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Improving in the Fasting, but Not the Postprandial, Glucose Level is Associated with Reduction of Plasma d-ROMs Level in Patients with Type 2 Diabetes

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Abstract

Aims: This study aimed to evaluate the relationship between improvement of glucose metabolism and plasma levels of diacron-reactive oxygen metabolites (d-ROMs) in patients with type 2 diabetes.

Methods: As the first daily profile, the plasma levels of glucose and d-ROMs were determined on admission. Then, after treatment to lower plasma glucose levels, the second daily profile of these levels was evaluated. Fasting plasma glucose (FPG), the total area under the curve (AUC) of the daily plasma glucose profile (AUC_{DP}), the AUC of the postprandial plasma glucose levels (AUC_{PP}), the AUC of the daily plasma d-ROMs profile ($AUC_{d\text{-}ROMs}$), the coefficient of variation (CV) of plasma glucose (CV_{PG}), and the mean amplitude of glycemic excursions (MAGE) were calculated. The relationship between the improvement of glucose metabolism and that of oxidative stress in patients with type 2 diabetes was evaluated.

Results: The second determinations of FPG, AUC_{DP}, AUC_{PP}, MAGE, and $AUC_{d\text{-}ROMs}$ were significantly lower than those of the first determinations, but no significant difference was observed in CV_{PG}. Linear regression analysis demonstrated significant associations between the changes in $AUC_{d\text{-}ROMs}$ and the changes in both FPG and AUC_{DP}, whereas no significant association was observed between the change in $AUC_{d\text{-}ROMs}$ and the change in AUC_{PP}, CV_{PG}, or MAGE.

Conclusions: This study has demonstrated that improvement of the FPG level, but not of the postprandial glucose level, is associated with a reduction of the plasma level of d-ROMs in patients with type 2 diabetes.

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Key words: oxidative stress, diacron-reactive oxygen metabolites, type 2 diabetes, daily profile of plasma glucose, and postprandial glucose

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Introduction

Hyperglycemia is the main risk factor for microvascular complications and accelerates atherosclerosis in diabetes[1,2]. In many mechanisms of the development and progression of diabetic complications, especially hyperglycemia-induced complications, oxidative stress plays an important role. Hyperglycemic damage results from reactive oxygen species (ROS)-induced activation of polyol, hexosamine, protein kinase C, and the advanced glycation endproduct pathway[3]. Oxidative stress has been implicated in the premature development of atherosclerosis associated with risk factors, such as diabetes and smoking[4–6]. Also, oxidative stress develops from hyperglycemia and induces β-cell dysfunction[7] and insulin resistance[8], which, in turn, cause further hyperglycemia in a vicious circle in which oxidative stress plays a central role. Furthermore, oxidative stress is related to the development of many diseases, such as heart failure[9] and cancer[10]. Therefore, reducing oxidative stress in patients with diabetes has an important role in the preventing not only diabetic complications, but also many diseases induced by oxidative stress. Many studies have reported associations between glycemic control and oxidative stress and between the reduction of oxidative stress and the improvement of glycemic control[11–14]. However, because previous studies have yielded conflicting results regarding a link between glucose variability and oxidative stress[15,16], any relationship between oxidative stress and glucose variability remains uncertain.

Recently, the serum level of diacron-reactive oxygen metabolites (d-ROMs) has been reported to be a reliable biomarker for quantifying oxidative status by measuring the hydroperoxidation of organic compounds[17,18]. Furthermore, the d-ROMs assay can be used in clinical settings because it is inexpensive and can be performed easily in 5 to 7 minutes.

The purpose of the present study was to examine the association between glucose variability and the serum level of d-ROMs by improving glycemic control in patients with type 2 diabetes.

Materials and Methods

Study Patients

The subjects were 47 inpatients with type 2 diabetes treated by the Divisions of Cardiology, Hepatology, Geriatrics, or Integrated Medicine, Department of Internal Medicine, Nippon Medical School Hospital. Patients were excluded if they used corticosteroids or nonsteroidal anti-inflammatory drugs, had an acute illness within 3 months before the start of the study, or had an estimated glomerular filtration rate of less than 50 mL/min/1.73 m^2 according to the Cockcroft-Gault formula, because of possible effects on oxidative stress production. The inclusion criteria were a diagnosis of type 2 diabetes mellitus for more than 6 months. All subjects had received a diagnosis of diabetes because they met at least 1 of the following criteria: 1) fasting plasma glucose (FPG) level of ≥126 mg/dL (≥7.0 mmol/L); 2) 2-hour value of ≥200 mg/dL (≥11.1 mmol/L) on the 75-g oral glucose tolerance test; or 3) casual plasma glucose level of ≥200 mg/dL (≥11.1 mmol/L)[19]. Patients were considered to have hypertension if they were being treated for hypertension or had a blood pressure ≥140/90 mm Hg measured in a hospital, on the basis of the Japanese Society of Hypertension Committee for Guidelines for the Management of Hypertension[20]. Blood pressure was measured in the sitting position in the morning after an overnight fast. We briefly recorded several background factors of 47 subjects including age, sex, body-mass index, smoking habit, duration of diabetes, statin use, and treatment of diabetes on admission. The patients were enrolled consecutively without any HbA1c level-based selection (range, 6.1%–18.5%; mean [SD], 9.67% ± 2.27%). The baseline clinical and laboratory characteristics of the patients are shown in **Table 1**.

Before the start of the study, written informed consent was obtained from all subjects after they had received a clear explanation of the study protocol. The study was designed in compliance with the ethics regulations set out by the Declaration of Helsinki.

Table 1 Baseline clinical characteristics of study subjects

Clinical characteristics	Means ± SD, n (%)	Range
Age (years)	64.5 ± 12.1	38–85
Gender (men)	25 (53.2)	
Body mass index	24.91 ± 4.54	17.1–41.1
Smoking habits		
Never	32 (68.1)	
Formerly	8 (17.0)	
Currently	7 (14.9)	
Duration of diabetes (years)	15.7 ± 9.2	1–40
Treatment of diabetes		
Diet and exercise	3 (6.4)	
Sulfonylureas	11 (23.4)	
Meglitinides	0 (0)	
α-glucosidase inhibitors	12 (25.5)	
Thiazolidindiones	11 (23.4)	
Biguanides	14 (29.8)	
DPP-4 inhibitors	6 (12.8)	
GLP-1 receptor agonists	1 (2.1)	
Insulins	30 (63.9)	
Hypertension	34 (72.3)	
Statin use	36 (76.6)	
Blood pressure (mmHg)		
Systolic	127.1 ± 14.8	98–173
Diastolic	70.5 ± 9.4	52–94
LDL-cholesterol (mmol /L)	2.76 ± 1.01	1.30–7.43
HDL-cholesterol (mmol /L)	1.33 ± 0.40	0.80–2.51
Triglycerides (mmol /L)	1.80 ± 1.42	0.56–7.44
Uric acid (μmol /L)	313.4 ± 98.5	71.4–607.1
Serum creatinine (μmol/L)	70.5 ± 40.9	29.2–318.2
A1C (%)	9.67 ± 2.27	6.1–18.5

DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like peptide-1; A1c, glycosylated hemoglobin.

Study Design

The daily profiles of plasma glucose and oxidative stress were recorded in 2 series of 10 time points each during the periods of hospitalization—in the preprandial period (8: 00, 12: 00, and 18: 00) and the postprandial period (10: 00, 14: 00, and 20: 00)—and then at 0: 00, 3: 00, 6: 00, and 8: 00 on the next day. In the first analysis, the daily measurements were made without altering the treatment, including that of diabetes, before admission. After the first analysis of the daily profile, we started to improve glycemic control by using antihyperglycemic medications, including basal-bolus insulin therapy, and the treatment of diabetes was appropriately changed during the study period to maintain steady glycemic control. The second analysis of the daily profile was performed with steady glycemic control 10 days or more after the first analysis because the glucose metabolism of study subjects appeared to be at a steady state, as previously reported[21]: the mean duration of the observation period from the first to second analyses was 13.4 ± 5.2 days. During the in-hospital observation period, a weight-maintaining diet (25–30 kcal/kg standard body weight), as previously reported, was prescribed for all patients[22]. Also, the physical activity of subjects was not altered during the observation period.

The area under the curve (AUC) for the daily profile was used to evaluate changes before and after treatment in the glycemic control of the daily profile and in postprandial glucose levels, because the contributions of fasting and postprandial hyperglycemia and the pattern of peak glucose levels of the postprandial state are changed by

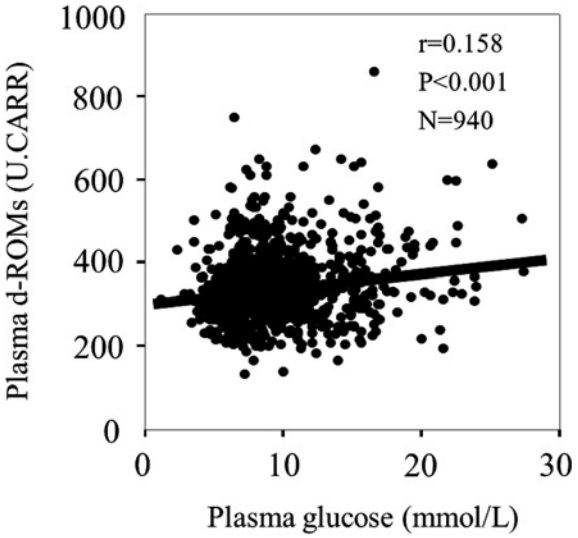


Fig. 1 Correlation between plasma glucose and d-ROMs.

worsening or improving glycemic control[23,24]. Furthermore, the change in oxidative stress was also assessed by the change in AUC for serum d-ROMs levels ($AUC_{d\text{-}ROMs}$) because significant correlations were observed between plasma levels of glucose and d-ROMs in the present subjects (**Fig. 1**). The AUC for the daily profile was measured as previously reported[22,25]. The daily profile AUC was determined with the trapezoidal rule. The AUCs for daily blood glucose (AUC_{DP}) and oxidative stress ($AUC_{d\text{-}ROMs}$) from 8.00 to 8.00 on the next day were calculated. The postprandial AUC (AUC_{PP}) was defined as the AUC greater than a baseline level equal to the 8: 00 plasma glucose value (before breakfast) from 8: 00 to 0: 00, ignoring the area below the baseline value, and was therefore considered a reflection of the postprandial glycemic responses to breakfast, lunch, and dinner[23]. The FPG was defined as the mean of plasma glucose levels determined at 3.00, 6.00, and 8.00 the next day and was assumed to be the nocturnal FPG level[23]. Glucose variability was evaluated with the coefficient of variation for the daily profile of plasma glucose (CV_{PG}). Furthermore, as a variable of postprandial glucose metabolism, the mean amplitude of glycemic excursions (MAGE) was calculated. The MAGE of each of the 2 series, each consisting of 10 time points over 24 hours, was defined with the following formula: $\Sigma\lambda/x$ (λ=extent of glycemic fluctuation exceeding 1 SD [=absolute value of the difference between variables and 1 SD when variables exceed 1 SD]; x=the number of variables showing a glycemic extent of fluctuation exceeding 1 SD)[21].

Laboratory Measurements

Activation of oxidative stress was estimated by measuring the plasma d-ROMs level as an index of products of ROS[26]. Plasma levels of d-ROMs were measured with the Free Radical Analytical System (FRAS4, Wismer LL Co., Tokyo, Japan). The d-ROMs test is a photometric method based on the radical reaction of Fenton and further elaborated in the biochemical field by Haber and Weiss[27,28]. This test, a kinetic spectrophotometric assay, measures serum levels of ROS, such as hydroperoxides, and takes advantage of the capacity of hydroperoxides to generate free radicals in the presence of transition metals, which act as catalysts. When free radicals react with a correctly buffered chromogenic substance, they develop a colored complex that is directly proportional to hydroperoxide levels. The measurement unit is the Carratelli unit (U.CARR), which corresponds to 0.08 mg/dL H_2O_2. Plasma levels of d-ROMs were measured by a single observer. The correlation coefficient for repeated measurements of plasma levels of d-ROMs was 0.966 ($P<0.001$), and the coefficient of variation was 3.2%. Concerning the relationship between plasma levels of d-ROMs and smoking habit in the present subjects, no significant difference in the $AUC_{d\text{-}ROMs}$ was observed in the 1st series between subjects with different smoking habits (8,620.7 ± 2,101.6 U.CARR for never smokers, 7,534.3 ± 652.2 U.CARR for former smokers, and 7,810.5 ± 1,675.6 U.CARR for current smokers, with the Kruskal-Wallis test).

Serum levels of total cholesterol, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, uric acid, and creatinine were measured with an automated analyzer (BM6070, Japan Electron Optics Laboratory, Tokyo, Japan). Plasma glucose was measured with the glucose oxidase method. The HbA1c (Japan Diabetes Society [JDS]) level was measured with high-performance liquid chromatography (JDS Lot3). The

Table 2 Plasma glucose and d-ROMs of the daily profiles in the 1st and 2nd series of measurements during the study period

Variables	1st series	2nd series
(1) Plasma glucose (mmol/L)		
8: 00	9.38 ± 2.95	6.91 ± 1.35***
10: 00	14.19 ± 3.62	9.77 ± 2.38***
12: 00	12.63 ± 3.91	8.06 ± 2.58***
14: 00	12.68 ± 5.31	8.22 ± 2.74***
18: 00	10.99 ± 4.08	8.24 ± 2.74***
20: 00	13.02 ± 4.73	8.59 ± 2.61***
0: 00	10.88 ± 4.29	7.63 ± 2.13***
3: 00	9.67 ± 3.83	6.90 ± 1.62***
6: 00	9.20 ± 3.09	6.73 ± 1.33***
8: 00 of the next day	9.21 ± 2.96	7.09 ± 1.56***
(2) d-ROMs (U.CARR)		
8: 00	366.5 ± 105.6	332.9 ± 76.7
10: 00	352.1 ± 84.0	332.0 ± 82.4
12: 00	348.3 ± 82.3	346.4 ± 70.9
14: 00	339.4 ± 89.9	326.8 ± 71.0
18: 00	342.3 ± 88.3	346.3 ± 76.7
20: 00	356.4 ± 96.6	343.1 ± 74.3
0: 00	339.5 ± 87.7	321.9 ± 71.0
3: 00	335.4 ± 81.8	318.6 ± 69.6
6: 00	346.9 ± 114.8	323.1 ± 75.9
8: 00 of the next day	371.7 ± 97.3	352.6 ± 76.6

mean ± SD

***p<0.001 for all measurements of plasma glucose during the 2nd series vs. those during the 1st series.

HbA1c (JDS) was transformed into A1C (National Glycohemoglobin Standardization Program [NGSP]) as follows: A1C (NGSP) (%)=HbA1c (JDS) + 0.4[29].

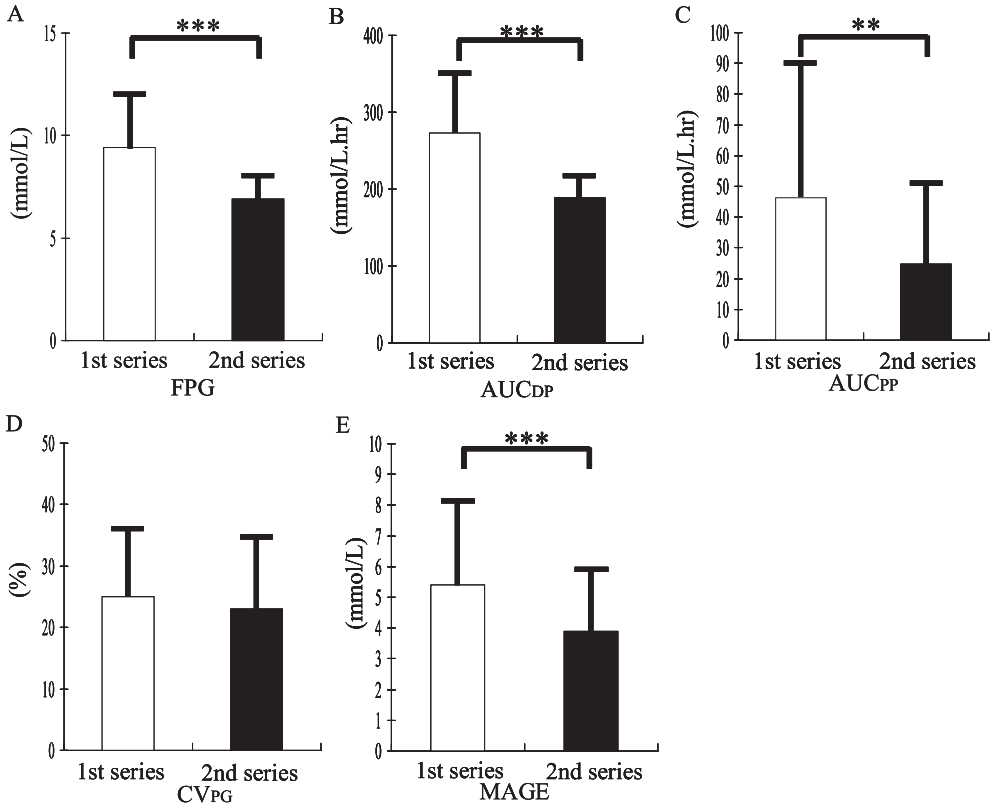
Statistical Analysis

Comparisons of clinical characteristics of study subjects at baseline and both the change in glucose metabolism variables and plasma d-ROMs were made with the Mann-Whitney U-test. Mean comparisons of AUC, CV_{PG}, MAGE, and d-ROMs between the 1st and 2nd series of measurements were made using the Wilcoxon test. Simple linear correlations between plasma levels of glucose and d-ROMs were calculated by determining the Pearson's correlation coefficient. Multiple regression models adjusted for the characteristics of subjects were used to explore the effect of different variables between the change in $AUC_{d\text{-}ROMs}$ ($\Delta AUC_{d\text{-}ROMs}$) and the changes in glucose metabolic variables. Analyses were performed with the software package SPSS version 12 for Windows (IBM SPSS Statistics, IBM Corp., Armonk, NY).

Results

Change in Glycemic Control

Plasma glucose levels at all 10 measurement points of the 2nd series were significantly lower than those of the 1st series (**Table 2**). Furthermore, values of FPG, AUC_{DP}, AUC_{PP}, and MAGE were significantly lower during the 2nd series than during the 1st series (9.36 ± 3.09 mmol/L vs 6.90 ± 1.37 mmol/L; P<0.001, 272.36 ± 81.46 mmol/L.hr vs 189.03 ± 33.57 mmol/Lhr; P<0.001, 46.17 ± 44.85 mmol/L.hr vs 24.78 ± 25.82 mmol/L.hr; P=0.006, 5.41 ± 2.80 mmol/L vs 3.85 ± 2.04 mmol/L; P<0.001) (**Fig. 2A, 2B, 2C, 2E**). However, CV_{PG} did not differ significantly between the 1st and 2nd series (**Fig. 2 D**). The change in MAGE from the 1st to 2nd series was significantly greater in subjects receiving



**P＜0.01, ***P＜0.001

Fig. 2 Comparisons of FPG, AUC, CV of plasma glucose and MAGE in the 1st and 2nd series. **A**: fasting plasma glucose, **B**: AUC of daily profile of plasma glucose. **C**: AUC of postprandial glucose, **D**: CV of plasma glucose. **E**: MAGE.

insulin treatment (n=17; 2.05 ± 2.56 mmol/L) than in subjects not receiving insulin treatment (n=30; 0.49 ± 2.36 mmol/L; P=0.023). In contrast, no significant difference was observed in the change in FPG, AUC_{DP}, AUC_{PP}, or CV_{PG} between subjects receiving insulin treatment and subjects not receiving insulin treatment.

Change in Plasma d-ROMs

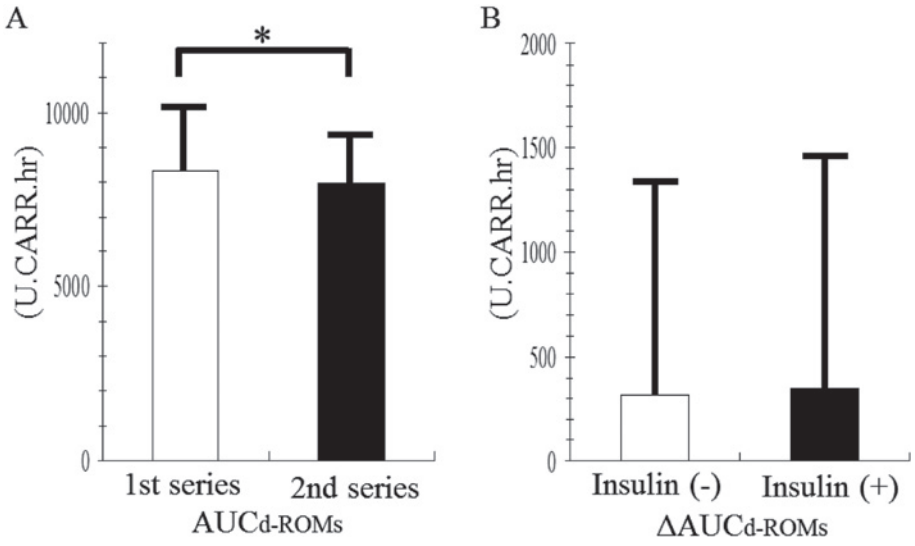
There were no significant differences between the 1st and 2nd series in plasma levels of d-ROMs in any of the 10 measurement times of the daily profile (**Table 2**). In contrast, the $AUC_{d\text{-}ROMs}$ was significantly less in the 2nd series (7,980.1 ± 1,532.1 U.CARR.hr) than in the 1st series (8,315 ± 1,901.8 U.CARR.hr; P=0.007) (**Fig. 3A**). Also, no significant difference was observed in the $\Delta AUC_{d\text{-}ROMs}$ from the 1st to 2nd series between subjects receiving and subjects not receiving insulin treatment (**Fig. 3B**).

Relationship between d-ROMs and Plasma Glucose

Significant, but weak, correlations were observed between $AUC_{d\text{-}ROMs}$ and both FPG (r=0.277; P=0.007) and AUC_{DP} (r=0.226; P=0.028), whereas no significant correlation was observed between $AUC_{d\text{-}ROMs}$ and the other glucose variables (AUC_{PP}, CV_{PG}, and MAGE) (**Table 3**).

In contrast, significant correlations were observed between $\Delta AUC_{d\text{-}ROMs}$ and both the change in FPG (ΔFPG, r=0.492; P<0.001) and the change in AUC_{DP} (ΔAUC_{DP}, r=0.384; P=0.008), whereas no significant correlation was observed between $\Delta AUC_{d\text{-}ROMs}$ and the changes in the AUC_{PP} (ΔAUC_{PP}), the CV_{PG} (ΔCV_{PG}), or the MAGE (ΔMAGE).

Significant associations were observed between $\Delta AUC_{d\text{-}ROMs}$ and both ΔFPG (β=196.470, t=4.277; P<0.001) and ΔAUC_{DP} (β=7.020, t=3.320; P=0.002) on multivariable linear regression analysis adjusted for clinical characteristics, including receiving insulin



*P＜0.05

Fig. 3 Comparison of $AUC_{d\text{-}ROMs}$ in the 1st and 2nd series (A), and the Δ $AUC_{d\text{-}ROMs}$ from the 1st to 2nd series between subjects receiving and not receiving insulin treatment (B). Δ $AUC_{d\text{-}ROMs}$: ($AUC_{d\text{-}ROMs}$ in the 1st series)-($AUC_{d\text{-}ROMs}$ in the 2nd series), Insulin (−): subjects not receiving insulin treatment, Insulin (+): subjects receiving insulin treatment.

Table 3 Correlations between glucose metabolism variables and d-ROMs

	FPG	AUC_{DP}	AUC_{PP}	CV_{PG}	MAGE
(1) $AUC_{d\text{-}ROMs}$	0.277**	0.226*	0.125	−0.078	0.081
	Δ FPG	Δ AUC_{DP}	Δ AUC_{PP}	Δ CV_{PG}	Δ MAGE
(2) Δ $AUC_{d\text{-}ROMs}$	0.492**	0.384**	−0.034	−0.262	0.138

*P<0.05, **P<0.01

Δ $AUC_{d\text{-}ROMs}$: ($AUC_{d\text{-}ROMs}$ in the 1st series)−($AUC_{d\text{-}ROMs}$ in the 2nd series)
Δ FPG: (FPG in the 1st series)−(FPG in the 2nd series)
Δ AUC_{DP}: (AUC_{DP} in the 1st series)−(AUC_{DP} in the 2nd series)
Δ AUC_{PP}: (AUC_{PP} in the 1st series)−(AUC_{PP} in the 2nd series)
Δ CV_{PG}: (CV_{PG} in the 1st series)−(CV_{PG} in the 2nd series)
Δ MAGE: (MAGE in the 1st series)−(MAGE in the 2nd series)

treatment and smoking habit (**Table 4**), whereas no association was observed between $\Delta AUC_{d\text{-}ROMs}$ and the changes in other variables of glucose variability (ΔAUC_{PP}, ΔCV_{PG} and ΔMAGE). Also, no significant association was found between $\Delta AUC_{d\text{-}ROMs}$ and either smoking habit or insulin treatment in these linear regression analysis models.

Discussion

We found an association between improving glucose metabolism and plasma levels of d-ROMs in patients with type 2 diabetes. However, no significant relationship was observed between improving plasma levels of d-ROMs and either postprandial glucose or the CV_{PG}, whereas there was a significant relationship between improving plasma levels of d-ROMs and FPG.

Several studies have established d-ROMs as a suitable marker of oxidative stress. In these studies, d-ROMs showed significant associations with cardiovascular risk factors, such as lipid metabolic variables, inflammatory markers, and metabolic syndrome[17,30,31]. Therefore, in the present study

Table 4 Linear regression analysis for evaluating associations between Δ $AUC_{d\text{-}ROMs}$ and changes of glucose metabolism variables.

	Dependent variables: Δ $AUC_{d\text{-}ROMs}$ (U.CARR.hr)			
	β co-efficient	95%CI	t-value	P-value
(1) Δ FPG				
non-adjusted	150.396	70.579–230.214	3.795	<0.001
adjusted*	196.470	103.264–89.737	4.277	<0.001
(2) Δ AUC_{DP}				
non-adjusted	4.837	1.349–8.324	2.793	0.008
adjusted*	7.020	2.723–11.317	3.320	0.002
(3) Δ AUC_{PP}				
non-adjusted	−0.821	−8.108–6.465	−0.227	0.821
adjusted*	−2.650	−13.099–7.799	−0.515	0.610
(4) Δ CV_{PG}				
non-adjusted	−24.318	−51.177–2.540	−1.824	0.075
adjusted*	−29.648	−63.858–4.563	−1.761	0.087
(5) Δ MAGE				
non-adjusted	59.949	−69.633–189.531	0.932	0.356
adjusted*	45.159	−117.651–207.969	0.564	0.577

*Adjusted for gender (women), age, BMI, smoking habit (current), duration of diabetes, hypertension, statin use in 2nd series, insulin use in 2nd series, change of LDL and UA from 1st series, and serum creatinine.

plasma levels of d-ROMs were used to evaluate the relationship between oxidative stress and glucose metabolism.

First, we assumed that plasma levels of d-ROMs are significantly related to postprandial glucose metabolism because the glycemic control level of our subjects was similar to that in the study of Monnier et al., which showed an association between the 24-hour urinary excretion rate of free 8-iso prostaglandin F_2 and the MAGE in the continuous glucose monitoring system[15]. However, our study found no evidence of association between plasma levels of d-ROMs and either the postprandial glucose level (AUC_{PP} and MAGE) or the glucose fluctuation (CV_{PG}).

Postprandial hyperglycemia is generally believed to induce oxidative stress and to interfere with normal endothelial function by overproduction of ROS, which results in diabetic complications through several molecular mechanisms[3,32,33]. Fluctuating glucose levels can be more deleterious to endothelial function and can generate more oxidative stress than can nonfluctuating glucose in healthy subjects and in patients with type 2 diabetes[15,34]. There is strong epidemiological evidence that postchallenge/postprandial plasma glucose levels independently predict cardiovascular disease events. However, little evidence suggests that FPG levels are predictive[35,36]. In fact, several interventional studies against postprandial hyperglycemia achieved better restriction of atherosclerosis such as carotid intima-media thickness[37] and postprandial attenuation of endothelial function[38]. The Hyperglycemia and Its Effect After Acute Myocardial Infarction on Cardiovascular Outcomes in Patients With Type 2 Diabetes Mellitus (HEART2D) trial was designed to compare the effects of postprandial and fasting glycemic control by insulin administration in relation to cardiovascular outcomes in patients with type 2 diabetes after acute myocardial infarction[39]. This trial showed that the risk of future cardiovascular events did not differ between prandial treatment strategies and basal treatment strategies. These divergent results for treating fasting hyperglycemia versus postprandial hyperglycemia provide an important piece of information. Monnier et al. have shown a significant relation between glucose variability and oxidative stress in patients treated with oral hypoglycemic drugs alone[15]. In contrast, no significant association was observed between the

MAGE and 8-iso prostaglanding F_2 levels in patients with type 1 diabetes[40] or in patients with type 2 diabetes treated with insulin[21,41]. The present study also showed no significant difference in $\Delta AUC_{d\text{-}ROMs}$ from the 1st to 2nd series of measurements between subjects receiving and subjects not receiving insulin treatment, whereas the ΔMAGE from the 1st to 2nd series in subjects receiving insulin treatment was significantly greater than that in subjects not receiving insulin treatment. Furthermore, the findings of Monnier et al. are compatible with our results, because insulin injection was more frequently used (63.9%) in our study. Insulin is thought to have an antioxidant effect independent of glucose metabolism. Monnier et al. investigated whether insulin injection reduces oxidative stress independently of glycemic control in patients with type 1 or type 2 diabetes and found that the association of glucose variability and oxidative stress is weaker in patients with insulin-treated type 2 diabetes than in non-insulin-treated patients[42].

The reason for our failure to find a relationship between the reduction in $AUC_{d\text{-}ROMs}$ and the reduction in postprandial glucose levels in patients with diabetes is unclear. Two studies have evaluated the effects of reducing postprandial glucose levels and oxidative stress with insulin injection. Tanaka et al.[43] have reported that monocyte adhesion to endothelial cells in the thoracic aorta was reduced by decreasing glucose fluctuation in Goto-Kakizaki rats treated with nateglinide or insulin and that nateglinide, but not insulin, reduced the intimal thickness of the thoracic aorta. In this study, nateglinide decreased postprandial hyperglycemia and prevented prolonged hyperinsulinemia more effectively than did insulin. Tanaka et al. suggested that hyperinsulinemia prolonged by insulin administration was a significant cause of intimal thickening of the thoracic aorta and canceled the beneficial effect of reduced postprandial hyperglycemia. Furthermore, Acarbo et al.[44] have reported that continuous insulin infusion with the euglycemic and hyperglycemic clamp techniques attenuated endothelium-dependent vasodilation of the femoral and brachial arteries in healthy subjects. Furthermore, this insulin-induced attenuation of flow-mediated vasodilation was inhibited by the addition of an antioxidant (vitamin C). They concluded that modest hyperinsulinemia abrogates endothelium-dependent vasodilation in large conduit arteries, probably by increasing oxidant stress. Thus, prolonged postprandial hyperinsulinemia might be a significant determinant of oxidative stress, which cancels out the beneficial effect of reduced postprandial hyperglycemia.

The glycemic control level of the present subjects provides a second important piece of information. Fasting hyperglycemia contributes more to overall diurnal hyperglycemia in patients with type 2 diabetes as glycemic control worsens[24]. Our subjects had high HbA1c levels (mean, 9.67%) at baseline. Furthermore, elevated FPG levels have been reported to induce oxidative damage, such as lymphocytic DNA damage, in patients with type 2 diabetes[45]. Also, in the present study, a significant relationship was observed between ΔFPG and $\Delta AUC_{d\text{-}ROMs}$ but not between ΔAUC_{PP} and $\Delta AUC_{d\text{-}ROMs}$. Hence, a significant decrease in the FPG level, but not in the postprandial glucose level, might have contributed to the reduction in $AUC_{d\text{-}ROMs}$ in the present study.

The present study had several limitations. The daily profile of plasma levels of d-ROMs in the 2nd series of measurements, excluding that at 18: 00, were lower, but not significantly so, than those in the 1st series, whereas $AUC_{d\text{-}ROMs}$ in the 2nd series was significantly lower than that in the 1st series. These results might be attributed to the length of the observation period. The observation period of the present study was determined on the basis of protocols of several studies that evaluated the relationship between oxidative stress and glucose variability in patients with diabetes[21,41]. In contrast, several interventional studies in which plasma levels of d-ROMs were used to assess the effect of treatment on oxidative stress required several months to evaluate the significant reduction of d-ROMS[46–48]. The reduction in the daily profile of plasma d-ROMs in the present study seems to be slight because the observation period was shorter than in other interventional studies. Further longitudinal studies are needed to investigate the

long-term effect of glycemic control on oxidative stress. Also, the present study could not assess the relationship between the change in glucose metabolism or other oxidative-stress variables, such as thiobarbituric acid reactive substances[49], oxidized phosphatidylcholine[50], and advanced glycation endproducts[51]. Thiobarbituric acid reactive substances and phosphatidylcholine reflect oxidative stress induced by lipid metabolism, and advanced glycation endproducts are believed to be related to postprandial hyperglycemia[51]. In contrast, the plasma d-ROMs level reflects the plasma concentration of all free radicals. Hence, to confirm the results of the present study, the relationship between glucose metabolism and oxidative stress should be evaluated with other oxidative stress variables.

In conclusion, this study has demonstrated that oxidative stress is reduced by the improvement in glucose metabolism in patients with type 2 diabetes as evidenced by the degree of AUC d-ROMs. Improvement in the FPG level, but not the postprandial glucose level, is associated with a reduction in plasma d-ROMs levels in patients with type 2 diabetes. Additional observation over an extended time and in a greater number of patients will provide more meaningful evidence.

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