135–140
22. Saisho Y, Tanaka C, Tanaka K et al (2015): Relationships among different glycemic variability indices obtained by continuous glucose monitoring. Prim Care Diabetes 9(4): 290–296
23. Koga M, Murai J, Morita S, Saito H & Kasayama S (2013): Comparison of annual variability in HbA1c and glycated albumin in patients with type 1 vs. type 2 diabetes mellitus. J Diabetes Complications 27(3): 211–213
24. Saisho Y, Tanaka K, Abe T et al (2011): Glycated albumin to glycated hemoglobin ratio reflects postprandial glucose excursion and relates to beta cell function in both type 1 and type 2 diabetes. Diabetol Int 2(3): 146–153
25. Sato Y, Nagao M, Asai A et al (2013): Association of glycated albumin with the presence of carotid plaque in patients with type 2 diabetes. J Diabetes Invest 4(6): 634–639
26. Pu LJ, Lu L, Shen WF et al (2007): Increased serum glycated albumin level is associated with the presence and severity of coronary artery disease in type 2 diabetic patients. Circ J 71(7): 1067–1073

27. Takahashi S, Uchino H, Shimizu T et al (2007): Comparison of Glycated Albumin (GA) and Glycated Hemoglobin (HbA1c) in type 2 diabetic patients: usefulness of GA for evaluation of short-term changes in glycemic control. Endocr J 54(1): 139–144