Associations between continuous glucose monitoring-derived metrics and diabetic retinopathy and albuminuria in patients with type 2 diabetes

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ABSTRACT

Introduction Preventing the development and progression of diabetic microvascular complications through optimal glucose control remains an important challenge. Whether metrics based on continuous glucose monitoring are useful for the management of diabetic microvascular complications is not entirely clear.

Research design and methods This is an exploratory analysis of an ongoing prospective, multicenter, 5-year follow-up observational study. Study participants included 999 outpatients with type 2 diabetes who underwent continuous glucose monitoring at baseline. Associations between continuous glucose monitoring-derived metrics and the severity of diabetic retinopathy or albuminuria were investigated using multivariable proportional odds models.

Results The overall prevalence of diabetic retinopathy was 22.2%. Multivariate analysis with proportional odds models demonstrated that continuous glucose monitoring-derived metrics related to intraday and interday glucose variability are significantly associated with the severity of diabetic retinopathy, even after adjusting for various possible risk factors. However, significant relationships were not observed after adjusting for hemoglobin A1c (HbA1c) levels. The prevalence of microalbuminuria and macroalbuminuria was 20.3% and 6.7%, respectively. Similarly, multivariate analysis demonstrated that these metrics are significantly associated with the severity of albuminuria. These relationships remained significant even after further adjusting for HbA1c levels.

Conclusions Continuous glucose monitoring-derived metrics related to intraday and interday glucose variability are significantly associated with the severity of diabetic retinopathy or albuminuria in patients with type 2 diabetes. Thus, evaluating these metrics might possibly be useful for risk assessment of diabetic microvascular complications.

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INTRODUCTION

Diabetic retinopathy (DR) is a main cause of visual impairment and blindness.1 Diabetic nephropathy (DN) is the main cause of end-stage renal disease.2 Accordingly, taking preventive measures against the development and progression of diabetic microvascular complications in patients with type 2 diabetes is an important task necessary for maintaining daily quality of life, extending healthy lifespan, and reducing healthcare costs.
caused by persistent hyperglycemia, optimal glycemic control is the best way to prevent the development and progression of microvascular complications.

Hemoglobin A1c (HbA1c) is recognized as a gold standard for assessment of glycemic management. Several studies have demonstrated strong associations between HbA1c levels and diabetic microvascular complications. In addition, previous studies have indicated that improvement in HbA1c levels is associated with risk reduction in the incidence and progression of diabetic complications in patients with type 2 diabetes. Based on these data, current guidelines recommend a target HbA1c level of 7% or less. On the other hand, another study failed to show glycemic control to HbA1c <7% has a beneficial effect on the prevention of microvascular complications. One possible explanation for the discrepant findings may be that frequent episodes of severe hypoglycemia counterbalanced the beneficial effects of glycemic control. This hypothesis is based on the fact that HbA1c reflects average glucose over the last few months, but it provides no information on intraday and interday glucose variability and hypoglycemia, both of which may play an important role in the development of macrovascular and microvascular complications. In addition, HbA1c has another limitation. HbA1c is affected by factors such as anemia, hemoglobinopathy, chronic kidney disease, and ethnicity. Thus, new metrics reflecting various aspects of glycemic status are needed for the management of diabetic microvascular complications.

Continuous glucose monitoring (CGM) has emerged as an optimal method to obtain a more comprehensive glycemic profile, including data on intraday and interday glucose variability and patterns of hyperglycemia and hypoglycemia. In particular, the Advanced Technologies and Treatments for Diabetes (ATTD) Congress recommends using 10 core CGM metrics that may be most useful in clinical practice. The CGM metrics include three key CGM measurements: (1) time in range (TIR), defined as the percentage of the time spent within the target glucose range; (2) time below range (TBR); and (3) time above range (TAR). These new metrics assessed with CGM could help improve clinical management by providing more information than HbA1c.

Nevertheless, until now, only limited data from cross-sectional studies investigating the relationship between CGM metrics and diabetic microvascular complications have been available. Intriguingly, two recent cross-sectional studies conducted by the same group demonstrated that SD and TIR are each significantly associated with the presence of DR in inpatients with type 2 diabetes. Another study showed that TIR is associated with the presence of albuminuria in patients with type 2 diabetes, but this relationship did not reach statistical significance after adjusting for HbA1c levels. On the other hand, a small retrospective cross-sectional study demonstrated that TAR is associated with the presence of DR, but other metrics of glucose variability were not associated with the presence of DR or DN in inpatients with type 2 diabetes. Thus, the association between metrics from CGM and the presence or severity of diabetic microvascular complications in patients with type 2 diabetes has not been fully elucidated yet.

In this exploratory cross-sectional study, we investigated the relationship between CGM-derived metrics and the severity of DR and albuminuria in 999 outpatients with type 2 diabetes.

**RESEARCH DESIGN AND METHODS**

**Study design**

This study is an exploratory subanalysis of an ongoing, observational, prospective cohort study that aims to investigate the relationships between glucose fluctuations evaluated with CGM and the incidence of composite cardiovascular events over a 5-year follow-up period as described previously. This study used baseline study from the cohort study. This study has been registered in the University Hospital Medical Information Network Clinical Trials Registry, which is a non-profit organization in Japan that meets the requirements of the International Committee of Medical Journal Editors.

**Study population**

The study population consists of Japanese patients with type 2 diabetes who regularly attend the outpatient diabetes clinics of 34 institutions across Japan (with investigator names in parentheses shown in online supplemental table 1). The inclusion criteria were as follows: (1) age ≥50 years and ≤80 years, regardless of gender; (2) receiving treatment for type 2 diabetes at one of the participating outpatient clinics; (3) informed consent for study participation; (4) no changes (including new prescriptions) in antidiabetic medications for 6 months before written informed consent was obtained (insulin dosage changes were allowed); and (5) no anticipated changes in antidiabetic medications from the time of enrollment until a CGM device was applied on the back of the upper arm (insulin dosage changes were allowed). The following exclusion criteria were also applied: (1) type 1 or secondary diabetes; (2) presence of severe infectious disease preoperatively, postoperatively, or associated with severe trauma; (3) history of myocardial infarction, angina pectoris, cerebral stroke, cerebral infarction, or arteriosclerosis obliterans; (4) current treatment with artificial dialysis; (5) moderate liver dysfunction defined as aspartate aminotransferase ≥100 IU/L; (6) moderate or severe heart failure (New York Heart Association stage III or worse); (7) pregnancy, lactation, possible pregnancy, or plans to become pregnant during the study period; (8) present or history of a malignant tumor; (9) use of a sensor-augmented insulin pump; (10) type 2 diabetes diagnosis within the past year; and (11) judged as ineligible by the clinical investigators. Patients not currently receiving medication for a malignant tumor, with no disease recurrence to date, and without recurrence risks during the study period were allowed to participate.
Consecutive subjects were screened. Patients who meet the eligibility criteria were asked to participate in the present study. A total of 1000 patients who met the eligibility criteria were recruited between May 2018 and March 2019. One patient withdrew consent. Written informed consent was obtained from all participants after a full explanation of the study.

**Biochemical tests**

Blood samples were obtained at visits after overnight fasting. Renal function tests, lipid levels, and HbA1c (National Glycohemoglobin Standardization Program) were measured with standard techniques. Urinary albumin excretion (UAE) was measured using a latex agglutination assay on a spot urine sample. Estimated glomerular filtration rate (eGFR) was calculated using a formula.21

**DR and DN assessment**

The presence and severity of DR were determined by trained ophthalmologists. The patients were grouped into four groups based on medical records: no diabetic retinopathy (NDR), simple diabetic retinopathy (SDR), preproliferative diabetic retinopathy (PPDR), or proliferative diabetic retinopathy (PDR). DN was defined according to the level of UAE: <30 mg/g creatinine was defined as normoalbuminuria, 30–299 mg/g creatinine was defined as microalbuminuria, and ≥300 mg/g creatinine was defined as macroalbuminuria.

**CGM with the FreeStyle Libre Pro device**

The FreeStyle Libre Pro (Abbott Japan, Tokyo, Japan) CGM (FLP-CGM) device, which measures glucose levels every 15 min for up to 14 days, was used in this study as previously reported.20 Other than wearing FLP-CGM, there were no restrictions on participants’ daily lives. Downloaded data sets were further analyzed. Glucose variability was assessed based on mean amplitude of glycemic excursions (MAGE).22 SD, and glucose coefficient of variation (CV). MAGE was calculated as the arithmetic mean of the differences between consecutive peaks and nadirs, provided that the differences are greater than 1 SD of the mean glucose value. CV (%) was calculated by dividing SD by the mean of the corresponding glucose readings. The original statistical analysis plan (SAP) for this study was reported in the initial study protocol.20 We added some CGM-derived metrics in this study since the ATTD Congress proposed some CGM-derived metrics as useful clinical targets that complement HbA1c.15 Thus, we updated the SAP prior to database lock. Mean glucose was measured from data collected during FLP-CGM. TIR was defined as the percentage of the time spent in the target range between 3.9 and 10.0 mmol/L (TIR<3.0–10.0 mmol/L), time above target glucose range (TAR<3.9 mmol/L, TAR<3.0 mmol/L), and time below target glucose range (TBR<3.9 mmol/L, TBR<3.0 mmol/L). Low blood glucose index (LBGI) and high blood glucose index (HBGI) formulae were implemented by converting glucose values into risk scores.23 In addition, mean of daily differences (MODD)24 in glucose levels and IQR were calculated to assess interday glucose variability. MODD was calculated as the mean of the absolute difference between glucose levels measured at the same time on 2 consecutive days. IQR was calculated using values from the same time of day during the monitoring period. Since a previous study demonstrated that FLP-CGM was less accurate during the first 24 hours (from the first day to the second day) after insertion and during the last 4 days of its 14-day lifetime,25 we analyzed FLP-CGM data over the middle 8-day period.

**Statistical analysis**

Results are presented as mean±SD for continuous variables or number (proportion) of patients for categorical variables. Several parameters were logtransformed to approximate the normal distribution. Continuous data were compared using analysis of variance, and categorical data were compared using χ² test or Fisher’s exact test as appropriate. Multivariate analysis with proportional odds models was performed to investigate whether FLP-CGM-derived metrics are associated with the severity of diabetic microvascular complications. Conventional possible risk factors evaluated by clinical, biochemical, and metabolic tests based on clinical judgment were included in the models. All statistical tests were two-sided with a 5% significance level. All analyses were performed using SAS software V.9.4 or above.

**RESULTS**

**Relationship between FLP-CGM-derived metrics and DR severity**

The baseline clinical characteristics of the 999 patients with type 2 diabetes are summarized in table 1. The mean age was 64.6±9.6 years, 60.9% were male, the mean HbA1c was 7.1%±0.8% (53.7±8.8 mmol/mol), and the estimated duration of type 2 diabetes was 12.9±8.5 years.

In this study, 222 of 999 (22.2%) were diagnosed as having DR. Subject characteristics by DR stage are presented in table 2. SDR was observed in 133 subjects (13.3%), PPDR in 50 (5.0%), and PDR in 39 (3.9%). Subjects with more severe DR were more likely to be older, have longer duration of diabetes mellitus, higher HbA1c levels, higher uric acid levels, higher UAE, and lower eGFR. All FLP-CGM-derived metrics except TBR<3.9 mmol/L and TBR<3.0 mmol/L were significantly different among the groups. Subjects with more severe DR were more likely to be treated with oral antidiabetic drugs and antihypertensive drugs, respectively.

Next, we investigated the relationship between FLP-CGM-derived metrics and DR severity in patients with type 2 diabetes. In a proportional odds model with the patients with NDR as the reference group, HbA1c and FLP-CGM-derived metrics except for CV, TBR<3.9 mmol/L, TBR<3.0 mmol/L, and LBGI were significantly associated with DR severity (model 1 in table 3). In models 2 and 3, the associations remained significant after adjusting for diabetes duration, UAE, eGFR, and other conventional risk factors.
Pathophysiology/complications

Table 1  Patient demographic and background characteristics

| Parameter                                             | Value                          |
|-------------------------------------------------------|--------------------------------|
| Age (years)                                           | 64.6±9.6 (n=999)               |
| Male gender (%)                                       | 608 (60.9)                     |
| BMI (kg/m²)                                           | 24.6±3.9 (n=999)               |
| Estimated duration of diabetes (years)                | 12.9±8.5 (n=999)               |
| Systolic blood pressure (mm Hg)                       | 131.2±14.8 (n=999)             |
| Diastolic blood pressure (mm Hg)                      | 75.5±11.0 (n=999)              |
| HbA1c (%)                                             | 7.1±0.8 (n=999)                |
| HbA1c (mmol/mol)                                      | 53.7±8.8 (n=999)               |
| Total cholesterol (mmol/L)                            | 4.81±0.82 (n=964)              |
| LDL cholesterol (mmol/L)                              | 2.67±0.69 (n=990)              |
| HDL cholesterol (mmol/L)                              | 1.56±0.41 (n=998)              |
| Triglycerides (mmol/L)                                | 1.4±0.9 (n=999)                |
| Uric acid (μmol/L)                                    | 307.4±73.0 (n=994)             |
| Estimated glomerular filtration rate (mL/min/1.73 m²) | 73.4±20.6 (n=999)              |
| Use of oral glucose-lowering agents (%)               | 894 (89.5)                     |
| Metformin (%)                                         | 543 (54.4)                     |
| Sulfonylureas (%)                                     | 127 (12.7)                     |
| Glinides (%)                                          | 68 (6.8)                       |
| Dipeptidyl peptidase-4 inhibitors (%)                 | 577 (57.8)                     |
| Sodium-glucose cotransporter-2 inhibitors (%)         | 231 (23.1)                     |
| Thiazolidinediones (%)                                | 143 (14.3)                     |
| α-glucosidase inhibitors (%)                          | 172 (17.2)                     |
| Glucagon-like peptide-1 antagonists (%)              | 74 (7.4)                       |
| Insulin (%)                                           | 158 (15.8)                     |
| Use of antihypertensive drugs                         | 483 (48.3)                     |
| ACE inhibitors (%)                                    | 28 (2.8)                       |
| Angiotensin II receptor blockers (%)                  | 390 (39.0)                     |
| Calcium channel blockers (%)                          | 273 (27.3)                     |
| Diuretic drugs (%)                                    | 57 (5.7)                       |
| α-adrenergic receptor antagonists (%)                 | 19 (1.9)                       |
| β-adrenergic receptor antagonists (%)                | 33 (3.3)                       |
| Use of lipid-lowering agents (%)                      | 595 (59.7)                     |
| Statins (%)                                           | 508 (51.0)                     |
| Ezetimibe (%)                                         | 107 (10.7)                     |
| Fibrates (%)                                          | 41 (4.1)                       |

Table 1  Continued

| Parameter                                             | Value                          |
|-------------------------------------------------------|--------------------------------|
| Use of antithrombotic agents (%)                      | 64 (6.4)                       |
| Antiplatelet agents (%)                               | 50 (5.0)                       |
| Anticoagulants (%)                                    | 15 (1.5)                       |
| FLP-CGM-derived metrics                              |                                |
| Mean glucose (mmol/L)                                 | 7.80±1.79 (n=999)              |
| SD (mmol/L)                                           | 2.04±0.63 (n=999)              |
| CV (%)                                                | 26.2±5.79 (n=999)              |
| MAGE (mmol/L)                                         | 5.46±2.00 (n=999)              |
| TIR<3.9–10 mmol/L (%)                                 | 78.9±18.6 (n=999)              |
| TAR<10 mmol/L (%)                                     | 19.0±19.2 (n=999)              |
| TAR<13.9 mmol/L (%)                                   | 3.85±9.31 (n=999)              |
| TBR<3.8 mmol/L (%)                                    | 2.16±4.71 (n=999)              |
| TBR<3.0 mmol/L (%)                                    | 0.33±1.53 (n=999)              |
| LBGI                                                  | 1.56±1.67 (n=999)              |
| HBGI                                                  | 5.58±4.64 (n=999)              |
| MODD (mmol/L)                                         | 1.73±0.64 (n=999)              |
| IQR (mmol/L)                                          | 2.14±0.81 (n=999)              |

Data are mean±SD or number of patients (%). BMI, body mass index; CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring device; HbA1c, hemoglobin A1c; HBGI, high blood glucose index; HDL, high-density lipoprotein; LBGI, low blood glucose index; LDL, low-density lipoprotein; MAGE, mean amplitude of glycemic excursions; MODD, mean of daily differences; TAR, time above range; TBR, time below range; TIR, time in range.

Relationship between FLP-CGM-derived metrics and albuminuria severity

Of 999 subjects, 729 (73.0%) were classified as having normoalbuminuria, 203 (20.3%) were classified as having microalbuminuria, and 67 (6.7%) were classified as having macroalbuminuria. The clinical characteristics of the study participants stratified by albuminuria status are summarized in table 4. Subjects with more severe albuminuria were more likely to have longer duration of diabetes mellitus, higher BMI, higher prevalence of DR, higher HbA1c levels, higher triglyceride levels, higher uric acid levels, lower high-density lipoprotein levels, and lower eGFR. Among the groups, there were significant differences in most FLP-CGM-derived metrics, except for...
## Table 2 Patient demographic and background characteristics by diabetic retinopathy stage

| Parameter                                       | NDR (n=777) | SDR (n=133) | PPDR (n=50) | PDR (n=39) | P value |
|-------------------------------------------------|-------------|-------------|-------------|------------|---------|
| Age (years)                                     | 64.1±9.8    | 65.3±9.6    | 67.3±8.5    | 66.6±7.2   | 0.039   |
| Male gender (%)                                 | 482 (62.0)  | 84 (63.2)   | 23 (46.0)   | 19 (48.7)  | 0.050   |
| BMI (kg/m²)                                     | 24.6±3.8    | 24.7±3.8    | 24.4±4.1    | 25.1±4.3   | 0.838   |
| Estimated duration of diabetes (years)          | 11.8±8.0    | 15.6±8.9    | 17.5±9.6    | 19.1±9.6   | <0.001  |
| Systolic blood pressure (mm Hg)                 | 130.9±15.1  | 131.3±14.0  | 132.3±13.9  | 130.3±14.4 | 0.403   |
| Diastolic blood pressure (mm Hg)                | 75.6±11.3   | 76.5±10.2   | 71.9±9.7    | 75.5±10.4  | 0.089   |
| HbA1c (%)                                       | 7.0±0.8     | 7.2±0.9     | 7.6±0.9     | 7.6±1.1    | <0.001  |
| HbA1c (mmol/mol)                                | 52.8±8.2    | 55.2±9.4    | 59.1±9.6    | 59.8±11.8  | <0.001  |
| Total cholesterol (mmol/L)                      | 4.87±0.81   | 5.05±0.79   | 4.51±0.63   | 4.81±0.96  | <0.001  |
| LDL cholesterol (mmol/L)                        | 2.71±0.68   | 2.54±0.67   | 2.45±0.78   | 2.57±0.65  | 0.005   |
| HDL cholesterol (mmol/L)                        | 1.57±0.41   | 1.51±0.33   | 1.57±0.37   | 1.57±0.49  | 0.450   |
| Triglycerides (mmol/L)                          | 1.4±0.9     | 1.1±0.6     | 1.5±1.2     | 1.5±1.0    | 0.029   |
| Uric acid (μmol/L)                              | 306.6±72.3  | 315.4±72.7  | 278.1±71.3  | 333.9±78.1 | 0.002   |
| Estimated glomerular filtration rate (mL/min/1.73 m²) | 75±20       | 72±24       | 68±18       | 58±25      | <0.001  |
| Urinary albumin excretion (mg/g creatinine)     | 67.9±254    | 73.6±206    | 212±464     | 607±1261   | <0.001  |
| Use of oral glucose-lowering agents              | 677 (87.1)  | 129 (97)    | 49 (98)     | 39 (100)   | <0.001  |
| Metformin (%)                                   | 402 (51.7)  | 93 (69.9)   | 34 (68.0)   | 14 (35.9)  | <0.001  |
| Sulfonylureas (%)                               | 89 (11.5)   | 20 (15.0)   | 8 (16.0)    | 10 (25.6)  | 0.044   |
| Glinides (%)                                    | 40 (5.1)    | 12 (9.0)    | 9 (18.0)    | 7 (17.9)   | <0.001  |
| Dipeptidyl peptidase-4 inhibitors (%)           | 435 (56.0)  | 87 (65.4)   | 31 (62.0)   | 24 (61.5)  | 0.188   |
| Sodium-glucose cotransporter-2 inhibitors (%)   | 167 (21.5)  | 34 (25.6)   | 19 (38.0)   | 11 (28.2)  | 0.038   |
| Thiazolidinediones (%)                          | 100 (12.9)  | 23 (17.3)   | 13 (26.0)   | 7 (17.9)   | 0.041   |
| α-glucosidase inhibitors (%)                    | 117 (15.1)  | 39 (29.3)   | 10 (20.0)   | 6 (15.4)   | <0.001  |
| Glucagon-like peptide-1 receptor agonists (%)   | 36 (4.6)    | 18 (13.5)   | 11 (22.0)   | 9 (23.1)   | <0.001  |
| Insulin (%)                                     | 94 (12.1)   | 31 (23.3)   | 13 (26.0)   | 20 (51.3)  | <0.001  |
| Use of antihypertensive drugs                   | 346 (44.5)  | 79 (59.4)   | 29 (58.0)   | 29 (74.4)  | <0.001  |
| ACE inhibitors (%)                              | 19 (2.4)    | 5 (3.8)     | 2 (4.0)     | 2 (5.1)    | 0.323   |
| Angiotensin II receptor blockers (%)            | 278 (35.8)  | 67 (50.4)   | 22 (44.0)   | 23 (59.0)  | <0.001  |
| Calcium channel blockers (%)                    | 193 (24.8)  | 44 (33.1)   | 19 (38.0)   | 17 (43.6)  | 0.005   |
| Diuretics (%)                                   | 35 (4.5)    | 13 (9.8)    | 3 (6.0)     | 6 (15.4)   | 0.006   |
| α-adrenergic receptor antagonists (%)           | 13 (1.7)    | 4 (3.0)     | 1 (2.0)     | 1 (2.6)    | 0.455   |
| β-adrenergic receptor antagonists (%)           | 26 (3.3)    | 4 (3.0)     | 1 (2.0)     | 2 (5.1)    | 1.000   |
| Use of lipid-lowering agents (%)                | 450 (58.1)  | 93 (69.9)   | 35 (70.0)   | 17 (43.6)  | 0.005   |
| Statins (%)                                     | 386 (49.8)  | 73 (54.9)   | 32 (64.0)   | 17 (43.6)  | 0.140   |
| Ezetimibe (%)                                   | 76 (9.8)    | 23 (17.3)   | 5 (10.0)    | 3 (7.7)    | 0.089   |
| Fibrates (%)                                    | 31 (4)      | 6 (4.5)     | 4 (8.0)     | 0 (0.0)    | 0.298   |
| Use of antithrombotic agents (%)                | 48 (6.2)    | 8 (6.0)     | 5 (10.0)    | 3 (7.7)    | 0.623   |
| Antiplatelet agents (%)                         | 36 (4.6)    | 8 (6.0)     | 5 (10.0)    | 1 (2.6)    | 0.297   |
| Anticoagulants (%)                              | 13 (1.7)    | 0 (0.0)     | 0 (0.0)     | 2 (5.1)    | 0.110   |

### FLP-CGM-derived metrics

| Parameter       | NDR (n=777) | SDR (n=133) | PPDR (n=50) | PDR (n=39) | P value |
|-----------------|-------------|-------------|-------------|------------|---------|
| Mean glucose (mmol/L) | 7.66±1.71   | 8.09±1.77   | 8.55±2.06   | 8.61±2.48  | <0.001  |
| SD (mmol/L)     | 2.00±0.60   | 2.07±0.61   | 2.20±0.72   | 2.46±0.80  | <0.001  |
| CV (%)          | 26.2±5.84   | 25.5±5.38   | 25.9±5.72   | 28.8±5.66  | 0.019   |
| MAGE (mmol/L)   | 5.36±1.93   | 5.43±1.76   | 5.93±2.39   | 6.82±2.99  | <0.001  |

Continued


### Table 2  Continued

| Parameter                               | NDR (n=777)     | SDR (n=133)     | PPDR (n=50)     | PDR (n=39)     | P value   |
|-----------------------------------------|-----------------|-----------------|-----------------|----------------|-----------|
| TIR<3.9-10 mmol/L (%)                   | 80.4±17.6       | 76.3±19.8       | 70.3±22.8       | 68.9±21.9      | <0.001    |
| TAR<3.0 mmol/L (%)                      | 17.4±18.0       | 22.2±20.7       | 27.6±24.5       | 27.8±22.8      | <0.001    |
| TAR>13.9 mmol/L (%)                     | 3.28±8.64       | 4.41±9.20       | 7.55±10.9       | 8.51±16.0      | <0.001    |
| TBR<3.9 mmol/L (%)                      | 2.21±4.93       | 1.56±3.33       | 2.08±4.28       | 3.29±4.72      | 0.210     |
| TBR<3.0 mmol/L (%)                      | 0.350±1.67      | 0.11±0.36       | 0.27±0.77       | 0.68±1.59      | 0.178     |
| LBGI                                    | 1.60±1.72       | 1.25±1.20       | 1.31±1.44       | 2.16±1.99      | 0.010     |
| HBGI                                    | 5.26±4.35       | 5.99±4.50       | 7.26±5.29       | 8.38±7.58      | <0.001    |
| MODD (mmol/L)                           | 1.69±0.61       | 1.78±0.56       | 1.95±0.66       | 2.34±1.00      | <0.001    |
| IQR (mmol/L)                            | 2.09±0.79       | 2.16±0.72       | 2.33±0.76       | 2.95±1.20      | <0.001    |

Data are mean±SD or number of patients (%). Continuous data were compared using analysis of variance. Categorical data were compared using χ² test or Fisher’s exact test as appropriate.

BMI, body mass index; CV, coefficient of variation; FLP-CGM, FreeStyle Libre Pro continuous glucose monitoring device; HbA1c, hemoglobin A1c; HBGI, high blood glucose index; HDL, high-density lipoprotein; LBGI, low blood glucose index; LDL, low-density lipoprotein; MAGE, mean amplitude of glycemic excursions; MODD, mean of daily differences; NDR, no diabetic retinopathy; PDR, proliferative diabetic retinopathy; PPDR, preproliferative diabetic retinopathy; SDR, simple retinopathy; TAR, time above range; TBR, time below range; TIR, time in range.

CV, TBR<3.0 mmol/L, TBR<3.0 mmol/L, and LBGI. Subjects with more severe albuminuria were more likely to be treated with sodium-glucose co-transporter-2 (SGLT-2) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and insulin, as well as antihypertensive drugs such as calcium channel blockers, ACE inhibitors, and ARBs.

Next, we investigated the relationship between FLP-CGM-derived metrics and albuminuria severity. In a proportional odds model with the patients with normoalbuminuria as the reference group, most FLP-CGM-derived metrics except for CV, TBR<3.0 mmol/L, TBR<3.9 mmol/L, and LBGI were significantly associated with albuminuria severity (model 1), as shown in table 5. In models 2 and 3, most FLP-CGM-derived metrics, except for CV, TBR<3.9 mmol/L, TBR<3.0 mmol/L, and LBGI were significantly associated with albuminuria severity after adjusting for age, gender, BMI, duration of diabetes, systolic blood pressure, lipid parameters, uric acid, eGFR, smoking, alcohol consumption, use of insulin therapy, use of ACE inhibitors and/or ARBs, use of statins, use of antiplatelet agents, and presence of DR. Associations between FLP-CGM-derived metrics and albuminuria severity remained significant after adjusting for HbA1c and parameters included in model 3 (model 4 in table 5). We performed multiple linear regression with logarithmic-transformed UAE as the dependent variable to further examine the association between UAE and CGM-derived metrics. Similar findings were obtained (data not shown). Thus, these metrics are predictive factors for the severity of albuminuria independent of HbA1c levels.

**DISCUSSION**

In this study, we demonstrated that most FLP-CGM-derived metrics related to intraday and interday glucose variability are significantly associated with the severity of DR or albuminuria, even after adjusting for various risk factors in 999 outpatients with type 2 diabetes. Notably, these metrics remain predictive factors for determining the severity of albuminuria after adjusting for HbA1c levels.

A pooled subanalysis of population-based studies demonstrated that the prevalence of any DR in patients with diabetes during 2000–2008 was substantially lower than the prevalence observed before 2000. The relative reduction in the prevalence of any DR from 49.6% to 24.8% may reflect improvements in medical care and management of diabetes and DR-related risk factors, including blood pressure, as well as early disease identification and medical provider awareness. In that study, the prevalence of any DR or PDR in patients with type 2 diabetes was 27.2% and 2.6%, respectively. Our study demonstrated that the prevalence of any DR was 22.2% and PDR was 3.9%. Given that risk factors for DR such as blood glucose and blood pressure were relatively well controlled in our study, the prevalence of DR in our study is reasonable.

The Japan Diabetes Complications Study demonstrated that HbA1c is the strongest risk factor for development and progression of DR, while longer duration of diabetes, systolic blood pressure, and BMI are positively associated with incident DR. In fact, previous studies have demonstrated that improvement in HbA1c levels is associated with reduced risk of DR development and progression in patients with type 2 diabetes. In accordance with those findings, our study indicated that HbA1c is positively associated with DR severity, even after adjusting for several risk factors. On the other hand, a recent study demonstrated that TIR<3.9-10 mmol/L based on...
seven-point glucose testing is inversely associated with the risk of DR progression in patients with type 1 diabetes.\textsuperscript{28}

Similarly, another cross-sectional study demonstrated that CGM-derived TIR\textsuperscript{3.9–10 mmol/L} is inversely associated with DR severity, independent of HbA1c levels in patients with type 2 diabetes.\textsuperscript{17} Our study also demonstrated that FLP-CGM-derived TIR\textsuperscript{3.9–10 mmol/L} is inversely associated with DR severity, even after adjusting for several possible risk factors in patients with type 2 diabetes.

### Table 3

| Parameter | OR (95% CI) | P value |
|-----------|-------------|---------|
| Mean glucose (1 mmol/L increase) | | |
| Model 1 | 1.21 (1.12 to 1.30) | <0.001 |
| Model 2 | 1.20 (1.10 to 1.29) | <0.001 |
| Model 3 | 1.22 (1.11 to 1.33) | <0.001 |
| Model 4 | 1.07 (0.94 to 1.22) | 0.311 |
| SD (mmol/L) (1 mmol/L increase) | | |
| Model 1 | 1.57 (1.26 to 1.96) | <0.001 |
| Model 2 | 1.36 (1.08 to 1.71) | 0.010 |
| Model 3 | 1.30 (1.00 to 1.69) | 0.049 |
| Model 4 | 0.97 (0.72 to 1.31) | 0.842 |
| CV (%) (1% increase) | | |
| Model 1 | 1.00 (0.98 to 1.03) | 0.747 |
| Model 2 | 0.99 (0.96 to 1.01) | 0.365 |
| Model 3 | 0.98 (0.95 to 1.00) | 0.089 |
| Model 4 | 0.98 (0.95 to 1.01) | 0.170 |
| MAGE (1 mmol/L increase) | | |
| Model 1 | 1.13 (1.05 to 1.21) | <0.001 |
| Model 2 | 1.10 (1.03 to 1.18) | 0.007 |
| Model 3 | 1.10 (1.02 to 1.19) | 0.015 |
| Model 4 | 1.03 (0.94 to 1.12) | 0.531 |
| TIR\textsuperscript{3.9–10 mmol/L} (10% increase) | | |
| Model 1 | 0.83 (0.77 to 0.89) | <0.001 |
| Model 2 | 0.85 (0.79 to 0.91) | <0.001 |
| Model 3 | 0.85 (0.78 to 0.93) | <0.001 |
| Model 4 | 0.97 (0.86 to 1.09) | 0.616 |
| TAR\textsuperscript{10 mmol/L} (1% increase) | | |
| Model 1 | 1.02 (1.01 to 1.04) | <0.001 |
| Model 2 | 1.02 (1.01 to 1.02) | <0.001 |
| Model 3 | 1.02 (1.01 to 1.03) | <0.001 |
| Model 4 | 1.01 (0.99 to 1.02) | 0.371 |
| TAR\textsuperscript{13.9 mmol/L} (1% increase) | | |
| Model 1 | 1.03 (1.01 to 1.04) | <0.001 |
| Model 2 | 1.03 (1.01 to 1.04) | <0.001 |
| Model 3 | 1.03 (1.01 to 1.05) | <0.001 |
| Model 4 | 1.01 (0.99 to 1.03) | 0.541 |
| TBR\textsuperscript{3.9 mmol/L} (1% increase) | | |
| Model 1 | 0.99 (0.96 to 1.03) | 0.677 |
| Model 2 | 0.99 (0.95 to 1.02) | 0.397 |
| Model 3 | 0.96 (0.93 to 0.99) | 0.028 |
| Model 4 | 0.98 (0.94 to 1.01) | 0.238 |
| TBR\textsuperscript{3.0 mmol/L} (1% increase) | | |
| Model 1 | 0.96 (0.85 to 1.08) | 0.499 |
| Model 2 | 0.94 (0.83 to 1.06) | 0.288 |
| Model 3 | 0.84 (0.72 to 0.97) | 0.020 |

Continued
**Table 4** Patient demographic and background characteristics stratified by albuminuria status

| Parameter                        | Normoalbuminuria (n=729) | Microalbuminuria (n=203) | Macroalbuminuria (n=67) | P value  |
|----------------------------------|--------------------------|--------------------------|-------------------------|----------|
| Age (years)                      | 64.1±9.5                 | 66.2±9.8                 | 64.6±10.0               | 0.026    |
| Male gender (%)                  | 441 (60.5)               | 120 (59.1)               | 47 (70.1)               | 0.256    |
| BMI (kg/m²)                      | 24.3±3.9                 | 25.2±3.6                 | 25.9±4.0                | <0.001   |
| Estimated duration of diabetes (years) | 12.0±8.2               | 14.6±8.7                 | 17.2±9.4                | <0.001   |
| Systolic blood pressure (mm Hg)  | 130.7±14.7               | 132.5±14.6               | 133.4±17.1              | 0.147    |
| Diastolic blood pressure (mm Hg) | 75.5±10.8                | 74.8±11.7                | 77.6±11.9               | 0.198    |
| HbA1c (%)                        | 7.0±0.7                  | 7.3±0.9                  | 7.5±1.1                 | <0.001   |
| HbA1c (mmol/mol)                 | 52.6±7.8                 | 55.8±10.0                | 58.5±12.3               | <0.001   |
| Total cholesterol (mmol/L)       | 4.83±0.81                | 4.76±0.74                | 4.72±1.05               | 0.437    |
| LDL cholesterol (mmol/L)         | 2.69±0.68                | 2.64±0.64                | 2.43±0.81               | 0.010    |
| HDL cholesterol (mmol/L)         | 1.59±0.41                | 1.49±0.41                | 1.49±0.37               | 0.004    |
| Triglycerides (mmol/L)           | 1.3±0.8                  | 1.4±0.9                  | 2.0±1.8                 | <0.001   |
| Uric acid (μmol/L)               | 300.6±69.9               | 314.8±79.0               | 358.7±65.0              | <0.001   |
| Estimated glomerular filtration rate (mL/min/1.73m²) | 76±19                   | 69±21                    | 58±25                   | <0.001   |
| Diabetic retinopathy (%)         | 123 (16.9)               | 63 (31.0)                | 36 (53.7)               | <0.001   |
| Use of oral glucose-lowering agents (%) | 639 (88)            | 188 (93)                 | 67 (100)                | 0.002    |
| Metformin (%)                    | 387 (53)                 | 120 (59)                 | 36 (54)                 | 0.311    |
| Sulfonylureas (%)                | 84 (12)                  | 32 (16)                  | 11 (16)                 | 0.177    |
| Glinides (%)                     | 44 (6)                   | 17 (8)                   | 7 (10)                  | 0.238    |
| Dipeptidyl peptidase-4 inhibitors (%) | 412 (57)            | 118 (58)                 | 47 (70)                 | 0.096    |
| Sodium-glucose cotransporter-2 inhibitors (%) | 149 (20)           | 59 (29)                  | 23 (34)                 | 0.003    |
| Thiazolidinediones (%)           | 99 (14)                  | 30 (15)                  | 14 (21)                 | 0.256    |
| α-glucosidase inhibitors (%)     | 125 (17)                 | 32 (16)                  | 15 (22)                 | 0.458    |
| Glucagon-like peptide-1 receptor agonists (%) | 40 (6)             | 22 (11)                  | 12 (18)                 | <0.001   |
| Insulin (%)                      | 92 (13)                  | 44 (22)                  | 22 (33)                 | <0.001   |
| Use of antihypertensive drugs (%) | 298 (41)              | 130 (64)                 | 55 (82)                 | <0.001   |
| ACE inhibitors (%)               | 13 (2)                   | 11 (5)                   | 4 (6)                   | 0.006    |
| Angiotensin II receptor blockers (%) | 245 (34)            | 101 (50)                 | 44 (66)                 | <0.001   |
| Calcium channel blockers (%)     | 154 (21)                 | 86 (42)                  | 33 (49)                 | <0.001   |
| Diuretic drugs (%)               | 33 (5)                   | 17 (8)                   | 7 (10)                  | 0.025    |
| α-adrenergic receptor antagonists (%) | 9 (1)               | 6 (3)                    | 4 (6)                   | 0.013    |
| β-adrenergic receptor antagonists (%) | 15 (2)              | 12 (6)                   | 6 (9)                   | <0.001   |
| Use of lipid-lowering agents (%)  | 426 (59)                 | 121 (60)                 | 48 (72)                 | 0.114    |
| Statins (%)                      | 360 (50)                 | 106 (52)                 | 42 (63)                 | 0.110    |
| Ezetimibe (%)                    | 75 (10)                  | 21 (10)                  | 11 (16)                 | 0.298    |
| Fibrates (%)                     | 29 (4)                   | 9 (4)                    | 3 (5)                   | 0.949    |
| Use of antithrombotic agents (%)  | 46 (6)                   | 14 (7)                   | 4 (6)                   | 0.948    |
| Antiplatelet agents (%)          | 36 (5)                   | 11 (5)                   | 3 (5)                   | 0.935    |
| Anticoagulants (%)               | 11 (2)                   | 3 (2)                    | 1 (2)                   | 1.000    |
| FLP-CGM-derived metrics          |                          |                          |                        |          |
| Mean glucose (mmol/L)            | 7.59±1.59                | 8.25±1.97                | 8.70±2.62               | <0.001   |
| SD (mmol/L)                      | 1.97±0.58                | 2.20±0.68                | 2.29±0.77               | <0.001   |
| CV (%)                           | 26.0±5.72                | 26.8±5.90                | 26.7±6.11               | 0.142    |
| MAGE (mmol/L)                    | 5.26±1.85                | 5.86±2.08                | 6.34±2.79               | <0.001   |

Continued
associations between TIR3.9–10 mmol/L as well as other FLP-CGM-derived metrics including SD, MAGE, TAR10 mmol/L, TAR13.9 mmol/L, HBGI, MODD, and IQR and DR severity did not reach statistical significance after adjusting for HbA1c levels.

A possible explanation for the discrepant findings may be differences in characteristics of patients between our studies and the other two previous studies. First, the subjects of a prior study28 were patients with type 2 diabetes treated with insulin, which is completely different from our subjects. In addition, TIR3.9–10 mmol/L despite substantially higher HbA1c levels. Accordingly, that data may not be generalizable to outpatients with type 2 diabetes under their usual living conditions.

Recently, SGLT-2 inhibitors, dipeptidyl peptidase-4 (DPP-4) inhibitors, and GLP-1 receptor agonists have been frequently used for patients with type 2 diabetes. These drugs are reported to decrease glucose fluctuations without increasing the risk of hypoglycemia.29 Not surprisingly, our subjects had lower HbA1c levels and were more likely to have lower SD and MAGE and higher TIR3.9–10 mmol/L, despite substantially higher HbA1c levels. Accordingly, that data may not be generalizable to outpatients with type 2 diabetes under their usual living conditions.

Table 4

| Parameter | Normoalbuminuria (n=729) | Microalbuminuria (n=203) | Macroalbuminuria (n=67) | P value |
|-----------|--------------------------|--------------------------|-------------------------|---------|
| TIR3.9–10 mmol/L (%) | 81.1±17.1 | 74.3±19.5 | 68.0±24.7 | <0.001 |
| TAR10 mmol/L (%) | 16.7±17.5 | 23.8±20.4 | 28.7±26.3 | <0.001 |
| TAR13.9 mmol/L (%) | 2.87±7.4 | 5.71±11.8 | 8.86±15.7 | <0.001 |
| TBR3.9 mmol/L (%) | 2.12±4.58 | 1.91±4.06 | 3.35±7.20 | 0.086 |
| TBR3.0 mmol/L (%) | 0.33±1.64 | 0.20±0.77 | 0.65±1.85 | 0.117 |
| LBGI | 1.58±1.68 | 1.41±1.44 | 1.79±2.07 | 0.226 |
| HBGI | 5.04±3.86 | 6.70±5.48 | 8.08±7.40 | <0.001 |
| MODD (mmol/L) | 1.66±0.59 | 1.88±0.64 | 2.13±0.90 | <0.001 |
| IQR (mmol/L) | 2.05±0.76 | 2.31±0.82 | 2.65±1.11 | <0.001 |

Data are mean±SD or number of patients (%). Continuous data were compared using analysis of variance. Categorical data were compared using χ² test or Fisher’s exact test as appropriate.

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**Pathophysiology/complications**

According to serial cross-sectional studies of patients with diabetes who participated in the National Health and Nutrition Examination Surveys, the prevalence of albuminuria declined progressively from 20.8% in 1988–1994 to 15.9% in 2009–2014.30 Higher prevalence of treatment with antidiabetic agents, renin–angiotensin system inhibitors, and statins may account for the reduction in the prevalence of albuminuria. On the other hand, an in vitro study showed that intermittent treatment of high blood glucose levels increases apoptosis of mesangial cells by increasing levels of inflammatory cytokines and oxidative stress, leading to the development of DN.31 However, there are limited data about the clinical impact of intraday and interday glucose variability on the presence or progression of albuminuria in patients with type 2 diabetes. Two previous studies conducted by the same group did not show a significant association between CGM-derived metrics of intraday and interday glucose variability and the presence or progression of albuminuria independent of HbA1c levels in patients with type 2 diabetes with HbA1c levels of more than 8%.32 33 In contrast, our study clearly showed close relationships between FLP-CGM-derived metrics related to intraday and interday glucose variability and the severity of albuminuria,
Pathophysiology/complications

Table 5 Associations between FLP-CGM-derived metrics and albuminuria severity

| Parameter                          | OR (95% CI) | P value |
|------------------------------------|-------------|---------|
| Mean glucose (1 mmol/L increase)   |             |         |
| Model 1: crude.                    | 1.26 (1.17 to 1.35) | <0.001  |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.24 (1.15 to 1.34) | <0.001  |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 1.24 (1.14 to 1.36) | <0.001  |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 1.28 (1.14 to 1.45) | <0.001  |
| SD (1 mmol/L increase)             |             |         |
| Model 1: crude.                    | 1.86 (1.50 to 2.31) | <0.001  |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.81 (1.45 to 2.27) | <0.001  |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 1.72 (1.33 to 2.21) | <0.001  |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 1.57 (1.18 to 2.08) | 0.002   |
| CV (1% increase)                   |             |         |
| Model 1: crude.                    | 1.02 (1.00 to 1.05) | 0.053   |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.02 (1.00 to 1.05) | 0.066   |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 1.02 (0.99 to 1.04) | 0.288   |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 1.02 (0.99 to 1.05) | 0.251   |
| MAGE (1 mmol/L increase)           |             |         |
| Model 1: crude.                    | 1.20 (1.12 to 1.28) | <0.001  |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.19 (1.11 to 1.27) | <0.001  |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 1.17 (1.09 to 1.26) | <0.001  |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 1.14 (1.05 to 1.24) | 0.001   |
| TIR (1% increase)                  |             |         |
| Model 1: crude.                    | 0.97 (0.74 to 0.85) | <0.001  |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 0.80 (0.75 to 0.86) | <0.001  |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 0.81 (0.75 to 0.89) | <0.001  |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 0.81 (0.72 to 0.90) | <0.001  |
| TAR (1% increase)                  |             |         |
| Model 1: crude.                    | 1.02 (1.02 to 1.03) | <0.001  |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.02 (1.01 to 1.03) | <0.001  |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 1.02 (1.01 to 1.03) | <0.001  |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 1.02 (1.01 to 1.03) | <0.001  |
| IQR (1% increase)                  |             |         |
| Model 1: crude.                    | 1.04 (1.02 to 1.05) | <0.001  |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.04 (1.02 to 1.05) | <0.001  |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 1.03 (1.02 to 1.05) | <0.001  |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 1.03 (1.01 to 1.05) | <0.001  |
| HbA1c (1% increase)                |             |         |
| Model 1: crude.                    | 1.01 (0.98 to 1.04) | 0.436   |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.01 (0.98 to 1.04) | 0.523   |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 0.99 (0.96 to 1.03) | 0.697   |
| Model 4: adjusted for variables in model 3 plus HbA1c. | 1.01 (0.97 to 1.03) | 0.751   |
| TBR (1% increase)                  |             |         |
| Model 1: crude.                    | 1.01 (0.92 to 1.10) | 0.914   |
| Model 2: adjusted for age, gender, BMI, and duration of diabetes. | 1.00 (0.91 to 1.09) | 0.927   |
| Model 3: adjusted for variables in model 2 plus systolic blood pressure, total cholesterol, HDL cholesterol, logarithm of triglycerides, serum uric acid, estimated glomerular filtration rate, smoker, alcohol consumption, presence of diabetic retinopathy, use of insulin therapy, use of ACE inhibitors and/or angiotensin II receptor blockers, use of statins, and use of antiplatelet agents. | 0.97 (0.87 to 1.07) | 0.502   |

continued

even after adjusting for various possible risk factors including HbA1c, in patients with type 2 diabetes. The reason that those studies yielded conflicting results is not clear, but it may be due to differences in the characteristics of subjects or use of antidiabetic agents among studies. Previous studies enrolled patients with inadequately controlled type 2 diabetes; thus, the prevalence of albuminuria was relatively high, approximately 40%. In contrast, our subjects had a substantially lower prevalence of albuminuria (27%) and were more likely to be
taking SGLT2 inhibitors, DPP-4 inhibitors, and GLP-1 receptor agonists. Taken together, our data suggest that intraday and interday glucose variabilities are important targets in terms of reducing the risk of albuminuria in patients treated according to the current consensus about diabetes treatment.34

HbA1c is recognized as a gold standard for treatment target. A few studies have demonstrated strong associations between HbA1c levels and diabetic microvascular complications.15 However, HbA1c alone may not adequately reflect an individual's glycemic variation and risk of hyperglycemia and hypoglycemia. In this regard, the ATTD Congress recommended TIR as a key metric of glycemic management in clinical practice.15 Our results showed that TIR<9.0–10 mmol/L, TAR>10 mmol/L, and TBR<3.9 mmol/L are significantly associated with the severity of albuminuria, even after adjusting for possible risk factors including HbA1c levels. On the other hand, a recent study demonstrated that severe hyperglycemia is a predictor of worsening renal dysfunction in patients with type 2 diabetes.35 However, in addition to LBGI, TBR<3.9 mmol/L and TBR<3.0 mmol/L were not associated with the severity of albuminuria in our study. Mild hypoglycemia, low frequency of hypoglycemic events, or both may not be involved in the development of albuminuria. Alternatively, the relatively low frequency of hypoglycemic events and short duration of hypoglycemia observed in our study may account for this finding. Taken together, based on FLP-CGM-derived metrics, focusing on improving hyperglycemia may be important to reduce the risk of albuminuria development. However, HbA1c alone does not provide enough information. Indeed, a previous study demonstrated that lower renal function (eGFR <60 min/1.73 m²) is strongly correlated with a higher prevalence of anemia in the general population.36 Modest reductions in hemoglobin due to a shorter erythrocyte lifespan may affect the accuracy of HbA1c. In patients with more advanced DN, evaluating FLP-CGM-derived metrics could serve as a therapeutic target complementary to HbA1c.

Our study found that FLP-CGM-derived metrics related to glucose variability are associated with the severity of albuminuria, which is different from DR, even after adjusting for HbA1c levels. Although the exact reason for these findings is not clear, we postulated one scenario. Atherosclerosis of the intrarenal and extrarenal arteries and microangiopathy of the glomerular capillaries, afferent arterioles, and efferent arterioles are considered to contribute to the progression of glomerular lesions in DN.37 38 Previous studies have demonstrated that glucose fluctuation is more significantly associated with atherosclerotic-related diseases than the degree of hyperglycemic exposure as indicated by HbA1c levels in patients with type 2 diabetes.39 40 Thus, it is possible that atherosclerosis of the intrarenal and extrarenal arteries caused by glucose variability may also accelerate renal damage. Therefore, glucose variability is more likely to be associated with the pathogenesis of DN than DR.

The strengths of this study included its relatively large sample size and multicenter study design. Our study had certain limitations. First, the cross-sectional study design made it impossible to evaluate whether FLP-CGM-derived metrics had a causal relationship with diabetic microvascular complications. In this regard, we are currently conducting a long-term follow-up study in the same cohort that focuses on FLP-CGM-derived metrics and onset of outcomes such as primary cardiovascular disease and diabetic microvascular complications. Second, FLP-CGM-derived metrics were evaluated based on FLP-CGM measurements during a limited time. Thus, FLP-CGM-derived metrics may not represent overall glycemic control of subjects. In order to attain the best measurements of glucose fluctuations with FLP-CGM at baseline, we only recruited patients with stable control. In addition, we employed a blind CGM system that prevented subjects from altering their lifestyle behaviors based on the results of glucose readings. Third, we did not assess the accuracy of interstitial glucose levels obtained with the FLP-CGM system by comparing them with capillary glucose levels or venous glucose levels. Previous studies demonstrated some discrepancies in glucose levels obtained with the FLP-CGM system and conventional glucose measurements.41-44 Thus, our findings should be interpreted with caution. Fourth, we only recruited Japanese patients with type 2 diabetes. These constraints may limit the generalizability of our results. Finally, some potential conventional risk factors for DR were not included in the multivariate regression analysis. Previous studies reported that inflammation and homocysteine contribute to DR progression.45 46 This point should also be addressed in a future study.

CONCLUSION
In conclusion, we demonstrated that FLP-CGM-derived metrics related to intraday and interday glucose variability are significantly associated with the severity of DR and albuminuria, even after adjusting for various risk factors in patients with type 2 diabetes. Thus, these derived metrics could provide medical professionals with useful information for assessing the risk of severe diabetic microvascular complications. CGM might identify treatment targets in addition to those based on HbA1c.

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Contributors All authors contributed to the study design and were involved in all stages of manuscript development. SW and TM drafted the manuscript. MG, a statistician, was primarily responsible for data analysis. SW, TM, NK, YO, TO, HY, KN, TS, KT, AK, MG, IS, and HW also collected, analyzed, and interpreted the data; reviewed and edited the manuscript; and approved the final manuscript. HW is the principal guarantor of this work; he has full access to all study data and takes responsibility for the integrity of the data and the accuracy of data analysis. All authors have read and agreed to the publication of the manuscript.

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REFERENCES

1. Klein BEK. Overview of epidemiologic studies of diabetic retinopathy. Diabet Med 2003;20:103–17.
2. Webster AC, Nagler EV, Morton RL, et al. Chronic kidney disease. Lancet 2017;389:1238–52.
3. Klein R, Klein BE, Moss SE, et al. Relationship of hyperglycemia to the long-term incidence and progression of diabetic retinopathy. Arch Intern Med 1994;154:2169–78.
4. Stratton IM, Adler AI, Neil HA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study, BMJ 2000;320:405–12.
5. The relationship of glycaemic exposure (HbA1c) to the risk of development and progression of retinopathy in the diabetes control and complications trial. Diabetes 1995;44:968–83.
6. Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). UK prospective diabetes study (UKPDS) group. Lancet 1998;352:837–53.
7. Ohkubo Y, Kishikawa H, Araki E, et al. Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. Diabetes Res Clin Pract 1995;28:103–17.
8. American Diabetes Association. 6. Glycemic Targets: Standards of Medical Care in Diabetes—2020. Diabetes Care 2020;43:S66–76.
9. Haneda M, Noda M, Ogihara H, et al. Japanese clinical practice guideline for diabetes 2016. Diabetol Int 2016;9:1–45.
10. Duckworth W, Abraira C, Moritz T, et al. Glucose control and vascular complications in veterans with type 2 diabetes. N Engl J Med 2009;360:129–39.
11. Boussageon R, Bejan-Angoulvant T, Saadaatian-Elahi M, et al. Effect of intensive glucose lowering treatment on all cause mortality, cardiovascular death, and microvascular events in type 2 diabetes: meta-analysis of randomised controlled trials. BMJ 2011;343:d4169.
12. Ceriello A, Esposito K, Picconi L, et al. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. Diabetes 2008;57:1349–54.
13. Quagliaro L, Piconi L, Assaloni R, et al. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cells: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes 2003;52:2795–804.
14. Welsh KJ, Kirkman MS, Sacks DB. Role of glyciated proteins in the diagnosis and management of diabetes: research gaps and future directions. Diabetes Care 2016;39:1299–306.
15. Battelino T, Danne T, Bergenstal RM, et al. Clinical targets for continuous glucose monitoring data interpretation: recommendations from the International consensus on time in range. Diabetes Care 2019;42:1593–603.
16. Lu J, Ma X, Zhang L, et al. Glycemic variability assessed by continuous glucose monitoring and the risk of diabetic retinopathy in latent autoimmune diabetes of the adult and type 2 diabetes. J Diabetes Invest 2019;10:753–9.
17. Lu J, Ma X, Zhou J, et al. Association of time in range, as assessed by continuous glucose monitoring, with diabetic retinopathy in type 2 diabetes. Diabetes Care 2018;41:2370–6.
18. Xin Z, Zhu Y, Wang Z, et al. Associations of subclinical atherosclerosis with nonalcoholic fatty liver disease and fibrosis assessed by non-invasive score. Liver Int 2020;40:806–14.
19. Sonoda S, Okada Y, Mori H, et al. Association between diabetic microangiopathies and glycemic variability assessed by continuous glucose monitoring. J UOEH 2018;40:11–18.
20. Mita T, Katakami N, Okada Y, et al. Protocol of a prospective observational study on the relationship between glucose fluctuation and cardiovascular events in patients with type 2 diabetes. Diabetes Ther 2019;10:1565–76.
21. Matsu S, Imai E, Honjo M, et al. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982–92.
22. Service FJ, Molnar GD, Rosevear JW, et al. Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes 1970;19:644–55.
23. Kovatchev BP, Cox DJ, Kumar A, et al. Algorithmic evaluation of metabolic control and risk of severe hypoglycemia in type 1 and type 2 diabetes using self-monitoring blood glucose data. Diabetes Technol Ther 2002;5:817–28.
24. Hill NR, Oliver NS, Choudhary P, et al. Normal reference range for mean tissue glucose and glycemic variability derived from continuous glucose monitoring for subjects without diabetes in different ethnic groups. Diabetes Technol Ther 2011;13:921–8.
25. Boboriski R, Galasso S, et al. Hexose-1,6-10 comparison of the accuracy of Abbott FreeStyle Libre and Dexcom G6 mobile. Nutr Metab Cardiovasc Dis 2018;28:425–7.
26. Yao JW, Rogers SL, Kawasaki R, et al. Meta-Analysys for eye disease study G, global prevalence and major risk factors of diabetic retinopathy. Diabetologia 2012;55:641–52.
27. Kawasaki R, Tanaka S, Tanaka S, et al. Incidence and progression of diabetic retinopathy in Japanese adults with type 2 diabetes: 8 year follow-up study of the Japan diabetes complications study (JDCS). Diabetologia 2011;54:2288–94.
28. Beck RW, Bergenstal RM, Riddelworth TD, et al. Validation of time in range as an outcome measure for diabetes clinical trials. Diabetes Care 2019;42:400–5.
29. Zhou Z, Sun B, Huang S, et al. Glycemic variability: adverse clinical outcomes and how to improve it? Cardiovasc Diabetol 2020;19:102.
30. Afkarian M, Zelnick LR, Hall VN, et al. Clinical manifestations of kidney disease among US adults, 1988–2014. JAMA 2016;316:602–10.
31. Ying C, Wang S, Lu Y, et al. Glucose fluctuation increased mesangial cell apoptosis related to Akt signal pathway. Arch Med Sci 2019;15:730–7.
32. Yoo JH, Choi MS, Ahn J, et al. Association between continuous glucose Monitoring-Derived time in range, other core metrics,
and albuminuria in type 2 diabetes. *Diabetes Technol Ther* 2020;22:768–76.

33 Jin S-M, Kim T-H, Oh S, et al. Association between the extent of urinary albumin excretion and glycaemic variability indices measured by continuous glucose monitoring. *Diabet Med* 2015;32:274–9.

34 Davies MJ, D’Alessio DA, Fradkin J, et al. Management of hyperglycaemia in type 2 diabetes, 2018. A consensus report by the American diabetes association (ADA) and the European association for the study of diabetes (EASD). *Diabetologia* 2018;61:2461–98.

35 Lee Y-L, Yen S-J, Shin S-J, et al. Severe hypoglycemia as a predictor of end-stage renal disease in type 2 diabetes: a national cohort study. *Int J Environ Res Public Health* 2019;16. doi:10.3390/ijerph16050681. [Epub ahead of print: 26 02 2019].

36 Astor BC, Munter P, Levin A, et al. Association of kidney function with anemia: the third National health and nutrition examination survey (1988–1994). *Arch Intern Med* 2002;162:1401–8.

37 Bohle A, Wehrmann M, Bogenschütz O, et al. The pathogenesis of chronic renal failure in diabetic nephropathy. investigation of 488 cases of diabetic glomerulosclerosis. *Pathol Res Pract* 1991;187:251–9.

38 Shimizu M, Furuichi K, Toyama T, et al. Association of renal arteriosclerosis and hypertension with renal and cardiovascular outcomes in Japanese type 2 diabetes patients with diabetic nephropathy. *J Diabetes Investig* 2019;10:1041–9.

39 Torimoto K, Okada Y, Mori H, et al. Relationship between fluctuations in glucose levels measured by continuous glucose monitoring and vascular endothelial dysfunction in type 2 diabetes mellitus. *Cardiovasc Diabetol* 2013;12:1.

40 Su G, Mi S, Tao H, et al. Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. *Cardiovasc Diabetol* 2011;10:19.

41 Danne T, Nimri R, Battelino T, et al. International consensus on use of continuous glucose monitoring. *Diabetes Care* 2017;40:1631–40.

42 Ji L, Guo X, Guo L, et al. A multicenter evaluation of the performance and usability of a novel glucose monitoring system in Chinese adults with diabetes. *J Diabetes Sci Technol* 2017;11:290–5.

43 Ajjan RA, Cummings MH, Jennings P, et al. Accuracy of flash glucose monitoring and continuous glucose monitoring technologies: implications for clinical practice. *Diab Vasc Dis Res* 2018;15:175–84.

44 Galindo RJ, Migdal AL, Davis GM, et al. Comparison of the FreeStyle Libre pro flash continuous glucose monitoring (CGM) system and point-of-care capillary glucose testing in hospitalized patients with type 2 diabetes treated with Basal-Bolus insulin regimen. *Diabetes Care* 2020;43:dc192073–735.

45 Platania CBM, Giuridanella G, Di Paola L, et al. P2X7 receptor antagonism: implications in diabetic retinopathy. *Biochem Pharmacol* 2017;138:130–9.

46 Malaguarnera G, Gagliano C, Giordano M, et al. Homocysteine serum levels in diabetic patients with non proliferative, proliferative and without retinopathy. *Biomed Res Int* 2014;2014:1–4.