Original Article

Correlation between the Amplitude of Glucose Excursion and the Oxidative/ Antioxidative System in Subjects with Different Types of Glucose Regulation*

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Abstract

Objective To investigate effects of glucose excursion on the oxidative/antioxidative system in subjects with different types of glucose regulation.

Methods A total of 30 individuals with normal glucose regulation (NGR), 27 subjects with impaired glucose regulation (IGR) and 27 subjects with newly diagnosed type 2 diabetes mellitus (T2DM) were selected and recruited for 3 days' continuous glucose monitor system (CGMS) assessment. The data from CGMS was used to calculate the mean amplitude of glycemic excursion (MAGE), mean blood glucose (MBG) and its standard deviation (SDBG), area under the ROC curve when the blood glucose >5.6 mmol/L within 24 h (AUC 5.6), mean of daily differences (MODD), and mean postprandial glucose excursion (MPPGE). In all groups, the content or activity of malondialdehyde (MDA), total antioxidation capacity (TAOC) and glutathione peroxidase (GSH-Px) were detected.

Results Glucose excursion parameters of subjects with T2DM or IGR were higher than those of NGR subjects (P<0.05 or 0.01). Moreover, Glucose excursion parameters of T2DM subjects were higher than those of IGR subjects (P<0.05 or 0.01). Subjects with T2DM or IGR had significant higher MDA levels and lower GSH-Px/MDA and TAOC/MDA levels compared to NGR subjects (P<0.01). T2DM subjects had even higher MDA levels and lower GSH-Px/MDA levels than IGR (P<0.05 or 0.01). According to the median of normal population for MAGE, T2DM and IGR subjects were divided into MAGE>2.6mmol/L Group and MAGE≤2.6mmol/L Group. MAGE>2.6mmol/L Group had higher levels of MDA and lower levels of GSH-Px/MDA than MAGE≤2.6mmol/L Group (P<0.05). There was no significant difference between the two groups (P>0.05) in terms of the levels of TAOC/MDA. Pearson correlation analysis showed that MDA was positively correlated with FPG, 2hPG, MAGE, and SBP. GSH-Px/MDA was negatively correlated with MAGE and TC. TAOC/MDA was negatively correlated with FPG. Partial correlation analysis showed that the relationship between MDA and MAGE, GSH-Px/MDA, and MAGE remained significant after adjustments for the other differences among groups.

Conclusion Glucose excursion contributed significantly to promoting lipid peroxidation and decreasing antioxidation capacity than chronic sustained hyperglycemia did in the subjects with different types of glucose regulation.

Key words: Glucose excursion; Oxidative stress; Total antioxidant capacity; Malondialdehyde; Glutathione peroxidase

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INTRODUCTION

Diabetes is characterized by development of specific microvascular complications and a high incidence of accelerated atherosclerosis. The costs of type 2 diabetes are enormous with attendant risks of cardiovascular disease, blindness, kidney disease, and neuropathy. It is estimated based on data from the American Diabetes Association that in 2007, the total cost of diabetes in the United States was $174 billion[1]. Early intensive treatment in diabetes patients can achieve long-term beneficial effects on the risk of complications. The most commonly used parameter to show the long-term glucose control is hemoglobin A1c protein (HbA1c). However, it can't reflect the frequency and magnitude of glycemic variability[2]. Glycemic variability is an important parameter used to resolve potential clinical problems in diabetic patients. It is known that glycemic variability contributes to the development of macro- and microvascular complications in diabetes[3]. Glycemic variability was an independent risk factor for diabetic complications; it can generate more reactive oxygen species (ROS) because hyperglycemia triggers activation of oxidative stress, resulting from the overproduction of ROS by the mitochondrial electron-transport chain[4]. More recently, many *in vitro* and *in vivo* studies have shown the importance of ROS as the main mechanism of glycemic variability-induced vascular complications[5]. The aim of this study was to use the continuous glucose monitor system (CGMS) to investigate glucose excursion and the correlation between glucose excursion and the oxidative/antioxidative system in subjects with different types of glucose regulation.

SUBJECTS AND METHODS

Subjects

Based on the criteria for diagnosis and classification of diabetes (WHO 1999), subjects were divided into 3 groups: (1) 27 patients (11 women, 16 men) with newly diagnosed type 2 diabetes mellitus (T2DM) of a mean age of 47.7±11.4 years, (2) 30 individuals (15 women, 15 men) with normal glucose regulation (NGR) of a mean age of 48.5±14.7 years, (3) 27 subjects (10 women, 17 men) with impaired glucose regulation (IGR) defined as IGT (n=18), IGT and IFG (n=9) with a mean age of 54.1±10.3 years. The subjects in the study were not taking drugs with recognized antioxidative properties. They were also on a diet excluding foods with recognized antioxidative properties. Informed consent was obtained from each individual. Permission to carry out the study was granted by the Ethics Committee of the Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University.

Methods

Assessment of glycemic excursions Each participant wore a CGMS (CGMS® System Gold™, Medtronic MiniMed) for 3 days. The sensor was placed subcutaneously and secured with adhesive. Subjects were taught to use the device at the time of the first insertion and were asked to enter at least four blood glucose values per day (measured with the OneTouch® Ultra® glucose meter, LifeS-can). Subjects were asked to enter meal event markers into the CGMS and remove the device after 3 days. The following parameters were analyzed: ① 24 h mean blood glucose (MBG) and standard deviation of mean blood glucose (SDBG); ② AUC5.6: area under the ROC curve when the blood glucose >5.6 mmol/L within 24 h; ③ mean amplitude of glycemic excursion (MAGE) and mean of daily differences (MODD) (The glucose profiles obtained from continuous monitoring for 48 h. Only increases of more than 1 SD of the mean glycemic values were taken into account. Calculation of the MAGE was conducted by measuring the arithmetic mean of the differences between consecutive peaks and nadirs; MODD was calculated as the mean of the absolute differences between glucose values at the same time on two consecutive days); ④ mean of postprandial glucose excursion (MPPGE): the mean of the absolute differences between 3 h postprandial blood glucose and the corresponding 1 h preprandial glucose.

Experimental procedures All the participants of the study underwent a subjective and an objective examination. They also had their blood tested. Fast whole blood was collected by venipuncture into EDTA-lined vacutainer tubes. Plasma was obtained by centrifugation at 1 000 g for 15 min at 4 °C, separated and stored at -70 °C. The content or activity of plasma malondialdehyde (MDA), total antioxidation capacity (TAOC) and glutathione peroxidase (GSH-Px) were measured with chemocolorimetry by Commercial Kit (Nanjing Jiancheng Bioengineering Research Institute, China).

Statistical Analysis

All statistical analyses were expressed as means ± standard deviations (SD). Because of skewness, we logarithmically transformed AUC5.6 to the natural logarithm in the analyses. Multiple groups of data were analyzed by one-way ANOVA; comparisons were made by t-test for dependent samples. Pearson correlation analysis and partial correlation analysis were used to examine relationships between glucose excursion and oxidative /antioxidative system parameters. All statistical evaluations were carried out by the SPSS Version 13.0 for Windows. P values <0.05 was considered significant.

RESULTS

Clinical and Biochemical Characteristics

There were no significant differences in age and gender distribution among all groups (P>0.05). The levels of BMI, FPG, 2hPG, SBP, and DBP were significantly higher in the T2DM and IGR groups than those in the NGR group (P<0.05 or 0.01). T2DM group had higher levels of BMI, FPG, 2hPG, and HbA1c than IGR group (P<0.05 or 0.01) (Table 1).

Table 1. Demographic and Clinical Characteristics of All the Participants ($\bar{x} \pm s$)

	NGR (n=30)	IGR (n=27)	T2DM (n=27)	F Value	P Value
Mean Age	48.5±14.7	54.1±10.3	47.7±11.4	2.178	0.120
Male/Female	15/15	17/10	16/11	/	0.778
Smokers (%)	2 (6.7)	1 (3.7)	1 (3.7)	/	0.614
HbA1c (%)	ND	5.88±0.59	6.51±0.96$^{\#\#}$	/	0.005
FPG (mmol/L)	5.23±0.27	6.27±0.47**	7.54±1.62**,$^{\#\#}$	40.244	0.000
2HPG (mmol/L)	5.70±1.14	8.13±1.65**	14.78±3.82**,$^{\#\#}$	115.056	0.000
BMI(kg/m^2)	21.69±2.00	23.44±2.73*	25.17±2.54**,$^{\#}$	14.340	0.000
TC (mmol/L)	4.30±0.67	4.68±1.20	4.59±1.13	1.086	0.343
LDL-c(mmol/L)	2.28±0.61	2.56±1.07	2.32±1.00	0.784	0.460
TG (mmol/L)	1.30±0.36	1.74±0.79	2.19±1.21**	4.391	0.016
SBP (mmHg)	115.3±11.2	134.7±18.6**	130.7±18.6**	11.373	0.000
DBP (mmHg)	75.2±6.5	80.9±9.5*	82.0±10.9**	4.571	0.013

Note. Compared with NGR Group* P<0.05,** P<0.01; Compared with IGR Group $^{\#}P$<0.05, $^{\#\#}P$<0.01.

CGM Measurements and Glycemic Excursions

T2DM and IGR subjects had higher levels of MBG, SDBG, MAGE, MODD and MPPGE than NGR subjects (P < 0.05 or 0.01). T2DM subjects had higher levels of MBG, SDBG, MAGE, MODD and MPPGE than IGR subjects (P < 0.05 or 0.01) (Table 2).

Comparison of Oxidative/Antioxidative System

T2DM and IGR subjects had significant higher MDA levels and lower GSH-Px/MDA and TAOC/MDA levels compared to NGR subjects (P < 0.01). T2DM subjects had even higher MDA levels and lower GSH-Px/MDA levels than IGR subjects (P < 0.05 or 0.01). The levels of TAOC/MDA were not significantly different between the two groups (P>0.05) (Table 2) (Figure 1).

Relationships between Glycemic Excursions and Oxidative/Antioxidative System

According to the data from the Chinese Glucose Monitoring Collaborative Group[6], the median of normal population for MAGE was 2.6 mmol/L. T2DM and IGR group was therefore divided into MAGE>2.6 mmol/L Group (n=31), and MAGE $\leqslant$ 2.6 mmol/L Group (n=23). MAGE>2.6 mmol/L Group had higher levels of MDA and lower levels of GSH-Px/MDA than MAGE $\leqslant$ 2.6 mmol/L Group (P<0.05). The levels of TAOC/MDA were not significantly different between the two groups (P>0.05) (Table 3). In Pearson correlation analysis, MDA was positively correlated with FPG, 2hPG, MAGE, and SBP. GSH-Px/MDA was negatively correlated with MAGE and TC. TAOC/MDA was negatively correlated with FPG (P<0.05 or 0.01) (Figure 2). HbA1c was not correlated with MDA, GSH-Px/MDA or TAOC/MDA in T2DM and IGR subjects (P>0.05). Considering that these significant parameters could be closely correlated with each other, partial correlation analysis was performed with the adjustments for the BMI, FPG, 2hPG, and HbA1c among groups. Results from the analysis showed that the correlation between MDA and MAGE (coefficient of partial correlation=0.535, P<0.01), the correlation between GSH-Px/MDA and MAGE (coefficient of partial correlation=-0.319, P<0.05) remained significant.

DISCUSSION

Diabetes is associated with increased coronary artery, cerebrovascular and peripheral vascular disease. It is also largely responsible for blindness, amputations and end stage renal disease. For that, it is important to identify and provide treatment to this devastating disease early in its progression to postpone or even prevent the complications. Type 2 Diabetes is recognized as the etiology of over 80 percent of all diabetics and is increasing in incidence as a result of changes in human behavior. Yet, the pathological consequences of these disorders that involve both the neuronal and vascular systems are intimately linked through the pathways that mediate oxidative stress[7]. Oxidative stress, through the production of reactive oxygen species (ROS), has been proposed as the root cause underlying the development of insulin resistance, β-cell dysfunct ion, impaired glucose tolerance and type 2 diabetes. It has also been implicated in the progression of long-term diabetes complications, including microvascular and macrovascular dysfunc tionn[8]. In both type 1 and type 2 diabetes, improvement of overall blood glucose levels and glycaemic variability can exert an inhibitory effect on oxidative stress[9].

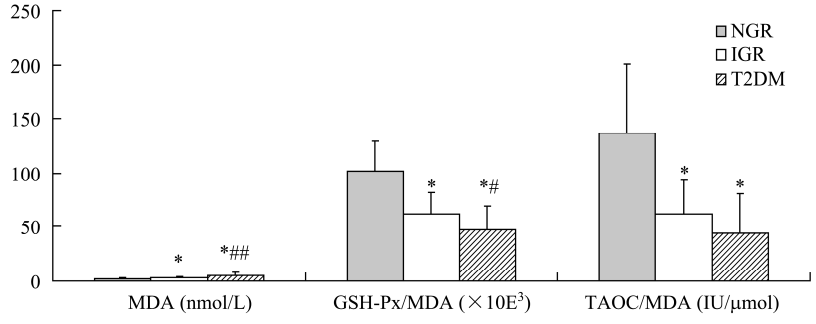

Figure 1. Oxidative/Antioxidative System Parameters of All Participants. NOTE. Compared with NGR Group *P<0.01; Compared with IGR Group $^{\#}P$<0.05, $^{\#\#}P$<0.01.

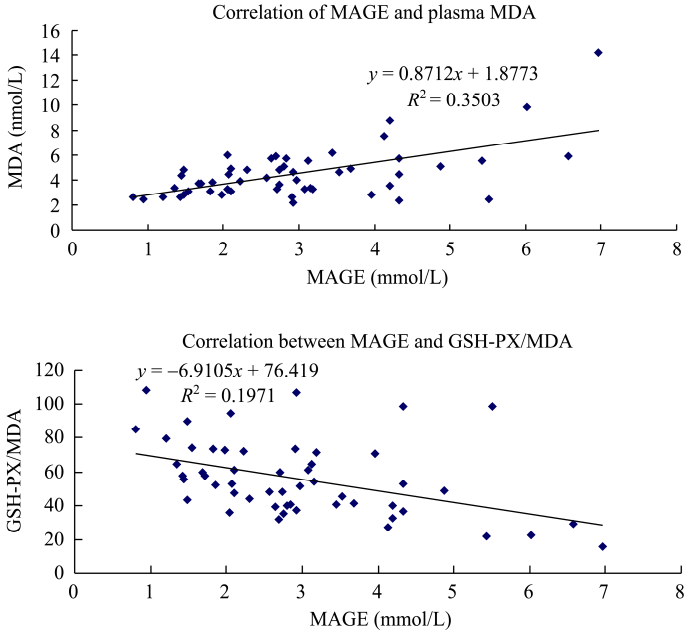

Figure 2. The relationship between MDA, GSH/MDA, and MAGE in IGR and T2DM subjects.

Table 2. Glucose Excursion and Oxidative/Antioxidative System Parameters of All Participants ($\bar{x} \pm s$)

	NGR (n=30)	IGR (n=27)	T2DM (n=27)	F Value	P Value
MBG (mmol/L)	5.67±0.52	6.76±0.62**	8.09±1.84**,##	32.136	0.000
Ln AUC 5.6(d · mmol · L^{-1})	-1.32±1.26	0.94±0.61**	0.73±0.71**,#	36.452	0.000
MAGE (mmol/L)	1.60±0.79	2.49±1.16**	3.46±1.51**,##	13.330	0.000
MODD (mmol/L)	0.68±0.27	1.07±0.46*	1.34±0.43**,##	19.842	0.000
SDBG (mmol/L)	0.80±0.35	1.20±0.52**	1.62±0.57**,##	20.253	0.000
MPPGE (mmol/L)	2.03±0.93	3.04±1.44**	4.12±1.57**,##	17.282	0.000
MDA (nmol/L)	2.31±0.56	3.56±1.01**	5.70±2.60**,##	31.739	0.000
GSH-Px/MDA (×10^3)	102.25±26.51	62.04±20.17**	46.85±22.59**,#	42.604	0.000
TAOC/MDA (IU/umol)	136.61±63.63	61.61±32.06**	44.24±36.24**	31.102	0.000

Note. Compared with NGR Group *P<0.05, **P<0.01; Compared with IGR Group #P<0.05, ##P<0.01.

Table 3. Oxidative/Antioxidative System Parameters of IGT and T2DM

	MAGE>2.6 mmol/L (n=31)	MAGE≤2.6mmol/L (n=23)	t Value	P Value
MDA (nmol/L)	5.04±2.46	3.64±0.93*	2.536	0.014
GSH-Px/MDA (×10^3)	49.75±22.53	64.88±18.30*	-2.627	0.011
TAOC/MDA (IU/μmol)	51.04±37.30	55.62±30.88	-0.472	0.639

Note. Compared with MAGE>2.6 mmol/L Group *P<0.05.

Plasma malondialdehyde, a product of lipid peroxidation, is a biomarker for oxidative stress. GSH-Px protects tissues from oxidative damage by removing peroxides resulting from free radical action. Total antioxidant capacity (TAOC) considers the cumulative effect of all antioxidants present in blood and body fluids[10]. In this study, T2DM and IGR subjects had significant higher MDA levels than NGR subjects; T2DM subjects had higher levels of MDA than IGR subjects. Other researches confirmed glucose toxicity and intensification of oxidative stress in diabetes and IGT [11-13]. Still some studies showed that diabetes patients had decreased plasma antioxidant concentration and total plasma antioxidant capacity[14-15]. In the present study, T2DM and IGR subjects had significant lower GSH-Px/MDA and TAOC/MDA levels compared to NGR subjects. T2DM subjects had even lower GSH-Px/MDA levels than IGR ones. Therefore, with the aggravation of abnormal glucose metabolism, the antioxidant capacity was relatively insufficient.

Oxidative stress can be defined as an imbalance between the oxidant and antioxidant system. Oxidative stress in diabetes is a result of the hyperproduction of reactive oxygen forms on the one hand and hypoactivity of the antioxidative system on the other. There was few data in patients with T2DM that make evident the existence of increased oxidative stress in response to glucose excursion. Several *in vitro* studies have demonstrated increased expression of markers of oxidative stress in cells exposed to fluctuating glucose concentrations[16]. Among individuals with or without diabetes, acute elevation in blood glucose during an oral glucose-tolerance test or hyperglycemic clamp increases measures of oxidative stress and lowers antioxidant concentrations in serum[17-18]. In this study, MAGE>2.6 mmol/L Group had higher levels of MDA and lower levels of GSH-Px/MDA than MAGE≤2.6 mmol/L Group. MDA was positively associated with FPG, 2hPG, MAGE, and SBP. GSH-Px/MDA was negatively associated with MAGE and TC. Further analysis showed that the relationship between MDA and MAGE, GSH-Px/MDA and MAGE remained significant after adjustments for the other differences among groups. And HbA1c was not associated with MDA, GSH-Px/MDA, and TAOC/MDA in T2DM and IGR subjects. These results showed that glucose excursion contributed significantly to promoting lipid peroxidation and decreasing antioxidation capacity than chronic sustained hyperglycemia did. Recently, it has been suggested that fluctuating blood glucose concentrations may

contribute significantly to oxidative stress even more than chronically elevated blood glucose[19].

An increased concentration of total cholesterol and triglycerides may have an influence on lowering the activity of the antioxidative system in patients[20]. However, the differences in the values of these parameters in the three groups were not statistically significant. The mechanism and relationships in the oxidative/antioxidative system of these patients would have been more comprehensible if the lipid peroxidation products concentration in erythrocytes had been determined, and this requires further and broader studies.

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