European Review for Medical and Pharmacological Sciences 2014; 18: 2025-2030

Blood glucose fluctuation aggravates lower extremity vascular disease in type 2 diabetes

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Abstract. – OBJECTIVES: Lower-extremity vascular diseases are important complication of diabetes. In the present study, we investigated the influence of blood glucose fluctuation in type 2 diabetes-associated lower-extremity vascular diseases, and explore the possible mechanism.

PATIENTS AND METHODS: Patients with type 2 diabetes was assigned to Group B (without lower-extremity vascular disease) and group C (with lower-extremity vascular disease). Healthy subjects (Group A) served as normal controls. All patients received dynamic blood glucose monitoring for 72 h. The mean amplitude of glycemic excursion (MAGE) and the largest amplitude of glycemic excursion (LAGE) were estimated. The levels of von Willebrand factor (vWF), ischemia-modified albumin (IMA), glycosylated hemoglobin (HbA1c), and biochemical indices were examined, and the lower-extremity vascular diseases were scored in patients from group C.

RESULTS: Groups B and C have higher systolic blood pressure (SBP), total cholesterol (TC) level, high-density lipoprotein cholesterol (HDL-C) level, HbA1c level, and vWF level and lower IMA level than those in Group A ($p < 0.05$). Elevated MAGE and LAGE were observed in groups B and C as compared with Group A. Correlation analysis revealed that the score of lower-extremity vascular diseases was associated with MAGE, LAGE, SBP, LDL-C, vWF, HbA1c, and IMA ($p < 0.05$). Stepwise multiple-linear regression analysis revealed that lower-extremity vascular diseases were involved with MAGE, IMA, and vWF.

CONCLUSIONS: Enhanced fluctuation in patients with type 2 diabetes may promote the occurrence and development of lower-extremity vascular diseases through aggravating vascular endothelial injury.

Key words:
Blood glucose fluctuation, Lower-extremity vascular disease, Type 2 diabetes, von Willebrand factor, Ischemia-modified albumin.

Introduction

The prevalence of diabetes mellitus shows an increasing tendency around worldwide[1]; the complications of diabetes, especially macrovascular complications, seriously affect life quality of patients[2]. The prevalence and disability rate of lower-extremity vascular diseases are extremely high, which are major causes of amputation. It is indicated that about 15% of the patients with diabetes develop foot ulcers over their lifetimes[3], and 11-24% of foot ulcers may ultimately lead to amputation[4]. Active prevention and control of lower-extremity vascular diseases is, therefore, a great challenge.

Dysglycemia is a major cause of chronic complications of diabetes, which mainly includes two types of complications, chronic persistent hyperglycemia and fluctuating hyperglycemia. The impact of chronic persistent hyperglycemia on chronic complications of diabetes mellitus has been clearly illustrated, and further recent research demonstrated that the blood glucose fluctuation is closely related with the vascular complications of diabetes; the elevated amplitude of blood glucose fluctuation may more likely to increase the risk of occurrence of chronic vascular complications[5,6]. Therefore, understanding the pathogenesis of blood glucose fluctuation on diabetic macroangiopathy could be very helpful for finding a strategy of prevention and treatment of macroangiopathy in diabetes.

In the current study, a continuous glucose monitoring system (CGMS) was employed for 24 h monitoring of blood glucose concentrations; the mean amplitude of glycemic excursion (MAGE) and the largest amplitude of glycemic excursion (LAGE) were measured. In addition, Doppler ultrasonography of the lower extremity was performed, and the lower-extremity vascular diseases were scored to evaluate the correlation between blood glucose fluctuation and lower-ex-

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tremity vascular diseases. The serum levels of von Willebrand factor (vWF) and ischemia-modified albumin (IMA) were also determined to investigate the pathogenesis of lower-extremity vascular diseases and blood glucose fluctuation.

Patients and methods

Patients and ethnic consideration

According to the 1999 WHO guidelines for the diagnosis and classification of diabetes mellitus[7], 86 patients with type 2 diabetes were recruited at Department of Endocrinology, General Hospital of Beijing PLA Military Region from January 2012 through April 2013. Their ages are between 18 and 75 years. The inclusion criteria involved: (1) a course of disease > 6 months; (2) a stable blood-glucose reducing scheme for at least 3 months; (3) no trauma or surgery within the past 6 months; (4) no severe heart, brain, liver, or kidney diseases, or diseases of the immune system; (5) metabolic stability for at least 6 months, with no acute or chronic infections, no administration of antioxidant drugs – including vitamins, and no tumors or connective tissue diseases; and (6) diagnosis of peripheral vascular disease of the lower extremities using vascular ultrasound, lower-extremity angiography, or magnetic resonance imaging within the past one month, and an ankle-brachial index (ABI) of < 0.9[8].

The study has been approved and registered in Ethics Committee of General Hospital of Beijing PLA Military Region in 2011, the Ethics Committee approved relating screening and data collection of these patients, all subjects signed written informed consent form. All works were undertaken following the provisions of the Declaration of Helsinki.

86 patients with type 2 diabetes were assigned to 2 groups: the only diabetes group (Group B, 46 cases), and the diabetes with lower-extremity vascular disease group (Group C, 40 cases); Another 40 healthy subjects during the study period were recruited as normal controls (Group A), these subjects have no diabetes and were tested by 75-g oral glucose tolerance test (OGTT), and they were confirmed no family history of diabetes or hypertension, and have normal blood pressure and blood lipid levels.

General clinical characteristics

Each subject's age, gender, course of disease, and body mass index (BMI) were recorded, as well as their systolic blood pressure (SBP) and diastolic blood pressure (DBP), and the levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and glycosylated hemoglobin (HbA1c) were detected. The HbA1c level was determined using low-pressure liquid chromatography on a DiaSTAT™ glycated HbA1c analyzer (Bio-Rad Laboratories, Inc.; Hercules, CA, USA), and the glucose level in capillary blood in the fingertip was determined by the glucose oxidase method on a SureStep® blood glucose monitor (Lifescan Inc.; Milpitas, CA, USA).

Dynamic blood glucose monitoring

All subjects received dynamic blood glucose monitoring with a CGMS (Medtronic Inc.; Minneapolis, MN, USA). The sensor of the CGMS was placed subcutaneously in the subjects' abdomen, and blood glucose concentrations in the interstitial fluid were measured every 5 min.

During the blood glucose monitoring, the patient's diet was unchanged, and the blood glucose-reducing scheme was not changed neither. The reference blood glucose concentrations were accurately recorded 4 times daily, and the interval between two inputs did not exceed 12 h. The MAGE and LAGE were calculated using the CGMS Solutions™ software version 3.0 (Medtronic Inc.; Minneapolis, MN, USA).

Scoring of lower-extremity vascular diseases

Lower-extremity vascular in group C was evaluated as following methods: (1) ABI: ABI > 0.9, score 0; 0.8 < ABI ≤ 0.9, score 1; 0.7 < ABI ≤ 0.8, score 2; 0.6 < ABI ≤ 0.7, score 3; and ABI ≤ 0.6, score 4. (2) Intermittent claudication (the longest distance at a walking speed of 70-80 m/min): ≥ 400 m, score 0; 300-399 m, score 1; 200-299 m, score 2; 100-199 m, score 3; and < 100 m or failure in walking, score 4. (3) Pain: no pain, score 0; pain after exercise, score 1; occasional rest pain, score 2; rest pain at night or regular rest pain, score 3; and persistent rest pain, score 4. (4) Sensation of numbness: no sensation of numbness, score 0; occasional sensation of numbness, score 1; regular sensation of numbness, score 2; regular sensation of numbness and no other abnormal sensations, score 3; and unbearable persistent sensation of numbness, score 4. (5) Ischemic ulcer: no open lesions, score 0; superficial ulcer, score 1; formation of ulcer, with involvement of tendon, ligament, and bone joint, score 2; ulcer and infection in the deep lower ex-

tremity, occurrence of osteomyelitis, and formation of abscess sinus, score 3; and toe gangrene or partial foot gangrene, score 4.

Determination of serum index

At the second morning of dynamic blood glucose monitoring, the fasting blood samples were collected and the serum IMA level (U/mL) was determined by an albumin cobalt binding (ACB) test on an Olympus AU2700 automatic biochemical analyzer (Olympus Corporation; Nishishinjuku Shinjuku-Ku, Tokyo, Japan) according to the manufacturer's instructions. Lower serum IMA levels indicated more severe disease. Meanwhile, the fasting blood samples were collected and the vWF level was determined with a double antibody sandwich enzyme-linked immunosorbent assay (ELISA) using the human vWF ELISA kit (Shanghai Yanxin Biological Technology Co. Ltd.; Shanghai, China).

Statistical analysis

All normally-distributed data were expressed as mean ± standard deviation (SD), while the non-normally distributed data were presented as median or interquartile range. The means of the normally-distributed data were compared using Student *t*-tests, while the means of the non-normally distributed data were compared using non-parametric tests. Differences of proportions were tested for statistical significance using chi-square tests. A p value of > 0.1 was indicative of homogeneity of variance, and a p value of < 0.05 was considered statistically significant. The correlation between two variables was evaluated using Pearson's correlation analysis, while the multi-factor analysis was performed using a multiple linear regression analysis. All statistical analyses were performed using the statistical software SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

Results

Comparisons among three groups

The SBP, HDL-C level, TC level, HbA1c level, and vWF level were significantly greater in groups B and C than in Group A ($p < 0.05$, Table I), and the IMA level in Group C was significantly lower than in Group B ($p < 0.05$).

However, no significant differences were found in the gender, BMI, course of disease, SBP, HDL-C level, and TG level between groups B and C ($p > 0.05$). Elevated MAGE and LAGE were detected in groups B and C compared to those in Group A, and a more significantly notable elevation was detected in group C ($p < 0.01$, vs group B).

Correlation analysis

Pearson's correlation analysis revealed that the score of lower-extremity vascular diseases was significantly associated with LDL-C, HbA1c, and vWF levels, MAGE, LAGE, SBP, and IMA level ($p < 0.05$, Table II).

Table I. Comparison of the observation factors among the 3 groups.

	Group A (N=40)	Group B (N=46)	Group C (N=40)
Age	59.2±5.7	59.9±5.9	61.5±7.0
Gender (male/female)	25/15	26/20	20/20
BMI (kg/m^2)	23.6±2.0	24.7±2.1	24.3±1.8
Course of Disease (year)	—	7.53 (5.35,9.11)	5.77 (4.61,6.78)
SBP (mmhg)	112±10	128±8	140±12*
DBP (mmhg)	76±4	78±6	80±8
HbA1C (%)	4.9±0.5	6.7±1.2**	7.7±1.4**
HDL-C (mmol/l)	0.88±0.18	1.05±0.20	1.09±0.18
LDL-C (mmol/l)	2.14±0.56	3.15±0.68 *	3.33±0.70*
TG (mmol/l)	1.45±0.47	1.48±0.50	1.55±0.24
TC (mmol/l)	4.08±0.78	5.04±0.88 *	5.75±0.79*
MAGE (mmol/l)	2.0±0.2	2.8±0.3**	4.3±0.4**
LAGE (mmol/l)	2.8±0.5	5.9±0.6**	9.3±0.5**
IMA (U/ml)	—	99.0±5.67	70.8±3.4
vWF (%)	—	105.34±6.28*	135.33±8.9*
Score of lower extremity vascular disease	—	0	12.3±2.4

*p <0.05, **p < 0.01, Compared with Group A; $p < 0.05$, $p < 0.01$, Compare to Group B.

Table II. Pearson correlation analysis of score of lower extremity vascular disease and the possible risk factors.

NIHSS	MAGE	LAGE	IMA	LDL-C	SBP	HbA1C	vWF
R	0.618	0.703	0.476	0.680	0.487	0.469	0.635
p	0.003	0.008	0.005	0.049	0.000	0.019	0.036

Stepwise multiple-linear regression analysis was performed, scores of lower-extremity vascular diseases was considered as a dependent variable and the detection parameters as independent variables, result revealed that vWF level, MAGE, and IMA level had the highest effect on the lower-extremity vascular diseases (Table III).

Discussion

The lower-extremity vascular disease associated with diabetes, a major cause of disability in diabetes patients, is one of the most common complications of diabetes. The major pathological changes of lower-extremity vascular diseases[9] include increased carotid artery intima-media thickness (IMT), lumen narrowing, reduction of lumen wall compliance, and formation of irregular atherosclerotic plaques in the vascular intima, which lead to the further narrowing of the lumen, followed by thrombotic formation and occlusion.

Studies have demonstrated that long-term persistent hyperglycemia may cause chronic complications of diabetes[10,11]; On the other hand, it has been shown that the risk of vascular complications differs greatly, even if the subjects have the same HbA1c levels[12]. Therefore, the HbA1c level cannot be used as the only parameter to evaluate the efficiency of blood glucose control[13,14]. In addition to HbA1c, blood glucose fluctuation may correlate with the occurrence and development of vascular complications of diabetes[7,8,12]. Many parameters have been used to assess blood glucose fluctuation, among them, MAGE is currently accepted as the gold standard[15]. The Diabetes Epidemiology: Collaborative analysis of Diagnostic criteria in Europe (DECODE) study showed that postprandial hyperglycemia closely correlated with macrovascular complications, and an independent correlation exists between postprandial hyperglycemia, cardiovascular events, and resultant mortality[16].

Our findings demonstrated that the SBP, HDL-C, TC, and HbA1c levels were significantly greater in groups B and C than in Group A, which proves that type 2 diabetes could produce severe disorder of metabolism in human body. Comparison of the blood glucose fluctuations among groups A, B, and C revealed elevated MAGE and LAGE in groups B and C than in Group A, and a more significantly notable elevation was detected in Group C ($p < 0.01$), which suggests that blood glucose fluctuation may be associated with the lower-extremity vascular diseases, and further validates that blood glucose fluctuation has an important effect on the vascular complications of diabetes.

Endothelial dysfunction is considered as the basis of macrovascular complications of type 2 diabetes. Hyperglycemia may induce vascular endothelial cell dysfunction, and endothelial dysfunction could accelerate the development of diabetes and its complications[17]. Recent study indicated that blood glucose fluctuation may aggravate oxidative stress in patients with type 2 diabetes, and further injures endothelial cells, thereby resulting in the occurrence of macrovascular complications of diabetes[18,19]. IMA, a recently identified biochemical marker to reflect the acute

Table III. Multiple step regression analysis of score of lower extremity vascular disease and the possible risk factors.

Variable	Partial regression coefficient	Standard error (SE)	Standard partial regression coefficient t	*t* value	*p* value
Constant	-5.670	1.1464		-4.264	0.001
MAGE	7.234	2.377	0.473	3.025	0.006
IMA	1.057	0.435	0.335	2.365	0.026
vWF	0.149	0.074	0.163	1.753	0.049

phase of myocardial infarction, is a novel ischemic index for early diagnosis and prognosis[20,21]. However, there are few studies regarding IMA in lower-extremity vascular diseases of diabetes. As a macromolecule glycoprotein, vWF is released directly into blood to promote coagulation if the vascular endothelial tissues are injured, thereby stop bleeding. The elevation of plasma vWF level is considered a marker of vascular endothelial injury[22].

Our findings showed that the vWF levels in group C were significantly higher than Group B, while the IMA level in Group C was significantly lower than in Group B, these suggesting greater blood glucose fluctuation and endothelial dysfunction occurred in diabetic patients with lower-extremity vascular diseases. Pearson's correlation analysis revealed that the score of lower-extremity vascular diseases was significantly associated with vWF and IMA levels, and stepwise multiple-linear regression analysis showed that the vWF and IMA levels had the greatest effect on lower-extremity vascular diseases. These findings suggest that blood glucose fluctuation may cause endothelial dysfunction in patients with diabetes, which may attribute to the elevated vWF secretion during endothelial injury, leading to endothelial platelet adhesion and platelet activation, followed by thrombotic formation. Thrombotic formation consequently aggravates the formation of arteriosclerosis, followed by stenosis and ischemia formation. In addition, our findings showed that an abnormal IMA concentration may correlate with the severity of hyperglycemia, blood glucose fluctuation, and severity of lower-extremity ischemia. It is, therefore, considered that the IMA level has a certain clinical value in identifying the severity of lower-extremity vascular diseases in type 2 diabetes.

Conclusions

Endothelial cell dysfunction is involved in the occurrence and development of type 2 diabetes associated vascular complications, while persistent glucotoxicity, lipotoxicity, and hypertension in type 2 diabetes aggravates endothelial cell dysfunction. Therefore, treatment of type 2 diabetes should include not only lowering blood glucose and blood pressure, but also reducing blood glucose fluctuation, as well as other intervention strategies to protect endothelial cell dysfunction.

Conflict of interest

The Authors declare that they have no conflict of interests.

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