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Relationship between blood glucose fluctuation and macrovascular endothelial dysfunction in type 2 diabetic patients with coronary heart disease

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Abstract. – OBJECTIVE: To evaluate the relationship between blood glucose fluctuation and macrovascular dysfunction.

PATIENTS AND METHODS: Eighty-eight type 2 diabetes mellitus (T2DM) patients with or without coronary heart disease (CHD) and 30 healthy control subjects were recruited. Glycosylated hemoglobin A1c (HbA1c), fasting insulin (FIns), and C-reaction protein (CRP) and some other general clinical variables were measured. A 72-hour continuous glucose monitoring (CGM) and brachial artery endothelium-dependent flow-mediated dilation (FMD) assessment were performed. The glucose excursion, MAGE (mean amplitude of glycemic excursions), LAGE (largest amplitude of glycemic excursions), MPPGE (mean postprandial glycemic excursions), MODD (absolute means of daily differences), and IAUC70 (incremental area under the curve below 70 mg/dl) during the CGM were analyzed. Correlations between the various variables were analyzed.

RESULTS: Enhanced blood glucose fluctuation was observed in T2DM patients with CHD as compared to other participants. And blood glucose fluctuation was correlated with FMD, CRP and HOMA-IR.

CONCLUSIONS: Blood glucose fluctuation is an important factor that affects inflammatory response and possibly induces CHD in T2DM patients.

Key Words:
Type 2 diabetes mellitus, Coronary heart disease, Continuous glucose monitoring system, Macrovascular endothelial dysfunction, C-reactive protein.

Introduction

Coronary heart disease (CHD) is one of the most common complications of Type 2 diabetes mellitus (T2DM). Evidence-based medicine studies have shown that control of glycosylated hemoglobin A1c (HbA1c) may reduce the occurrence of microvascular complications significantly but not cardiovascular diseases. This suggests that the prevalence of macrovascular complications may not be evaluated by measuring HbA1c alone[1]. It has been reported that blood glucose fluctuation is significantly associated with complications of T2DM[2-5]. Research demonstrated by Jiao et al indicated that blood glucose fluctuation influence lower-extremity vascular disease in type 2 diabetes and related with diabetic macroangiopathy. Mean amplitude of glycemic excursion (MAGE), firstly proposed in 1970 by Service et al[5], changes the overall level of blood glucose independently. As a result, MAGE is constantly used to assess glycemic variability[7,8].

It has been demonstrated that an intermittent exposure to high glucose induces more pronounced metabolic changes and cytotoxicity than a constant exposure[8]. This is because intermittent high glucose is more effective in triggering the generation of nitrotyrosine, activating the expression of protein kinase C (PKC) and inducing the expression of adhesion molecules (ICAM-1, VCAM-1 and E-selectin) than constant high glucose[9,10]. Additionally, intermittent high glucose is capable of enhancing oxidative stress, inducing cell apoptosis as well as reducing the synthesis of vascular relaxing factor (e.g. NO) as demonstrated in cultured cells[12-14].

Changes in vessel wall shear stress induced by increased blood flow may result in NO release from vascular endothelial cells. NO subsequently activates guanylate cyclase in smooth muscle cells, leading to an elevation in cyclic guanosine monophosphate (cGMP). Eventually, brachial artery flow-mediated endothelium-dependent va-

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sodilation (FMD) occurs in the smooth muscle. Previous studies have demonstrated that brachial artery FMD is closely related to vasodilation of the coronary artery and the injury severity of the brachial artery is closely related to vasodilation of the atherosclerotic carotid and the atherosclerotic coronary, these suggesting that brachial artery FMD may be used as an indirect indicator of the coronary and systemic vascular functions[15,16].

In the present study, we observed that blood glucose fluctuation was more pronounced in T2DM patients with CHD. Glycemic fluctuation may be one of the factors that influence brachial artery FMD. An increase in C reactive protein (CRP) concentration associated with glucose fluctuation may result in the decline of brachial artery FMD.

Patients and Methods

Subjects

Type 2 diabetes mellitus (T2DM) patients aged between 50 and 70 years were consecutively admitted to the General Hospital of Beijing Military Area December 2010 to November 2011. The study protocol including screening, treatment, and data collection were approved by the Institutional Ethics Committee. Written informed consent was obtained from all subjects. The provisions of the Declaration of Helsinki were strictly followed.

Inclusion and Exclusion Criteria

Coronary arteriography was adopted if the examination performed in the last 2 months. Coronary heart disease was accordingly diagnosed if the left main artery showed ≥ 30% stenosis or at least one branch of three major coronary arteries showed ≥ 50% stenosis. The diagnosis of T2DM was adopted according to the diagnostic criteria of American Diabetes Association (ADA) in 2010. Patients should have regular diet, exercise and medication, stable hypoglycemic scheme in the last three months and no extreme blood biochemical indicators of hepatic and renal function. The exclusion criteria were: (1) acute complication of diabetes in the last six months; (2) postmenopausal women taking estrogen; (3) eye disease caused by hypertension, thromboangioitis obliterans or Takayasu arteritis (caused by the non-diabetic vascular disease) and other diseases that could affect vascular endothelial function; (4) acute coronary syndrome or acute brain damage in the last month; (5) hepatic or renal dysfunction; (6) MI (myocardial infarction), unstable angina, stroke or a transient ischaemic attack; and (7) evidence of severe hepatic or renal disease.

Demographic Data Collection

Demographic data and information on medications prior to admission were recorded. After fasting for 12 hours, 3-5 ml venous blood was sampled, mixed and placed at room temperature. Hepatic and renal function parameters and lipid metabolism indicators were determined with an automatic biochemical analyzer (Dxc600, Beckman, Fremont, CA, USA). Fasting plasma glucose (FPG) was measured by glucose oxidase method. Affinity chromatography detection was carried out on a glycated hemoglobin analyzer (D10, Bio-Rad, Hercules, CA, USA) to determine HbA1c concentration. Radioimmunoassay was used for fasting insulin (FIns) determination. C-reaction protein (CRP) in the plasma was measured using an enhanced turbidimetric immunoassay. Non-anticoagulant blood was centrifuged at 3,500 rpm for 10 min and the same indicators in the serum were detected for calibration and quality control. Insulin resistance index (IR) was calculated by the Homeostasis Model as previously described[17]: HOMA-IR=Fins (μIU/mL) × FPG (mmol/L)/22.5. Coronary angiography was performed by coronary angiography equipment (General Electric, USA).

Continuous Glucose Monitoring

Continuous glucose monitoring (CGM) was performed at least one week after coronary angiography. A CGM system (CGMS) sensor (Medtronic, Northridge, CA, USA) was inserted into all participants by the same specialized nurse at 8:00-9:00 AM on the first day of hospitalization. First CGMS calibration by finger stick blood glucose was performed 1 h after the procedure initialization. Subsequently, calibration was performed four times daily for each subject. The interval between two calibrations was not exceeding 8 hours. Events such as eating, exercise, taking hypoglycemic drugs and low blood glucose reactions that might affect blood glucose fluctuation were digitally recorded. If no abnormal CGMS situation was observed, CGM was performed for 72 consecutive hours. Data on mean blood glucose (MBG), standard deviation of blood glucose (SDBG), mean amplitude of glycemic excursions (MAGE), largest amplitude of glycemic excur-

sions (LAGE), mean postprandial glycemic excursions (MPPGE), absolute means of daily differences (MODD), incremental area under the curve below 70 mg/dl ($IAUC_{70}$), endothelium-dependent flow-mediated dilation (FMD), and C-reaction protein (CRP) were extracted and analyzed using the CGMS system solutions software (MMT-7310 Version 3.0C 3.0.128).

Brachial Artery FMD Examination

Brachial artery FMD examination was carried out following fasting or low-fat diet for 8-12 h during the course of CGM. Patients were told not to drink coffee or tea at least 2 h before the examination. Vasoactive drugs, antihypertensive drugs, nitrates and statins drugs were also avoided for at least3 days before the test. The test was performed in a quiet environment at a comfortable temperature (18 to 24°C). BP (blood pressure) was monitored during a 20 min supine rest. The process of pressurization was performed by a specialized nurse using an electric pressure pump. Ultrasonic examination was performed using a high-resolution ultrasound system (ProSound α10, Aloka, Tokjo, Japan) with a 13-Hz probe, which was placed 2-5 cm above the elbow to detect brachial artery. The depth of investigation was adjusted so that the boundaries of the vessel lumen and vessel wall were clearly distinguishable in the longitudinal section image. The baseline value of the internal diameter of the brachial artery was recorded and referred to as D_0 (mm). Reactive hyperemia was induced by rapid pressurization to 300 mmHg for 5 min. The peak value of the internal diameter of the brachial artery after releasing was recorded and referred to as D_1 (mm). FMD = (D1-D0) / D0 × 100%.

Statistical Analysis

Statistical analysis was performed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). Data were expressed as mean ± standard deviation and analyzed by Student's *t*-test, one-way ANOVA, Pearson correlation analysis or multivariate regression analysis. Results were considered significant when $p < 0.05$.

Results

A total of 88 T2DM patients were enrolled in the study. Of them, 36 (16 male, 20 female) did not have CHD and were allocated to T2DM1 group and 52 (26 male, 26 female) had CHD and were allocated to T2DM2 group. Thirty (15 male, 15 female) healthy individuals were included as control subjects (NC group).

Clinical Characteristics, CGM, FMD and CRP

In the NC group, blood glucose was within the normal range with a minor fluctuation. In contrast, significant blood glucose fluctuations were observed in the two (T2DM1 and T2DM2) disease groups. When the two disease groups were compared, a larger degree of blood glucose fluctuation was detected in the T2DM2 group than that in T2DM1 group ($p < 0.05$).

Showed in Table I are the clinical data of subjects in the 3 groups. There was no significant difference ($p > 0.05$) either in the average age (or in the use of hypoglycemic drugs between the 3 groups. No extreme blood pressure was measured in any of the subjects. Compared with the NC group, T2DM1 and T2DM2 groups had significantly higher levels of SDP, LDL-C, FBG, HbA1c, HOMA-IR, MAGE, LAGE, MPPGE, MODD, IAUC70 and CRP ($p < 0.05$), and LDL-C, FBG, HbA1c, HOMA-IR, MAGE, LAGE, MODD and CRP ($p < 0.01$). In contrast, levels of LDL-C and FMD were significantly lower in the two disease groups than in the NC group ($p < 0.01$).Compared with patients in the T2DM1 group, those in the T2DM2 group had higher levels MAGE, LAGE, MPPGE, MODD, IAUC70, CRP and lower level of FMD ($p < 0.01$). A larger degree of blood glucose fluctuation was observed in the T2DM2 group than in the T2DM1 group and the NC group.

Negative Correlation Between FDM and LAGE, MPPGE, MODD and IAUC70 in T2DM2 Patients

FDM decreased significantly in T2DM2 patients. In these patients, FDM was negatively correlated with MAGE ($p = 0.003$), LAGE ($p = 0.029$), MPPGE ($p = 0.033$), MODD ($p = 0.025$) and IAUC70 ($p = 0.042$) (Table II).

Inversely, CRP was positively correlated with MAGE ($p = 0.002$), LAGE ($p = 0.001$), MPPGE ($p = 0.010$), MODD ($p = 0.020$) and IAUC70 ($p = 0.182$); HOMA-IR was positively correlated with MAGE ($p = 0.025$), LAGE ($p = 0.013$), MPPGE and MODD ($p = 0.045$). No significant correlation between HbA1c and the blood glucose fluctuation was detected (Table II). Taken together, these observations suggest that blood

Table I. Clinical characteristics, CGMS parameters and FMD in the three study groups.

Group	NC	T2DM1	T2DM2
Age (yr)	59.30 ± 6.06	58.17 ± 5.34	61.73 ± 4.36
Duration of diabetes (yr)	–	3.68 ± 0.82	3.95 ± 0.90
Hypoglycemic drugs			
Metformin (%)	–	61.1% (22/36)	53.8% (28/52)
Acarbose (%)	–	16.7% (6/36)	25% (13/52)
Linagliptin (%)	–	5.6% (2/36)	11.5% (6/52)
TCM (%)	–	16.7% (6/36)	9.6% (5/52)
Lipid-lowering drugs			
Statins (%)		25% (9/36)	32.7% (17/52)
Bates (%)		17% (6/36)	15.4% (8/52)
Others (%)		5.6% (2/36)	7.7% (4/52)
SBP (mmHg)	108.50 ± 13.13	125.06 ± 10.74*	123.19 ± 16.74*
DBP (mmHg)	72.00 ± 11.83	71.17 ± 12.20	76.31 ± 10.67
TG (mmol/l)	1.04 ± 0.47	1.58 ± 1.11	1.91 ± 1.14**
LDL-C (mmol/l)	2.13 ± 0.17	2.86 ± 0.58**	3.03 ± 0.70**
HDL-C (mmol/l)	1.37 ± 0.15	1.02 ± 0.20**	0.91 ± 0.49**
FBG (mmol/l)	5.11 ± 0.35	8.37 ± 2.86**	9.09 ± 2.28**
HbA1c (%)	5.29 ± 0.32	7.52 ± 2.46**	7.61 ± 1.47**
HOMA-IR	0.45 ± 0.08	1.41 ± 0.42**	1.46 ± 0.44**
MAGEa (mmol/l)	1.54 ± 0.43	3.52 ± 1.12**	4.95 ± 1.38**##
LAGEa (mmol/l)	3.17 ± 0.88	5.52 ± 1.16**	6.54 ± 1.62**
MPPGE[a] (mmol/l)	1.58 ± 0.47	2.40 ± 0.70*	3.31 ± 1.15**##
MODD[a] (mmol/l)	0.56 ± 0.21	1.43 ± 0.56**	2.27 ± 1.05**##
$IAUC_{70}$[a] (mmol·d/l)	–	0.04 ± 0.04	0.15 ± 0.08##
FMD[b] (%)	10.17 ± 1.35	7.12 ± 1.22**	4.71 ± 1.13**##
CRP (mg/l)	1.64 ± 0.46	3.81 ± 1.43**	6.21 ± 1.17**##

[a]Measured by continuous glucose monitoring system; [b]Measured by high-resolution ultrasound system. *$p < 0.05$; **$p < 0.01$: compared with NC group; #$p < 0.05$; ##$p < 0.01$: compared with T2DM1 group.

glucose fluctuation might be a result of changes in FMD, CRP and HOMA-IR in T2DM patients with CHD.

Relationship Among Multi-Variables in T2DM2 Patients

When FMD was considered as the dependent variable, it could be calculated using the following regression equation: FMD = 11.217-0.369 MAGE-0.346 (HOMA-IR)-0.447 SBP (Table III). When CRP was chosen as the dependent variable, the regression equation was as follows: CRP = 3.527 + 0.566 LAGE (Table IV). These further suggest that FMD was strongly correlated with MAGE, HOMA-IR and SBP while CRP was strongly correlated with LAGE.

Discussion

The effect of blood glucose fluctuation on the function of vascular endothelial cells has become a subject of extensive research in recent years. It is reported that postprandial hyperglycemia is an

Table II. Correlations between the indicated variables in T2DM2 group.

Dependent variable	MAGE		LAGE		MPPGE		MODD		$IAUC_{70}$	
	r	*p*	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
FMD	-0.520	0.003	-0.378	0.029	-0.367	0.033	-0.389	0.025	-0.344	0.042
CRP	0.543	0.002	0.566	0.001	0.454	0.010	0.406	0.020	0.185	0.182
HOMA-IR	0.390	0.025	0.480	0.007	0.438	0.013	0.339	0.045	0.163	0.212
HbA1c	0.196	0.169	0.198	0.166	0.102	0.311	0.313	0.060	0.193	0.172

[a]Measured by continuous glucose monitoring system; [b]Measured by high-resolution ultrasound system.

Table III. Results of multivariate regression analysis of factors affecting FMD in T2DM2 group.

Variable	Regression coefficient	Standard error	Standardized regression coefficient (β)	*t*	*p* value
Constant term	11.217	1.406	–	7.987	0.000
MAGE	-0.302	0.129	-0.369	-2.350	0.028
HOMA-IR	-0.877	0.404	-0.346	-2.196	0.039
SBP	-0.030	0.010	-0.447	-3.078	0.006

independent predictor of cardiovascular events and death in diabetic patients[18]. Moreover, it has been shown that postprandial hyperglycemia is a risk factor for patients with or without diagnosed by diabetes[19]. In our study, brachial artery FMD in patients with T2DM was significantly decreased compared with healthy control subjects. Correlation analysis showed that FMD was correlated with TG, SBP, HbA1c and blood glucose fluctuation. This suggests that decreased brachial artery FMD may be associated with aberrant glucose and lipid metabolism in patients with T2DM. Macrovascular complications occurred earlier in the T2DM2 group than in the T2DM1 group. Blood glucose fluctuation parameters (MAGE, LAGE, MODD, MPPGE and $IAUC_{70}$) in T2DM2 patients were remarkably increased as compared with those in T2DM1 patients. In other words, when the course of the disease, blood glucose, blood pressure, cholesterol and other factors were controlled, increased blood glucose fluctuation became an important factor causes the decrease of brachial artery FMD in T2DM patients with CHD.

Vascular dysfunction may result from blood glucose fluctuation, but the underlying mechanism remains unclear. Many studies indicated that oxidative stress play an important role in the process of vascular dysfunction caused by blood glucose fluctuation. Quagliaro et al[11] have reported that temporary hyperglycemia accelerates the damage of vascular endothelial cells, and promotes apoptosis and oxidative DNA damage as compared with persistent hyperglycemia. Furthermore, *in vivo* studies have demonstrated that rapidly elevated glucose concentration may activate P65 subunit of NF-kB of arterial endothelial cells in non-diabetic rats, and elevated glucose concentration induce the expression of monocyte chemoattractant protein-1 (MCP-1) and vascular cell adhesion molecule-l (VCAM-1). Inhibition of mitochondrial superoxide ion may attenuate the aberrant expression of these factors[20]. In addition to vascular endothelial cells, a variety of other cell types including renal cortex fibroblast cells and human peripheral blood mononuclear cells can also be injured after intermittent high glucose exposure. Through analyzing changes in the intracellular nitrotyrosine and 8-hydroxy deoxyguanosine (8-OHdG) expression and activity of Bcl-2 and caspase-3, Piconi et al[21] found intermittent elevated blood glucose could induce endothelial cell apoptosis through reactive oxygen species (ROS)-associated oxidative stress. These observations indicate that oxidative stress is involved in blood glucose fluctuation.

Previous clinical studies[22] have reported that the excretion rate of 8-iso-prostaglandin F2 alpha (8-isoPGF2α) is highly correlated with MAGE ($r = 0.86$, $p < 0.001$) but not the average blood glucose, fasting blood glucose or HbA1c. MAGE is considered to be the "gold standard" indicator of blood glucose fluctuation and 8-isoPGF2α (a peroxidation product) *in vivo.* The correlation between these two factors suggest that oxidative stress may be an important mechanism underlying the blood glucose fluctuation-associated vascular injury. In the present study, MAGE was the variable that showed the strongest correlation with brachial artery FMD in T2DM2 patients. It

Table IV. Results of multiple regression analysis of factors affecting CRP in T2DM2 group.

Variable	Regression coefficient	Standard error	Standardized regression coefficient (β)	*t*	*p* value
Constant term	3.527	0.817	–	4.317	0.000
LAGE	0.409	0.121	0.566	3.367	0.003

is possible that blood glucose fluctuation causes vascular endothelial injury through decreasing both NO synthesis and FMD. MAGE includes all valid blood glucose fluctuation indicators and, therefore, is closely related to endothelial function in FMD. Parameters such as LAGE, MODD, MPPGE and IAUC70 were also correlated with FMD in the linear regression analysis but failed to perform the multivariate regression analysis. This might be due to the incomprehensive representativeness of these parameters to blood glucose fluctuation.

Another potential mechanism is the inflammation hypothesis. It is believed that type 2 diabetes is a chronic inflammatory disease. Our study found that CRP was significantly higher in T2DM patients as compared to healthy control subjects. This observation is consistent with results from previous studies[23-25]. CRP can cause vascular endothelial dysfunction in a variety of ways[26,27]. To date, it remains inconclusive regarding whether blood glucose fluctuation plays a role in increasing inflammation in T2DM patients. Tanaka et al[28] reported that postprandial hyperglycemia could increase IL-L and TNF-α in peripheral blood. And excessive secretion of IL-1 and TNF-α can increase CRP synthesis[29], which indicating that blood glucose fluctuation may lead to increased inflammatory factors such as IL-l, TNF-α and CRP. In this study, the CRP level was apparently increased in T2DM patients, particularly those with CHD as compared with control subjects. Correlation analysis showed that CRP was correlated with MAGE, LAGE, MPPGE, and MODD. The maximal correlation coefficient was obtained between LAGE and CRP, indicating that an increase in CRP may be related to excessive blood glucose drift in T2DM patients with CHD. LAGE was the maximal acute fluctuation during the entire observation period (24 hours) and CRP is an acute phase protein. Our findings showed that increased blood glucose fluctuation could decrease the brachial artery FMD and elevate CRP in T2DM patients with CHD. This indicates that elevated CRP may be one of the mechanisms responsible for vascular endothelial dysfunction in T2DM patients with CHD.

In addition to blood glucose fluctuation, HOMA-IR was another important factor that affected the brachial artery FMD in T2DM patients with CHD. Insulin-dependent phosphatidylinositol 3 kinase (PI-3K) signal transduction pathway regulates the expression of endothelial nitric oxide synthase (eNOS) gene[30], thereby regulating NO production. IR leads to injuries of endothelial cells in many ways while damaged endothelial cells may exacerbate IR by changing the distribution and function of insulin receptors on the cell surface.

Furthermore, this study showed that in T2DM patients with CHD, systolic blood pressure was one of the important factors that affect brachial artery FMD in addition to blood glucose fluctuations. Woodman et al[31] found that there was a significant increase in CRP and von willebrand facotr (vWF) in T2DM patients with hypertension. And von Willebrand Factor (vWF) is an important indicator of vascular endothelial function. This indicates an endothelial dysfunction in T2DM patients with hypertension. The United Kingdom Prospective Diabetes Study (UKPDS) has demonstrated that a strict control of blood pressure may reduce the incidence of vascular complications in T2DM patients[32]. Elevated systolic blood pressure is commonly seen in elderly patients. An increase in blood pressure may lead to injury of vascular endothelial cells of the affected vessels through a flow-associated mechanical mechanism. In addition, high blood pressure may cause endothelial cell dysfunction through insufficient L-arginine (NO precursors), NO inactivation induced by increased superoxide anion generation, activation of vascular renin-angiotensin-aldosterone system (RAS), and imbalance of NO/ET-1 synthesized by endothelial cells[33]. The relationship between systolic blood pressure and brachial artery FMD in our study might result from a mechanical stimulation and the interaction of FMD with the vascular endothelial cells.

Conclusions

This study demonstrated that blood glucose fluctuation increased significantly in T2DM patients with CHD as compared with those without CHD. Blood glucose fluctuation was found to be an important factor that affected the brachial artery FMD, possibly through a CRP elevation-associated mechanism. Moreover, IR and systolic blood pressure were also important factors that could affect brachial artery FMD. All these observations suggest that vascular endothelial dysfunction in T2DM patients with CHD may be protected through an effective control of blood pressure and extenuation of blood glucose fluctuation.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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