Relationship between daily and day-to-day glycemic variability and increased oxidative stress in type 2 diabetes

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ABSTRACT

Aims: To determine the association of daily and day-to-day glucose variability with oxidative stress.

Methods: This was a cross-sectional analysis of 68 patients with type 2 diabetes mellitus (T2DM) over 72 h of continuous glucose monitoring. Fasting plasma glucose (FPG) and hemoglobin A1c (HbA1c) were measured before breakfast on day 1. Glucose variability, mean glucose level (MGL), mean amplitude of glycemic excursions (MAGE), mean of daily differences (MODD) in glucose levels and area under the postprandial plasma glucose curve (AUC$_{PP}$) were measured on days 2 and 3. Plasma oxidant capacity against N,N-diethylpara phenylenediamine was measured with the diacron-reactive oxygen metabolites (d-ROMs) test on day 1.

Results: Overall, 66.2% males with the mean age of 63.2 ± 12.6 years, diabetes duration of 12.9 ± 10.4 years, and HbA1c level of 8.1 ± 1.6% (65 ± 17 mmol/mol) were included. MGL ($r = 0.330$), HbA1c ($r = 0.326$), MAGE ($r = 0.565$), MODD ($r = 0.488$), and AUC$_{PP}$ ($r = 0.254$) exhibited significant correlations with d-ROMs and not FPG; these correlations remained significant after adjustment for clinical factors (sex, age, duration of diabetes, smoking habit, insulin use, statin use, angiotensin II receptor blocker use, BMI, LDL-C, HDL-C, TG, eGFR, and systolic blood pressure) ($R^2 = 0.268$, $R^2 = 0.268$, $R^2 = 0.417$, $R^2 = 0.314$, and $R^2 = 0.347$, respectively). MAGE was significantly correlated with MODD ($r = 0.708$) and MAGE and MODD were independently correlated with d-ROMs by multivariate analysis.

Conclusions: Therefore, oxidative stress is associated with daily and day-to-day glucose variability in patients with T2DM.

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1.   Introduction

The United Kingdom Prospective Diabetes Study (UKPDS) demonstrated that chronic hyperglycemia, as measured by glycated hemoglobin (HbA1c), is the main risk factor for diabetes-related complications [1]. However, HbA1c does not inform us about short-term glycemic variability, which refers to swings in blood glucose levels throughout the day, including the possibilities of hypoglycemic and hyperglycemic periods within and between days. Recently, continuous glucose monitoring (CGM) has become increasingly relevant when evaluating such variability and can detect glucose variability in greater detail than conventional self-monitoring methods.

Various studies have been conducted on the relationship between glucose variability and diabetic complications. Postprandial plasma glucose (PPG) is more closely related to cardiovascular disease than fasting plasma glucose (FPG) [2], with glucose variability considered important in patients with type 2 diabetes mellitus (T2DM) [3]. In addition, FPG variability is reportedly associated with the 10-year survival of this patient group [4], with both intra-day glucose variability and HbA1c variability being independent risk factors for microangiopathy [5,6]. Therefore, glucose variability may be an additional risk factor for diabetic complications, independent of hyperglycemia [7]. On the other hand, HbA1c and mean blood glucose are related to cardiovascular disease in addition to PPG and glucose variability [8]. Furthermore, a decrease in glucose variability dose does not reduce the risk of cardiovascular disease in patients with T2DM after acute myocardial infarction [9]. Furthermore, HbA1c variability is not associated with microvascular complications in type 1 diabetes [10]. Based on the abovementioned findings, the relationship between glucose variability and diabetic complication is controversial.

Oxidative stress appears important in the development and progression of diabetic complications [11]. Hyperglycemic damage results from reactive oxygen species (ROS)-induced activation of polyol, hexosamine, protein kinase C, and the advanced glycation end-product pathway [12]. Because atherosclerosis can result when acute glucose variability induces endothelial dysfunction through oxidative stress [13], the activation of oxidative stress could be a risk factor for diabetic complications. There are various markers of oxidative stress [14], but 8-hydroxydeoxyguanosine (8-OHdG) and 8-iso-prostaglandin F2α (8-iso-PGF2α) are particularly useful in diabetes: 8-OHdG has often been used as a biomarker of oxidative DNA damage [15], whereas 8-iso-PGF2α is a major product of the peroxidation of unsaturated fatty acids and can predict oxidative stress [16].

Direct measurement of ROS and free radicals is difficult in a standard laboratory owing to their biochemical instability, which requires that the oxidation products of biological components be used as markers of oxidative stress. However, such assays are complex and unsuitable when analyzing a large number of subjects. Recently, a method of measuring reactive oxygen metabolites (ROMs) in the blood has been developed that uses diacron (i.e., the d-ROMs test) [17]. This photometric test measures the total oxidant capacity of serum or plasma against the chromogenic substrate N,N-die thylparaphenylenediamine. ROMs mainly comprise organic hydroperoxide; despite its moderate oxidative power, serum levels are detectable because of its relative stability compared with other free radicals. Not only is the d-ROMs test quick and inexpensive for use in clinical settings, it is also predictive of morbidity and mortality [18,19]. Moreover, the test correlates positively with plasma glucose and HbA1c levels [20], and levels reduce after antioxidant supplementation in patients with T2DM [21].

Therefore, we aimed to determine whether daily and day-to-day glucose variability measured by CGM were associated with plasma oxidative stress measured by d-ROMs in patients with T2DM.

2.   Subjects, materials and methods

2.1.   Subjects

We recruited 68 patients (45 inpatients and 23 outpatients) with T2DM among those treated at Showa University Hospital (from October 2013 to May 2015) and Nippon Medical School (from March 2012 to December 2012). The reasons for hospital admission were to achieve glycemic control because of poor control or to evaluate for glucose variability. The inclusion criteria were a diagnosis of T2DM and stable oral hypoglycemic and/or insulin treatment, both for ≥3 months before the study. The exclusion criteria were the use of steroids or anti-inflammatory drugs, any febrile illnesses within 3 months before the study, and an estimated glomerular filtration rate (eGFR) <30 mL/min/1.73 m^2 according to the Cockcroft–Gault formula [22].

2.2.   Study design

This was a cross-sectional analysis of patients with T2DM over a 72-h period of (CGM).

The following clinical and laboratory parameters were measured before breakfast on day 1: body mass index, low-density lipoprotein cholesterol (LDLc), high-density lipoprotein cholesterol (HDLc), triglycerides, eGFR, blood pressure, FPG, and HbA1c. Plasma oxidant capacity against N,N-diethyl paraphenylenediamine was also measured using the d-ROMs test on day 1. Glucose variability, mean glucose level (MGL), mean amplitude of glycemic excursions (MAGE), mean of daily differences (MODD) in glucose levels, and area under the PPG curve (AUC$_{PP}$) were measured on days 2 and 3. Clinical data (age, sex, smoking, and duration of diabetes in years) were retrieved from medical records.

The study protocol was approved by the ethics committee of the Showa University School of Medicine. Informed consent was obtained from all subjects after receiving a clear explanation of the study protocol. The study was designed in compliance with the Declaration of Helsinki.

2.3.   Procedures and measurements

The CGM sensor (CGMS System Gold; Medtronic MiniMed, Northridge, CA, USA or ipro2; Medtronic MiniMed, Northridge, CA) was inserted subcutaneously on day 1 and removed on

day 4, and glucose variability was only calculated on days 2 and 3 to avoid bias. Venous blood samples were drawn for laboratory measurements on day 1 before breakfast. All patients received a weight-maintaining diet (25–30 kcal/kg ideal body weight). Glucose variabilities were calculated using CGM data; the AUC_{PP} was calculated using the incremental areas above preprandial glucose values beyond 4 h after each meal [23]. The MGL was measured from the date recorded on CGM, adjusted for self-monitored blood glucose. The mean amplitude of glycemic excursions (MAGE) [24] was calculated to assess glucose variability (Fig. 1). The mean of the daily differences (MODD) [25] was calculated as the mean of the absolute difference between corresponding glucose values on days 2 and 3 (Fig. 2).

2.4. Diagnosis of complications

The presence and severity of diabetic retinopathy was assessed by ophthalmologists. Fundal assessment was performed by indirect mydriatic examination and slit-lamp biomicroscopy using a precorneal lens and fluorescein angiography if needed. Diabetic nephropathy was defined as follows using the urinary albumin:creatinine ratio (UACR) based on the first urine sample obtained in the morning: normoalbuminuria, 0–29.9 mg/g creatinine; microalbuminuria, 30–299 mg/g creatinine; and nephropathy, ⩾300 mg/g creatinine [26]. Diabetic neuropathy was defined as the presence of two or more clinical symptoms (bilateral spontaneous pain, hypoesthesia, or paraesthesia of the legs), decreased or absent ankle reflexes, and decreased vibration sensation with respect to a standard 128-Hz tuning fork.
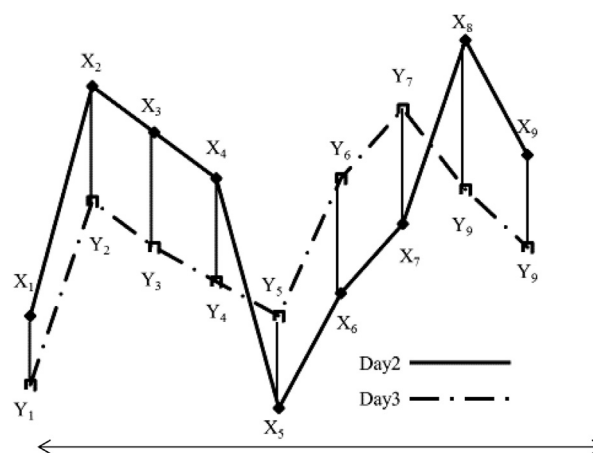
2.5. Laboratory measurements

Oxidative stress was measured using a d-ROMs test and dedicated photometer (F.R.E.E. System; imported by LTD Tokyo from Diacron International s.r.l. Grosseto, Italy), as previously reported [27]. According to the Wismerll kinetic procedure, the change of absorbance per minute was expressed as arbi-
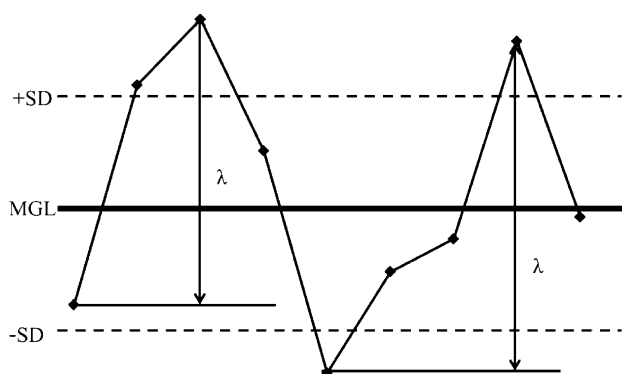
$$K = Count$$
$$d_n = X_n - Y_n$$
$$MODD = \frac{Sum (d)}{K}$$

Fig. 2 – Graphical illustration of mean of daily difference of blood glucose (MODD).

trary units after correction (U.CARR, where 1 U.CARR = the oxidant capacity of a 0.08 mg/dL H_2O_2 solution, and the normal range = 250–300 U.CARR). The intra- and inter-assay coefficients of variation were 2.1% and 3.1%, respectively. We also measured serum total cholesterol, LDLc, HDLc, triglyceride, and creatinine levels by an automated analyzer (BM6070; Japan Electron Optics Laboratory, Tokyo, Japan). Plasma glucose was measured by the glucose oxidase method, and HbA1c was measured by high-performance liquid chromatography [28].

2.6. Statistical analysis

Simple linear correlations were calculated by determining Pearson's correlation coefficients with Bonferroni correction to compare glucose variability and markers of diabetic control between quartiles of d-ROMs. Multiple stepwise regression analysis, adjusted for subject characteristics, was performed to explore the effects of different variables between the d-ROMs test values and glucose variability as well as to investigate the influence of different variables on d-ROMs test values. The level of significance was determined as $p < 0.05$. Analyses were performed using IBM SPSS, Version 22, for Windows (IBM Corp., Armonk, NY). Data are expressed as means ± standard deviations.

3. Results

3.1. Clinical characteristics

Clinical and laboratory characteristics of the 68 participants are shown in Table 1, and their treatments are summarized in Table 2. The study group included more men than women, and men were slightly overweight. Most participants ($n = 64$) were treated with oral glucose-lowering agents or insulin.

$$MAGE = \frac{\lambda > 1SD}{n} \quad n = Count (\lambda)$$

Fig. 1 – Graphical illustration of mean amplitude of glycemic excursions (MAGE).

3.2. Relationship of d-ROMs with glucose metabolism variables and non-glycemic clinical and laboratory variables

Table 3 shows the correlations between glucose metabolic variables and d-ROMs. Significant correlations were observed between d-ROMs and MGL ($r = 0.330$; $p = 0.006$), HbA1c ($r = 0.326$; $p = 0.007$), MAGE ($r = 0.565$; $p < 0.001$), MODD ($r = 0.488$; $p < 0.001$), and AUC_{PP} ($r = 0.254$; $p = 0.037$). However, d-ROMs did not correlate with FPG. Among the non-glycemic variables, there was a significant correlation between d-ROMs and LDLc ($r = 0.282$; $p = 0.020$).

Table 4 shows that MGL, HbA1c, and MAGE levels were significantly higher in the second to fourth quartiles than those in the first quartile of d-ROM values. MAGE levels increased with increasing quartile to a maximum of 130.0 ± 30.5 mg/dL, whereas MGL and HbA1c levels peaked in the third quartile (175.4 ± 35.4 mg/dL and $8.6\% \pm 1.0\%$, respectively). MODD levels were also significantly higher in the fourth quartile than those in the first quartile of the d-ROM results (MODD; fourth quartile: $p = 0.002$).

3.3. Linear regression analysis of the associations between the d-ROMs test results and glucose variability

Multivariate analyses were used to assess the effects of different clinical variables and glucose variability parameters on the activation of oxidative stress. As shown in Table 5, significant associations existed between d-ROMs and MGL, HbA1c, MAGE, MODD, and AUC_{PP} but not FPG.

Table 2 – Diabetes treatments and other treatments.

Diabetes treatment	n (%)
Diabetes therapy	
Diet alone	4 (5.9)
Metformin	17 (25.0)
Sulfonylurea	22 (32.4)
Glinide	1 (1.5)
α-glucosidase inhibitor	12 (17.6)
Thiazolidine	10 (14.7)
Dipeptidyl peptidase 4 inhibitor	31 (45.6)
Glucose-like peptide 1 receptor agonist	14 (20.6)
Insulin	24 (35.3)
Other treatments	
Lipid-lowering drugs (Statin)	35 (51.5)
Antihypertensive drugs	
Angiotensin II receptor blocker	35 (51.5)
Calcium channel blocker	22 (32.4)
Diuretic	6 (8.8)
β blocker	3 (4.4)
α blocker	10 (14.7)

Table 1 – Baseline clinical characteristics of subjects.

Clinical characteristics	Means ± SD, n (%)
Age (years)	63.2 ± 12.6
Sex (male)	45 (66.2)
Body mass index (kg/m^2)	25.2 ± 5.7
Smoking (%)	13 (19.1)
Duration of diabetes (years)	12.9 ± 10.4
Hypertension	48 (70.6)
Dyslipidemia	49 (72.1)
Blood pressure (mm Hg)	
Systolic	122.6 ± 17.2
Diastolic	72.3 ± 9.3
Low-density lipoprotein cholesterol (mg/dL)	99.5 ± 29.2
High-density lipoprotein cholesterol (mg/dL)	51.4 ± 19.0
Triglycerides (mg/dL)	130.7 ± 82.3
Estimated glomerular filtration rate (ml/min/1.73 m^2)	79.3 ± 25.8
HbA1c (%; mmol/mol)	8.1 ± 1.6 (65 ± 17)
Mean glucose level (mg/dL)	162.2 ± 40.7
Markers of glucose variability	
MAGE (mg/dL)	105.4 ± 34.9
MODD (mg/dL)	26.6 ± 9.9
Fasting plasma glucose state (mg/dL)	141.6 ± 39.9
AUCPP (mg/dL/h)	320.3 ± 246.0
d-ROMs (U.CARR)	336.0 ± 49.9
Macroangiopathy	16 (23.5)
Nephropathy	31 (45.6)
Neuropathy	43 (63.2)
Retinopathy	27 (39.7)

AUC_{PP}: the total area under the curve of the postprandial plasma glucose, d-ROMs: diacron-reactive oxygen metabolites, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursions, MGL: mean glucose level over 24 h, MODD: mean of daily difference of blood glucose.
1 U.CARR (arbitrary unit) = the oxidant capacity of a 0.08 mg/dL H_2O_2 solution.

Table 3 – Correlations between d-ROMs and markers of diabetic control and non-glycemic metabolic variables.

	FPG	MGL	HbA1c	MAGE	MODD	AUC$_{PP}$	HDL-C	LDL-C	TG
MGL	0.764[**]								
HbA1c	0.636[**]	0.693[**]							
MAGE	0.243[*]	0.482[**]	0.443[**]						
MODD	0.370[**]	0.470[**]	0.319[**]	0.708[**]					
AUC$_{PP}$	−0.017	0.264[*]	0.292[*]	0.606[**]	0.218				
HDL-C	0.064	0.163	0.215	0.182	0.090	0.287[*]			
LDL-C	0.253[*]	0.342[**]	0.479[**]	0.323[**]	0.226	0.358[**]	0.209		
TG	0.282[*]	0.243[*]	0.199	0.185	0.272[*]	−0.039	−0.440[**]	0.178	
d-ROMs	0.213	0.330[**]	0.326[**]	0.565[**]	0.488[**]	0.254[*]	0.129	0.282[*]	0.096

FPG: fasting plasma glucose, MGL: mean glucose level over 24 h, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursions, MODD: mean of daily difference of blood glucose, AUC$_{PP}$: the AUC of the postprandial glucose, HDL-C: high-density lipoprotein cholesterol, LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, d-ROMs: diacron-reactive oxygen metabolites.

[*] $p < 0.05$.
[**] $p < 0.01$.

3.4. Independent effect of markers of diabetic control on d-ROMs test results

Finally, we performed multiple linear regression analyses with MGL, HbA1c, AUC$_{PP}$, MAGE, and MODD. In the univariate analysis, a strong correlation was observed between MAGE and MODD ($r = 0.708$, $p < 0.001$, Fig. 3); therefore, we designed two independent models: model 1 included MAGE and model 2 included MODD. As shown in Table 6, the substitution of MODD by MAGE resulted in a significant increase in the coefficient of determination (multiple R^2) from 0.226 to 0.309 (i.e., an increase of 26.9%).

4. Discussion

To the best of our knowledge, there have been no investigations on the relationship between oxidative stress and day-to-day glucose variability in patients with T2DM. This study is, therefore, the first to demonstrate that oxidative stress is associated with not only daily but also day-to-day glucose variability in patients with T2DM; both variabilities correlated with oxidative stress when compared with sustained hyperglycemia. Our findings are clinically relevant and indicate that therapies should now be evaluated for their potential to minimize glucose variability and the resulting oxidative stress.

Previous clinical studies have reported that daily glucose variability increased oxidative stress in patients with T2DM [23,29]. In in vitro studies with human endothelial cells, it has been reported that intermittent high glucose levels stimulated ROS overproduction and increased cellular apoptosis when compared with a stable high-glucose environment [30]. Experiments in animals also support the hypothesis that glucose variability results in endothelial damage [31]. Azuma et al. showed that repeated glucose variability resulted in significant induction of monocyte–endothelial adhesion compared with sustained hyperglycemia [32]. Blood glucose variability likely accelerated macrophage adhesion to endothelial cells and promoted the formation of fibrotic arteriosclerotic lesions. The same group also showed that reducing glucose "swings" was associated with a significant decrease in monocyte–endothelial adhesion [33,34]. In

Table 4 – Glucose variability and markers of diabetic control according to d-ROMs test results of quartile.

	First quartile	Second quartile	Third quartile	Fourth quartile	p
d-ROMs (U.CARR)	279.9 (245–300)	315.8 (301–329)	342.3 (330–360)	405.8 (361–459)	
N	17	17	17	17	
FPG (mg/dL)	125.8 ± 34.6	148.7 ± 36.2	149.7 ± 41.5	142.4 ± 45.3	0.274
MGL (mg/dL)	133.7 ± 26.9	173.5 ± 46.7[*]	175.4 ± 35.4[*]	166.4 ± 39.7	0.006
HbA1c (%; mmol/mol)	6.9 ± 1.5; (52 ± 17)	8.5 ± 1.9; (69 ± 21[*])	8.6 ± 1.0; (71 ± 11[**])	8.6 ± 1.4; (70 ± 15[*])	0.003
MAGE (mg/dL)	73.7 ± 16.5	106.1 ± 30.9[*]	111.7 ± 34.3[**]	130.0 ± 30.5[**]	<0.001
MODD (mg/dL)	21.7 ± 6.4	24.9 ± 9.1	26.2 ± 7.2	33.5 ± 12.5[**]	0.003
AUCpp (mg/dL/h)	187.7 ± 188.6	348.9 ± 286.0	354.6 ± 250.8	389.8 ± 246.0	0.073

Specific values when comparing with the first quartile are as follows: MGL: second quartile: $p = 0.019$, third quartile: $p = 0.012$; HbA1c: second quartile: $p = 0.020$, third quartile: $p = 0.007$, fourth quartile: $p = 0.011$; and MAGE: second quartile: $p = 0.010$, third quartile: $p = 0.002$, fourth quartile: $p < 0.001$.
AUC$_{PP}$: the total area under the curve of the postprandial plasma glucose, d-ROMs: diacron-reactive oxygen metabolites, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursions, MGL: mean glucose level over 24 h, MODD: mean of daily difference of blood glucose.

[*] $p < 0.05$; comparison to first quartile.
[**] $p < 0.01$; comparison to first quartile.

Table 5 – Linear regression analysis for evaluating associations between d-ROMs test results and glucose variability.

	Dependent variables: d-ROMs (U.CARR)			
	β coefficient	t value	p value	Full-model R^2
(1) FPG				
		0.767	0.446	0.267
(2) MGL				
	0.276	2.595	0.012[*]	0.268
(3) HbA1c				
	0.253	2.585	0.012[*]	0.268
(4) MAGE				
	0.480	4.972	<0.001[**]	0.417
(5) MODD				
	0.423	4.087	<0.001[**]	0.314
(6) AUCpp				
	0.409	3.896	<0.001[**]	0.347

AUC$_{PP}$: the total area under the curve of the postprandial plasma glucose, d-ROMs: diacron-reactive oxygen metabolites, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursions, MGL: mean glucose level over 24 h, MODD: mean of daily difference of blood glucose.
Adjusted for sex (female), age, duration of diabetes, smoking habit (current), insulin use, statin use, angiotensin II receptor blocker use, body mass index, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, estimated glomerular filtration rate, systolic blood pressure.
[*] $p < 0.05$.
[**] $p < 0.01$.

Table 6 – Independent effect of markers of diabetic control on d-ROMs test results.

	Dependent variables: d-ROMs (U.CARR.)			
	β coefficient	t value	p value	Full-model R^2
Model 1			<0.001[*]	0.309
MGL		0.645	0.521	
HbA1c		0.829	0.410	
AUC$_{PP}$		−1.092	0.279	
MAGE	0.565	5.560	<0.001[*]	
Model 2			<0.001[*]	0.226
MGL		1.062	0.292	
HbA1c		1.699	0.094	
AUC$_{PP}$		1.422	0.160	
MODD	0.488	4.540	<0.001[*]	

AUC$_{PP}$: the total area under the curve of the postprandial plasma glucose, d-ROMs: diacron-reactive oxygen metabolites, FPG: fasting plasma glucose, HbA1c: hemoglobin A1c, MAGE: mean amplitude of glycemic excursions, MGL: mean glucose level over 24 h, MODD: mean of daily difference of blood glucose.
[*] $p < 0.01$.

humans, data show that repeated glucose variation increases the levels of proinflammatory cytokines in normal subjects and induces endothelial dysfunction in both normal subjects and patients with T2DM [35].

Although the relationship between oxidative stress and day-to-day glucose variability in patients with T2DM has never been reported, Chang et al. demonstrated that variability in HbA1c, which reflects longer-term glucose variability, is associated with oxidative stress in these patients [15]. In addition, they reported a strong correlation between daily glucose and HbA1c variability ($r = 0.730$); this was consistent with our results that show a strong correlation between daily and day-to-day glucose variability ($r = 0.703$). Thus, short- (daily and day-to-day glucose variability) and long-term glucose variabil-
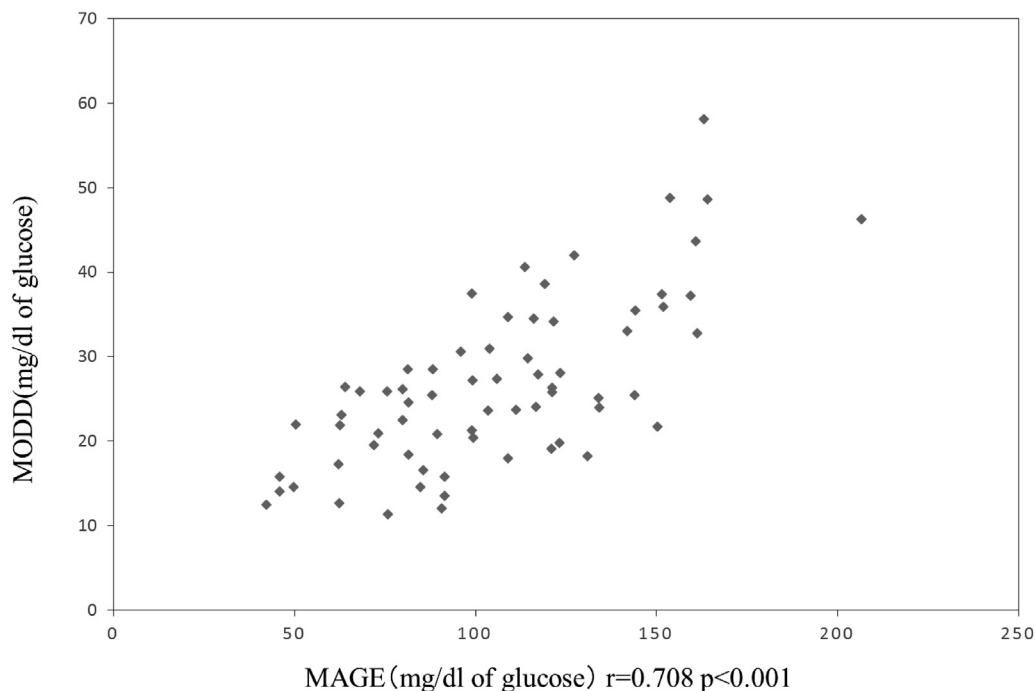

Fig. 3 – Correlation between mean of daily difference of blood glucose (MODD) and mean amplitude of glycemic excursions (MAGE).

ity (HbA1c and FPG variability) may be associated. Therefore, acute and chronic variability in blood glucose levels appear to contribute to increased oxidative stress.

In patients with T2DM, glycemic control has previously been associated with d-ROMs [20], but no reports have indicated a relation between oxidative stress and glucose variability using this test. Although other oxidative stress markers reflect *in vivo* metabolites produced by reactive oxygen, the d-ROMs test reflects the plasma concentration of all free radicals, as validated against the gold standard method of electron spin resonance spectrometry [36]. We showed that the d-ROMs test, with other markers of oxidative stress, correlated with glucose variability in patients with T2DM.

Monnier et al. reported that the three main components that contribute to tissue exposure in hyperglycemia are HbA1c, FPG, and PPG [37]. Elevated FPG levels have long been reported to induce oxidative damages, such as lymphocytic DNA damage, in patients with T2DM [38], but PPG is also believed to induce oxidative stress and interfere with normal endothelial function via ROS overproduction [39]. Furthermore, correlations between blood concentrations of HbA1c and antioxidant values have been reported [40]. However, we found no association between FPG and d-ROMs, with the multivariate analysis showing that MAGE and MODD were most effective in inducing oxidative stress. Although PPG has been shown to be an independent factor for risk stratification for cardiovascular events and total mortality [2,3,41], our results could explain the differences observed between the influences of daily glucose variability and PPG. Furthermore, we showed that daily glucose variability was more effective than PPG in the development of oxidative stress. Therefore, we suggest that the triggering effect of acute (daily and day-to-day) glycemic variability on oxidative stress should be investigated beyond acute post-meal spikes [42,43].

We showed that glucose variability and oxidative stress increased in tandem, but there was no corresponding decrease in glycemic control (HbA1c and MGL). Ikebuchi et al. demonstrated that glycemic control was independently associated with increasing oxidative stress levels in patients with T2DM [20], with statistically significant differences between groups with HbA1c levels of 6.2–6.8% and either 6.9–8.3% or 8.4–10.3%; however, there was no significant difference between groups with HbA1c levels of 6.9–8.3% and 8.4–10.3%. Thus, there may be a threshold above which oxidative stress occurs during poor glycemic control. Another study has reported that HbA1c levels of >7% and >6.5% were associated with increased risks of macrovascular and microvascular events, respectively [44]. The onset and progression of diabetic complications may only occur when the HbA1c exceeds 7%, corresponding with the onset of oxidative stress. Our analysis of quartiles also showed that the average HbA1c in the first quartile of d-ROMs value (the first quartile corresponds to normal values of d-ROMs) is 6.9%. This emphasizes the need to maintain HbA1c levels below 7%, with minimal glucose variability.

The present study had several limitations. First, we included inpatients and outpatients, with the possibility that diets differed between these groups. Second, this study was cross-sectional, precluding evaluation of any cause–effect relationship between glucose variability and oxidative stress.

Whether intervention aimed at reducing glucose variability should be administered needs further examination. Third, the sample size was relatively small; therefore, any subgroup comparisons may lack statistical power. Fourth, we did not measure other markers of oxidative stress or antioxidant potential for comparison. Fifth, we calculated MODD by measuring glucose levels continuously only for 2 days, as reported by Wentholt et al. [45–47]. In future, MODD should be calculated based on the absolute difference between the minimum and maximum daily glycemic variability using CGM for 7 days.

In conclusion, this is the first study to show that oxidative stress is associated with both daily and day-to-day glucose variability in patients with T2DM. Further well-designed studies are necessary to investigate not only the relationship between glucose variability and oxidative stress in patients with T2DM but also the relationship and between glucose variability and endothelial function.

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Conflicts of interest

None.

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REFERENCES

[1] UK Prospective Diabetes Study. (UKPDS) Group. 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 1998;352:837–53.

[2] Nakagami TDECODA Study Group. Hyperglycaemia and mortality from all causes and from cardiovascular disease in five populations of Asian origin. Diabetologia 2004;47:385–94.

[3] Su G, Mi S, Tao H, Li Z, Yang H, Zheng H, Zhou Y, Ma C. Association of glycemic variability and the presence and severity of coronary artery disease in patients with type 2 diabetes. Cardiovasc Diabetol 2011;10:19.

[4] Muqgen M, Zoppini G, Bonora E. Fasting plasma glucose variability predicts 10-year survival of type 2 diabetic patients: the verona diabetes study. Diabetes Care 2000;23:45–50.

[5] Xu F, Zhao LH, Su JB, Chen T, Wang XQ, Chen JF, Wu G, Jin Y, Wang XH. The relationship between glycemic variability and diabetic peripheral neuropathy in type 2 diabetes with well-controlled HbA1c. Diabetol Metab Syndr 2014;6:139.

[6] Sugawara A, Kawai K, Motohashi S, Saito K, Kodama S, Yachi Y, Hirasawa R, Shimano H, Yamazaki K, Sone H. HbA1c variability and the development of microalbuminuria in type

2 diabetes: Tsukuba Kawai Diabetes Registry 2. Diabetologia 2012;55:2128–31.

[7] Brownlee M, Hirsch IB. Glycemic variability: a hemoglobin A1c-independent risk factor for diabetic complications. JAMA 2006;295:1707–8.

[8] Borg R, Kuenen JC, Carstensen B, Zheng H, Nathan DM, Heine RJ, Nerup J, Borch-Johnsen K. Witte DR; ADAG Study Group. HbA$_1$(c) and mean blood glucose show stronger associations with cardiovascular disease risk factors than do postprandial glycaemia or glucose variability in persons with diabetes: the A1C-Derived Average Glucose (ADAG) study. Diabetologia 2011;54:69–72.

[9] Siegelaar SE, Kerr L, Jacober SJ, Devries JH. A decrease in glucose variability does not reduce cardiovascular event rates in type 2 diabetic patients after acute myocardial infarction: a reanalysis of the HEART2D study. Diabetes Care 2011;34:855–7.

[10] Kilpatrick ES, Rigby AS, Atkin SL. A1C variability and the risk of microvascular complications in type 1 diabetes: data from the diabetes control and complications trial. Diabetes Care 2008;31:2198–202.

[11] Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;19:257–67.

[12] Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. Diabetes 2005;54:1615–25.

[13] Ceriello A, Esposito K, Piconi L, Ihnat MA, Thorpe JE, Testa R, Boemi M, Giugliano D. 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.

[14] Dalle-Donne I, Rossi R, Colombo R, Giustarini D, Milzani A. Biomarkers of oxidative damage in human disease. Clin Chem 2006;52:601–23.

[15] Chang CM, Hsieh CJ, Huang JC, Huang IC. Acute and chronic fluctuations in blood glucose levels can increase oxidative stress in type 2 diabetes mellitus. Acta Diabetol 2012;49: S171–7.

[16] Nakhjavani M, Ghazizadeh Z, Nargesi AA, Mokhtari A, Asgarani F, Imani M, Davoudi Z, Esteghamati A. Prostaglandin F2 alpha plasma concentration predicts glycemic control and oxidation status in patients with type 2 diabetes mellitus. Clin Lab 2014;60:2073–80.

[17] Gerardi G, Usberti M, Martini G, Albertini A, Sugherini L, Pompella A, Di Lorenzo D. Plasma total antioxidant capacity in hemodialyzed patients and its relationship to other biomarkers of oxidative stress and lipid peroxidation. Clin Chem Lab Med 2002;40:104–10.

[18] Vassalle C, Boni C, Di Cecco P, Landi P. Elevated hydroperoxide levels as a prognostic predictor of mortality in a cohort of patients with cardiovascular disease. Int J Cardiol 2006;110:415–6.

[19] Vassalle C, Bianchi S, Battaglia D, Landi P, Bianchi F, Carpeggiani C. Elevated levels of oxidative stress as a prognostic predictor of major adverse cardiovascular events in patients with coronary artery disease. J Atheroscler Thromb 2012;19:712–7.

[20] Ikebuchi M, Nishio Y, Maegawa H, Kashiwagi A. Effects of hyperglycemia on oxidative stress and antioxidant potential in patients with type 2 diabetes. Diabetol Int 2010;1:72–7.

[21] Sekhar RV, McKay SV, Patel SG, Guthikonda AP, Reddy VT, Balasubramanyam A, Jahoor F. Glutathione synthesis is diminished in patients with uncontrolled diabetes and restored by dietary supplementation with cysteine and glycine. Diabetes Care 2011;34:162–7.

[22] Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatine. Nephron 1976;16:31–41.

[23] Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006;295:1681–7.

[24] Service FJ, Molnar GD, Rosevear JW, Ackermann E, Gatewood LC, Taylor WF. Mean amplitude of glycemic excursions, a measure of diabetic instability. Diabetes 1970;19:644–55.

[25] Molnar GD, Taylor WF, Ho MM. Day-to-day variation of continuously monitored glycaemia: a further measure of diabetic instability. Diabetologia 1972;8:342–8.

[26] Gross JL, de Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, Zelmanovitz T. Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes Care 2005;28:164–76.

[27] Cesarone MR, Belcaro G, Carratelli M, Cornelli U, De Sanctis MT, Incandela I, Barsotti A, Terranova R, Nicolaides A. A simple test to monitor oxidative stress. Int Angiol 1999;18:127–30.

[28] Schnedl WJ, Lahousen T, Wallner SJ, Krause R, Lipp RW. Silent hemoglobin variants and determination of HbA1c with the high resolution program of the HPLC HA-8160 hemoglobin analyzer. Clin Biochem 2005;38:88–91.

[29] Zheng F, Lu W, Jia C, Li H, Wang Z, Jia W. Relationships between glucose excursion and the activation of oxidative stress in patients with newly diagnosed type 2 diabetes or impaired glucose regulation. Endocrine 2010;37:201–8.

[30] Risso A, Mercuri F, Quagliaro L, Damante G, Ceriello A. Intermittent high glucose enhances apoptosis in human umbilical vein endothelial cells in culture. Am J Physiol Endocrinol Metab 2001;281:E924–30.

[31] Azuma K, Watada H, Niihashi M, Otsuka A, Sato F, Kawasumi M, Shimada S, Tanaka Y, Kawamori R, Mitsumata M. A new En face method is useful to quantitate endothelial damage in vivo. Biochem Biophys Res Commun 2003;309:384–90.

[32] Azuma K, Kawamori R, Toyofuku Y, Kitahara Y, Sato F, Shimizu T, Miura K, Mine T, Tanaka Y, Mitsumata M, Watada H. Repetitive fluctuations in blood glucose enhance monocyte adhesion to the endothelium of rat thoracic aorta. Arterioscler Thromb Vasc Biol 2006;26:2275–80.

[33] Azuma K, Toyofuku Y, Iesaki T, Otsuka A, Tanaka A, Mita T, Hirose T, Tanaka Y, Daida H, Kawamori R, Watada H. Acarbose, an a-glucosidase inhibitor, improves endothelial dysfunction in Goto-Kakizaki rats exhibiting repetitive blood glucose fluctuation. Biochem Biophys Res Commun 2006;345:688–93.

[34] Tanaka A, Azuma K, Toyofuku Y, Kurokawa A, Otsuka A, Mita T, Hirosea T, Fujitani Y, Miyauchi K, Daida H, Kawamori R, Watada H. Insulin and nateglinide reduce monocyte adhesion to endothelial cells in Goto-Kakizaki rats exhibiting repetitive blood glucose fluctuation. Biochem Biophys Res Commun 2006;350:195–201.

[35] Esposito K, Nappo F, Marfella R, Giugliano G, Giugliano F, Ciotola M, Quagliaro L, Ceriello A, Giugliano D. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: role of oxidative stress. Circulation 2002;106:2067–72.

[36] Alberti A, Bolognini L, Macciantelli D, Carratelli M. The radical cation of N, N-diethyl-para-phenylendiamine: a possible indicator of oxidative stress in biological samples. Res Chem Intermed 2000;26:253–67.

[37] Monnier L, Colette C, Boniface H. Contribution of postprandial glucose to chronic hyperglycemia: from the 'glucose triad' to the trilogy of 'sevens'. Diabetes Metab 2006;32. 2S12–7.

[38] Choi SW, Benzie IF, Lam CS, Chat SW, Lam J, Yiu CH, Kwan JJ, Tang YH, Yeung GS, Yeung VT, Woo GC, Hannigan BM, Strain JJ. Inter-relationships between DNA damage, ascorbic acid and glycaemic control in Type 2 diabetes mellitus. Diabet Med 2005;22:1347–53.

[39] Piconi L, Quagliaro L, Ceriello A. Oxidative stress in diabetes. Clin Chem Lab Med 2003;41:1144–9.

[40] Annadurai T, Vasanthakumar A, Geraldine P, Thomas PA. Variations in erythrocyte antioxidant levels and lipid peroxidation status and in serum lipid profile parameters in relation to blood haemoglobin A1c values in individuals with type 2 diabetes mellitus. Diabetes Res Clin Pract 2014;105 (1):58–69.

[41] Cavalot F, Pagliarino A, Valle M, Di Martino L, Bonomo K, Massucco P, Anfossi G, Trovati M. Postprandial blood glucose predicts cardiovascular events and all-cause mortality in type 2 diabetes in a 14-year follow-up: lessons from the San Luigi Gonzaga. Diabetes Care 2011;34:2237–43.

[42] Chiasson JL, Josse RG, Gomis R, Hanefeld M, Karasik A. Laakso M; STOP-NIDDM Trial Research Group. Acarbose treatment and the risk of cardiovascular disease and hypertension in patients with impaired glucose tolerance: the STOP-NIDDM trial. JAMA 2003;290:486–94.

[43] Monnier L, Colette C, Rabasa-Lhoret R, Lapinski H, Caubel C, Avignon A, Boniface H. Morning hyperglycemic excursions: a constant failure in the metabolic control of non-insulin-using patients with type 2 diabetes. Diabetes Care 2002;25:737–41.

[44] Zoungas S, Chalmers J, Ninomiya T, Li Q, Cooper ME, Colagiuri S, et al. Association of HbA1c levels with vascular complications and death in patients with type 2 diabetes: evidence of glycaemic thresholds. Diabetologia 2012;55:636–43.

[45] Wentholt IM, Kulik W, Michels RP, Hoekstra JB, DeVries JH. Glucose fluctuations and activation of oxidative stress in patients with type 1 diabetes. Diabetologia 2008;51:183–90.

[46] Chen T, Xu F, Su JB, Wang XQ, Chen JF, Wu G, Jin Y, Wang XH. Glycemic variability in relation to oral disposition index in the subjects with different stages of glucose tolerance. Diabetol Metab Syndr 2013;5:38.

[47] Su JB, Chen T, Xu F, Wang XQ, Chen JF, Wu G, Jin Y, Wang XH. Glycemic variability in normal glucose regulation subjects with elevated 1-h postload plasma glucose levels. Endocrine 2014;46:241–8.