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Association of glycemic variability assessed by continuous glucose monitoring with subclinical diabetic polyneuropathy in type 2 diabetes patients

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Keywords
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ABSTRACT

Aims/Introduction: Diabetic peripheral neuropathy is a common diabetes-related microvascular complication. The relationship between peripheral nerve function and glucose variability is unclear. We investigated the association of glucose variability with subclinical diabetic polyneuropathy in a large-scale sample of patients with type 2 diabetes.

Materials and Methods: We enrolled 509 individuals with type 2 diabetes who were screened for diabetic peripheral neuropathy and monitored using a continuous glucose monitoring system. Multiple glycemic variability parameters, including the mean amplitude of glycemic excursions, glucose standard deviation (SD_{gluc}) and glucose coefficient of variation were calculated from 3-day glucose profiles obtained from continuous glucose monitoring. All participants underwent nerve conduction studies, and the composite *Z*-scores for nerve conduction parameters were calculated.

Results: Multivariate logistic regression analyses showed that SD_{gluc} and the conventional risk factor hemoglobin A1c (HbA1c) were independently associated with abnormal nerve function, and the corresponding odds ratios (95% confidence interval) were 1.198 (1.027–1.397, SD_{gluc}) and 1.182 (1.061–1.316, HbA1c), respectively. The composite *Z*-score of nerve conduction velocity and response amplitude obviously decreased with greater SD_{gluc}, and the composite *Z*-score of distal latency significantly increased with increasing tertiles of SD_{gluc} (all P trend <0.05). After adjusting for age, sex, body mass index, diabetes duration and HbA1c, SD_{gluc} was independently associated with nerve conduction velocity ($\beta = -0.124$, $P = 0.021$).

Conclusions: The SD_{gluc} is a significant independent contributor to subclinical diabetic polyneuropathy, in addition to conventional risk factors including diabetes duration and HbA1c.

INTRODUCTION

Diabetes mellitus is a metabolic disorder with a significantly increasing prevalence in China in recent decades, which has contributed to the increase in diabetes-related complications, including chronic kidney disease, diabetic retinopathy, diabetic peripheral neuropathy (DPN), diabetic foot and cardiovascular disease in China[1,2]. Typical DPN is a chronic, symmetrical, distant sensorimotor polyneuropathy and is considered the most common type of heterogeneity[3]. It might be silent, asymptomatic, undetectable or present with clinical symptoms. DPN is usually associated with a loss or diminished sensation in the foot and leads to an increased incidence of foot ulcers[4,5], resulting in amputation in patients with a long duration of diabetes[6].

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Up to 50% of DPN might be asymptomatic[7]. Subclinical diabetic neuropathy frequently occurrs at the early stage of DPN with abnormal electrophysiological features and no typical clinical manifestations of diabetic neuropathy[8]. As the early diagnosis and timely interventions are essential to prevent the progression of diabetic neuropathy, the reliance on clinical symptoms or signs alone might lead to decreased diagnostic accuracy for subclinical diabetic polyneuropathy. Therefore, as a surrogate measurement, nerve conduction study is widely used as an evaluation method of DPN. Of all the feasible evaluation methods to date, nerve conduction studies are considered the most objective, sensitive and reproducible method of early detection and quantification of DPN, especially appropriate for detecting patients without classic DPN symptoms.

Glycemic dysregulation is a risk factor for the onset or progression of DPN. Glycemic disorders are not only confined to traditional markers reflecting glycemic levels, such as plasma glucose levels, glycated albumin and hemoglobin A1c (HbA1c), but also contain markers of glycemic variability (GV), such as standard deviation of glucose levels (SD_{gluc}), coefficient of variation of glucose levels (CV_{gluc}) and mean amplitude of glycemic excursions (MAGE). Most GV parameters can be examined and presented in detail by using a continuous glucose monitoring (CGM) system[9,10]. In recent years, a few studies have reported a positive association between GV and diabetic macrovascular and microvascular complications[11]. Hu *et al.*[12] found that MAGE, as an indicator of glycemic variability, independently contributed to the presence of diabetic peripheral neuropathy. In contrast, Lachin *et al.*[13] found that GV within a day, as determined from daily glucose levels by point-of-care testing, did not have a significant effect on the development of diabetic microvascular complications. Thus, the uncertain association between GV and DPN requires further investigation. In the present study, we designed a cross-sectional study to determine the correlation of GV, assessed using CGM, with nerve conduction function in patients with type 2 diabetes mellitus and subclinical diabetic neuropathy.

MATERIALS AND METHODS

Study population

All participants were admitted to the Endocrinology Department of Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China, for screening of diabetic chronic complications and to optimize their antidiabetic regimens. A flow diagram of patient selection is shown in Figure 1. We enrolled 509 individuals with type 2 diabetes mellitus who had undergone a nerve conduction study for DPN screening and 3-day CGM from April 2013 to August 2014. The individuals satisfied the following inclusion criteria: (i) were aged 25–75 years; (ii) had valid and available data of nerve conduction studies; and (iii) had received the current antidiabetic medication regimen for at least 3 months. Individuals were excluded if they had any of the following criteria: (i) no complete data of sex, age, HbA1c and diabetes duration; (ii) history of neurological diseases that could influence nerve conducting function; (iii) history of severe kidney or liver disease; (iv) history of mental disorder or malignancy; (v) presence of symmetrical distant neuropathy symptoms or signs; (vi) use of vitamin B_1, vitamin B_{12} or folic acid in the previous 6 months; and (vii) acute diabetic complications. Diabetes was diagnosed according to the 1999 World Health Organization criteria. The diagnostic criteria of subclinical diabetic polyneuropathy are defined as the presence of no neuropathy signs or symptoms and abnormal nerve conduction[14]. The present study protocol was approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. Written informed consent was obtained from all participants.

Anthropometric measurements

During recruitment, all participants were surveyed, and their age, sex, lifestyle behaviors, past medical history and somatometric parameters were recorded. Weight (kg) divided by height squared (m^2) was calculated to determine body mass index (BMI). Blood pressure was calculated as the average of three measurements.

Biochemical measurements

Blood samples from all participants were collected for laboratory biochemical measurements. HbA1c was measured using an analyzer (Tosoh HLC-723 G7, Yamaguchi, Japan) using high-performance liquid chromatography. Fasting lipid profiles were measured using an automatic biochemical analyzer (Hitachi 7600, Tokyo, Japan). The Modification of Diet in Renal Disease equation was used to obtain the estimated glomerular filtration rate. More than two 24-h urinary samples were collected to determine urinary albumin excretion (UAE) as the mean 24-h urinary albumin level using a turbidimetric immunoassay.

The participants were defined as having cardiovascular disease if they had at least one cardiovascular event. We used standardized non-mydriatic fundus photography to determine diabetic retinopathy. Diagnosis of diabetic retinopathy was determined according to the International Classification of Diabetic Retinopathy criteria by an ophthalmologist. At least two 24-h UAE measurements were made, and the mean level was recorded for each participant. The participants were diagnosed with diabetic nephropathy if the mean UAE was ≥30 mg/24 h.

Nerve conduction study

All enrolled patients underwent an electrophysiological evaluation system using the Dantec Keypoint Focus EMG System (Natus, Taastrup, Denmark). Both the motor and sensory nerves on the non-dominant side were detected. Motor nerve studies were carried out on the ulnar, median and tibial nerves. Sensory nerve studies were carried out in the ulnar, median and sural nerves. Distal latency, response amplitude and nerve conduction velocity (NCV) were measured. Upper body

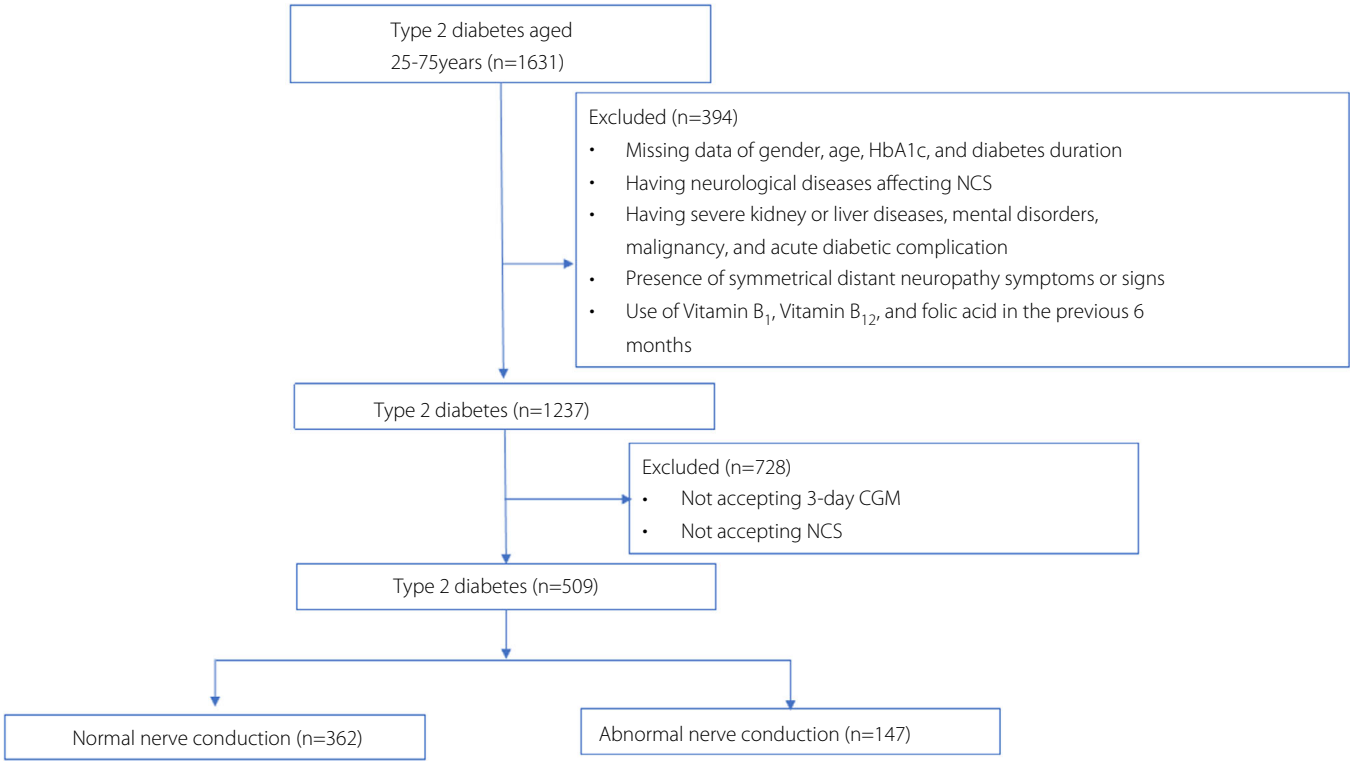

Figure 1 | Flow diagram of patient selection. CGM, continuous glucose monitoring; NCS, nerve conduction study.

temperature was maintained at ≥35°C, and lower body temperature was maintained at ≥32°C[15].

All measurements were collected and compared with laboratory reference values. Nerve conduction function was regarded as abnormal if one or more parameters in more than one of the tested motor or sensory nerves were beyond the reference values[16].

Additionally, the composite *Z*-scores of the tested nerve conduction parameters, including NCV, response amplitude and distal latency, were estimated. *Z*-score = (individual value of patient − mean value of control group) / SD of the control group[1]. The composite NCV *Z*-score was then calculated as [(*Z*-score motor median NCV) + (*Z*-score sensory median NCV) + (*Z*-score motor ulnar NCV) + (*Z*-score sensory ulnar NCV) + (*Z*-score tibial NCV) + (*Z*-score sural NCV)] / 6. The composite *Z*-scores for distal latency and response amplitude were then determined using a similar equation[17].

CGM examination

All participants underwent retrospective 3-day CGM (Medtronic Inc., Northridge, CA, USA) beginning on the first day of admission in accordance with clinical indications for retrospective CGM technology[18]. The sensor of the CGM system was inserted on day 0 and removed 72 h later, generating a daily record of 288 continuous sensor values. All participants adhered to the preadmission therapy regimen during the 3-day CGM and followed a standard dietary pattern at the same time. The CGM system was managed and operated by specialized staff according to standard operation procedures to guarantee the accuracy and validity of the results. The CGM system was calibrated four times daily using capillary blood glucose measurements, including fasting, 2 h after breakfast, before dinner and at 09.00 hours using a SureStep blood glucose meter (LifeScan, Milpitas, CA, USA). If three or more pairs of sensor glucose values and capillary blood glucose values matched per day, the CGM data were considered accurate. We applied three parameters to assess GV: SD_{gluc}, CV_{gluc} and MAGE to reflect intraday GV. All parameters were calculated using specific computer software after the 3-day CGM. CV_{gluc} was determined as SD_{gluc} divided by the mean glucose level. In addition, the arithmetic mean of the differences between consecutive nadirs and peaks was computed to determine the MAGE value.

Statistical analysis

Data of normally distributed variables are presented as the mean ± SD. Skewed data are expressed as the median (25 and 75% interquartile range). Student's *t*-test was used to test the differences in continuous variables, and the χ^2-test was used to compare the proportions of categorical variables. The differences in composite *Z*-score of nerve conduction parameters

among tertiles of SD_{gluc} were analyzed by a test for trend. One-way analysis of variance was used to compare nerve conduction parameters among the tertiles of SD_{gluc}. Taking into account potential confounders, binary logistic regression was carried out to investigate associations between GV parameters and nerve conduction function, and the corresponding odds ratios (ORs) and 95% confidence intervals are provided. Five binary logistic regression models were constructed for HbA1c (model 1), SD_{gluc} (model 2), MAGE (model 3), CV_{gluc} (model 4) and average glucose (model 5) after controlling for clinical risk factors including age, sex, BMI and diabetes duration. Additionally, a multiple linear regression analysis was applied to investigate associations between GV parameters and the continuous composite *Z*-score of nerve conduction parameters, including NCV, distal latency and response amplitude as dependent variables. Five multivariate linear regression models were carried out for HbA1c (model 1), HbA1c and SD_{gluc} (model 2), HbA1c and MAGE (model 3), HbA1c and CV_{gluc} (model 4), and HbA1c and average glucose (model 5) after controlling for clinical risk factors. Covariates in the models included age, BMI, diabetes duration, HbA1c, CV_{gluc}, SD_{gluc}, MAGE and average glucose as continuous variables, and sex as a categorical variable. All statistical analyses were carried out using SPSS software (version 25.0). Statistical differences were considered significant if the two-tailed *P*-value was <0.05.

RESULTS

A total of 509 patients with type 2 diabetes mellitus, including 278 men and 231 women aged 43–71 years, were enrolled. All participants were classified into two groups according to whether they had normal or abnormal nerve conduction functions. We have presented the reference values and mean values of nerve conduction study parameters in Table 1. As shown in Table 1, 147 patients (28.9%) had abnormal nerve conduction results, and they were relatively older than the normal nerve conduction group ($P = 0.003$). In addition, the abnormal nerve conduction group had higher HbA1c values ($P = 0.021$), longer diabetes duration ($P = 0.002$), and higher average glucose ($P = 0.041$) and SD_{gluc} ($P = 0.008$) values than the normal nerve conduction group. The lipid profile, MAGE, CV_{gluc}, UAE, estimated glomerular filtration rate and blood pressure were not significantly different between the normal and abnormal nerve conduction groups. Diabetic retinopathy, diabetic nephropathy, cardiovascular disease, smoking status and alcohol consumption were similar between the two groups.

Logistic regression analysis was carried out to evaluate the relationship between GV parameters and abnormal nerve conduction (Table 2). HbA1c and SD_{gluc} were associated with abnormal nerve conduction. The ORs of HbA1c and SD_{gluc} were 1.182 ($P = 0.002$) and 1.198 ($P = 0.021$), respectively, after adjusting for age, sex, BMI and diabetes duration. Although MAGE, CV_{gluc} and average glucose were considered potential risk factors, these parameters were not associated with abnormal nerve conduction (OR for MAGE = 1.057, $P = 0.173$; OR for CV_{gluc} = 1.017, $P = 0.311$; OR for average glucose = 1.102, $P = 0.06$).

Next, to further evaluate the association of nerve conduction function and SD_{gluc}, composite *Z*-scores of NCV, distal latency and response amplitude were calculated. Figure 2 shows that the composite *Z*-scores of NCV and response amplitude obviously decreased with increasing tertiles of SD_{gluc} (*P* for trend <0.01), whereas the composite *Z*-score of distal latency was significantly prolonged in the groups with higher SD_{gluc} (*P* for trend <0.01).

Finally, we carried out multivariate linear regression to assess the correlation between GV parameters and peripheral nerve conduction function by applying five different models. Model 1 showed that after adjusting for age, sex, BMI and duration of diabetes, HbA1c was associated with all nerve conduction parameters (NCV: $\beta = -0.313$, $P < 0.001$; latency: $\beta = 0.229$, $P < 0.001$; amplitude: $\beta = -0.180$, $P < 0.001$). Further adjustment for SD_{gluc}, but not MAGE, CV_{gluc} or average glucose, attenuated the relationship between HbA1c and NCV (SD_{gluc}: $\beta = -0.124$, $P = 0.02$; HbA1c: $\beta = -0.245$, $P < 0.001$). Additionally, after controlling for age, BMI, sex, duration of diabetes and HbA1c, NCV, but not distal latency or amplitude, was associated with SD_{gluc} (Table 3).

DISCUSSION

In the present study, we investigated the correlation of GV parameters in individuals with type 2 diabetes mellitus and subclinical diabetic polyneuropathy. The results showed that increased GV, revealed as SD_{gluc}, was significantly associated with abnormal nerve conduction. The higher the SD_{gluc}, the lower the composite *Z*-scores of NCV and response amplitude, and the higher the composite *Z*-score of latency. After controlling for potential confounders, elevated SD_{gluc} was still significantly associated with a slower NCV.

DPN is a common disease with a complicated pathogenesis and diverse mechanisms, including hypertension, dyslipidemia, diabetes duration and alcohol consumption[19–21]. Unlike other microvascular complications of diabetic retinopathy and nephropathy, there is little information regarding the relationship between GV and DPN, and whether GV is an independent contributor to DPN remains controversial. A prospective study found that quarterly point-of-care glucose values, reflecting within-day GV, did not contribute to the development of microvascular complications independent of the mean glucose level in type 1 diabetes[13]. Nevertheless, Pai showed that individuals with a higher CV of fasting plasma glucose had an obviously greater risk of DPN and no interaction effects between CV of fasting plasma glucose and HbA1c[22,23]. Hu *et al.*[12] found that GV, assessed as MAGE, had a strong relationship with DPN in individuals with type 2 diabetes mellitus, and the MAGE threshold of 4.60 mmol/L was considered the cut-off point to identify DPN. In the present study, GV assessed by SD_{gluc} was an independent factor correlated with nerve conduction function in patients with subclinical diabetic neuropathy.

Table 1 | Characteristics of patients in the study

Variable	Total	Normal nerve conduction	Abnormal nerve conduction	*P*-value
Samples (*n*)	509	362	147	
Age (years)	57.68 ± 13.88	56.52 ± 13.83	60.52 ± 13.65	0.003
Sex (male/female)	278/231	203/159	75/72	0.299
BMI (kg/m^2)	24.35 ± 3.65	24.40 ± 3.74	24.24 ± 3.44	0.662
Duration of diabetes (years)	9.02 ± 7.65	8.33 ± 7.33	10.70 ± 8.18	0.002
Alcohol consumers, *n* (%)	69 (13.56%)	56 (15.50%)	13 (8.84%)	0.062
Current smokers, *n* (%)	167 (13.56%)	122 (33.70%)	45 (30.61%)	0.533
Systolic blood pressure (mmHg)	130.60 ± 17.86	129.83 ± 17.84	132.49 ± 17.83	0.194
Diastolic blood pressure (mmHg)	78.36 ± 10.37	78.21 ± 10.77	78.74 ± 9.35	0.640
HbA1c (%)	8.61 ± 2.04	8.47 ± 2.03	8.97 ± 2.01	0.021
HDL-C (mmol/L)	1.08 ± 0.31	1.09 ± 0.30	1.07 ± 0.33	0.665
LDL-C (mmol/L)	2.74 ± 0.85	2.74 ± 0.80	2.74 ± 0.97	0.999
Triglyceride (mmol/L)	1.65 ± 1.49	1.64 ± 1.39	1.68 ± 1.72	0.776
Total cholesterol (mmol/L)	4.59 ± 1.02	4.58 ± 0.96	4.62 ± 1.16	0.738
Average glucose (mmol/L)	9.37 ± 1.90	9.26 ± 1.78	9.64 ± 2.13	0.041
SD_{gluc} (mmol/L)	5.96 ± 1.27	5.86 ± 1.18	6.19 ± 1.43	0.008
MAGE (mmol/L)	6.33 ± 2.48	6.23 ± 2.43	6.60 ± 2.59	0.129
CV_{gluc} (%)	63.72 ± 6.00	63.47 ± 5.87	64.32 ± 6.29	0.150
Diabetic retinopathy, *n* (%)	79,15.5	50, 13.8	29,19.7	0.064
Diabetic nephropathy, *n* (%)	87, 17.1	56, 15.5	31, 21.1	0.083
CVD (*n*, %)	83, 16.3	53, 14.6	30, 20.4	0.073
eGFR (mL/min/1.73 m^2)	80.86 ± 48.96	80.33 ± 49.14	82.18 ± 48.64	0.607
UAE (mg/24 h)	21.00 (15.00, 44.00)	20.44 (15.00, 40.00)	22.50 (16.00, 57.00)	0.097
Nerve conduction parameters (reference values)				
Motor median conduction velocity, m/s (>50.0)	54.65 ± 5.22	55.30 ± 4.63	53.05 ± 6.18	<0.001
Motor median latency, m/s (<4.2)	3.50 ± 0.57	3.36 ± 0.46	3.83 ± 0.67	<0.001
Motor median amplitude, mv (>5.0)	6.43 ± 3.33	7.17 ± 3.22	4.60 ± 2.88	<0.001
Motor ulnar conduction velocity, m/s (>50.0)	58.21 ± 6.39	58.51 ± 6.02	57.46 ± 7.16	0.095
Motor ulnar latency, ms (<3.1)	2.44 ± 0.36	2.39 ± 0.33	2.58 ± 0.44	<0.001
Motor ulnar amplitude, mv (>5.0)	5.09 ± 2.33	5.42 ± 2.52	4.27 ± 1.48	<0.001
Motor tibial conduction velocity, m/s (>37.0)	44.74 ± 5.43	45.11 ± 5.189	43.83 ± 5.90	0.016
Motor tibial latency, ms (<5.8)	3.72 ± 0.78	3.73 ± 0.77	3.70 ± 0.79	0.674
Motor tibial amplitude, mv (>4.8)	6.38 ± 3.90	6.95 ± 4.09	4.95 ± 2.96	<0.001
Sensory median conduction velocity, m/s (>45.0)	55.51 ± 8.58	57.16 ± 7.20	51.46 ± 10.24	<0.001
Sensory median latency, ms (<3.5)	2.55 ± 0.40	2.48 ± 0.31	2.74 ± 0.50	<0.001
Sensory median amplitude, mv (>20.0)	10.33 ± 5.85	11.68 ± 5.95	7.02 ± 3.99	<0.001
Sensory ulnar conduction velocity, m/s (>44.0)	57.34 ± 7.65	57.47 ± 7.04	57.01 ± 9.00	0.573
Sensory ulnar latency, ms (<2.8)	2.17 ± 0.32	2.17 ± 0.31	2.17 ± 0.35	0.974
Sensory ulnar amplitude, mv (>17.0)	9.14 ± 4.83	5.42 ± 2.52	4.27 ± 1.48	<0.001
Sensory sural conduction velocity, m/s (>40.0)	46.95 ± 8.54	47.81 ± 7.95	44.79 ± 9.57	<0.001
Sensory sural latency, ms (NA)	2.04 ± 0.92	1.93 ± 1.00	2.31 ± 0.56	<0.001
Sensory sural amplitude, mv (NA)	13.25 ± 9.57	14.94 ± 9.86	8.95 ± 7.18	<0.001

Data are expressed as mean ± standard deviation (SD) for normal distribution variables. Skewed data are expressed as the medians (25 and 75% interquartile ranges). Categorical variables are expressed as numbers (percentage). BMI, body mass index; CVD, cardiovascular disease; CV_{gluc}, glucose coefficient of variation; eGFR, estimated glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-c, high density lipoprotein cholesterol; LDL-c, low density lipoprotein cholesterol; MAGE, mean amplitude of glycemic excursions; NA, not available; SD_{gluc}, glucose standard deviation; UAE, urinary albumin excretion.

Although the known influence of HbA1c on the onset and development of diabetic vascular complications is definite, GV, as a pattern of glycemic disorders, is complementary to the development of diabetic vascular complications. Several mechanisms, including altered peripheral blood flow, damage to small fibers and central sensitization, have been implicated in the psychophysiological processes of DPN[24].

The lifetime incidence of diabetic neuropathy is approximately 45% in patients with type 2 diabetes mellitus, and even higher in patients with type 1 diabetes mellitus. Studies of nerve

Table 2 | Association of potential risk factors with abnormal nerve conduction after adjustment

Variables	Abnormal nerve conduction	
	OR (95% CI)	*P*
Model 1		
HbA1c	1.182 (1.061–1.316)	0.002
Model 2		
SD_{gluc}	1.198 (1.027–1.397)	0.021
Model 3		
MAGE	1.057 (0.976–1.144)	0.173
Model 4		
CV_{gluc}	1.017 (0.984–1.051)	0.311
Model 5		
Average glucose	1.102 (0.996–1.219)	0.060

Model 1: adjusted for age, sex, body mass index (BMI), diabetes duration and hemoglobin A1c (HbA1c); model 2: adjusted for age, sex, BMI, diabetes duration and glucose standard deviation (SD_{gluc}); model 3: adjusted for age, sex, BMI, diabetes duration and mean amplitude of glycemic excursions (MAGE); model 4: adjusted for age, sex, BMI, diabetes duration and glucose coefficient of variation (CV_{gluc}); model 5: adjusted for age, sex, BMI, diabetes duration and average glucose.

conduction tests carried out at the time of diabetes mellitus diagnosis showed that diabetic neuropathy was already present in patients with subclinical neuropathy; that is, without symmetrical distal numbness and pain[25,26]. Accordingly, a nerve conduction study is a commonly used method to detect nerve conduction abnormalities, even in patients with subclinical diabetic polyneuropathy. In the present study, electrophysiological assessments of peripheral nerves were carried out, which provided multiple markers of peripheral nerve function. Therefore, composite *Z*-scores were constructed to reflect overall neurological function, because composite nerve conduction test *Z*-scores have been shown to be sensitive and reproducible to correlate with neuropathic impairment[17]. Nerve conduction function has a positive correlation with the composite NCV and amplitude *Z*-scores, and a negative correlation with the composite *Z* score for distal latency.

CV_{gluc} and SD_{gluc} are the most common parameters of GV because of their availability, simplicity and certainty. In general, CV_{gluc} is correlated with a risk of hypoglycemia and has a weak association with the average glucose level. The SD_{gluc} can reflect both within- and between-day variability. Thus, SD_{gluc} has a moderate correlation with the average glucose level, but a weak relationship with the risk of hypoglycemia. MAGE is another GV metric that is applied to access the intraday GV by computing the mean height of the glycemic fluctuations between consecutive nadirs and peaks that were >1 SD_{gluc}. MAGE is estimated by computer programs, resulting in discrepancies due to differences in algorithms, definitions and the degree of initial smoothing of the blood glucose curve over time[27]. Hu *et al.*[12] found that increased GV was a significant independent risk factor for DPN in patients with type 2 diabetes mellitus. The enrolled participants presented with neuropathic symptoms and signs, an abnormal nerve conduction test, and a higher HbA1c level (10.18% in the DPN group). In the present study, we enrolled individuals with subclinical diabetic polyneuropathy and moderately elevated HbA1c levels (8.97% in the abnormal nerve conduction group). We hypothesized that SD_{gluc} might reflect inter- and intraday GV, and have a close relationship with subclinical diabetic polyneuropathy, the early stage of DPN.

Several limitations to our research should be addressed. First, this was a cross-sectional observational study to show the association between GV and DPN based on a large-scale sample of individuals with type 2 diabetes mellitus. A prospective follow-up study is required to further examine the effect of GV on DPN. Second, it has recently been shown that long-term GV, as determined by the variability of HbA1c values, has an effect on micro- and macrovascular complications[28]. Although the present study assessed only short-term GV through CGM, long-term GV, such as HbA1c variability, could be assessed

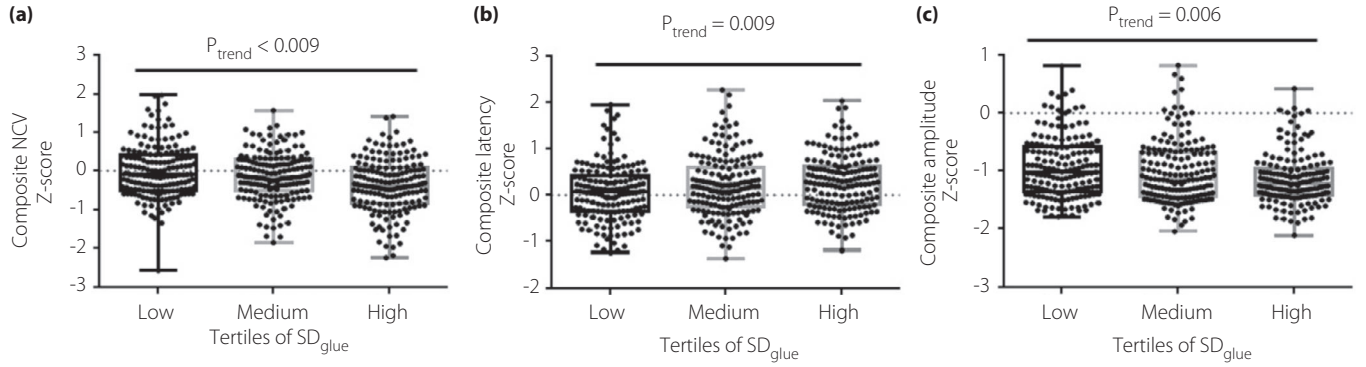

Figure 2 | Dot plots for composite *Z*-score of nerve conduction parameters, according to tertiles of glucose standard deviation (SD_{gluc}). Results were represented as the mean ± standard error. (a) Composite *Z*-score of nerve conduction velocity across SD_{gluc} tertiles; (b) Composite *Z*-score of latency across SD_{gluc} tertiles; (c) Composite *Z*-score of amplitude across SD_{gluc} tertiles. NCV, nerve conduction velocity.

Table 3 | Association of glucose variability parameters with nerve conduction parameters after adjustments

Variable	Composite *Z*-score of NCV		Composite *Z*-score of latency		Composite *Z*-score of amplitude	
	β	*P*-value	β	*P*-value	β	*P*-value
Model 1						
HbA1c	−0.313	<0.001	0.229	<0.001	−0.180	<0.001
Model 2						
SD_{gluc}	−0.124	0.021	0.048	0.367	−0.082	0.146
HbA1c	−0.245	<0.001	0.202	<0.001	−0.135	0.018
Model 3						
MAGE	−0.066	0.181	0.045	0.354	−0.030	0.555
HbA1c	−0.291	<0.001	0.213	<0.001	−0.170	0.001
Model 4						
CV_{gluc}	−0.040	0.384	0.049	0.284	0.012	0.806
HbA1c	−0.310	<0.001	0.225	<0.001	−0.181	<0.001
Model 5						
Average glucose	−0.100	0.058	0.017	0.747	−0.099	0.074
HbA1c	−0.258	<0.001	0.220	<0.001	−0.126	0.026

Model 1: adjusted for age, sex, body mass index (BMI), diabetes duration and hemoglobin A1c (HbA1c); model 2: adjusted for age, sex, BMI, diabetes duration, HbA1c and glucose standard deviation (SD_{gluc}); Model 3: adjusted for age, sex, BMI, diabetes duration and HbA1c, mean amplitude of glycemic excursions (MAGE); model 4: adjusted for age, sex, BMI, diabetes duration, HbA1c and glucose coefficient of variation (CV_{gluc}); model 5: adjusted for age, sex, BMI, diabetes duration, HbA1c and average glucose. NCV, nerve conduction velocity.

further. Third, this study was carried out in a single clinical center with a Chinese population, and investigation in other races and countries is required for further generalization. Finally, concomitant medication data were not available in the electronic medical records for this study. However, to our knowledge, there is a limited number of studies focusing on the direct effect of antidiabetic medication on DPN, and we excluded subjects using vitamin B_1, vitamin B_{12} and folic acid, which can affect nerve conduction function.

In conclusion, increased GV showed that SD_{gluc} was a significant independent contributor to subclinical diabetic polyneuropathy, in addition to conventional risk factors, including diabetes duration and HbA1c. It is suggested that blood SD_{gluc} might be another potential target for the management of subclinical diabetic polyneuropathy.

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DISCLOSURE

The authors declare no conflict of interest.

Approval of the research protocol: The present study protocol was approved by the Institutional Review Board of Shanghai Jiao Tong University Affiliated Sixth People's Hospital in accordance with the principles of the Helsinki Declaration (approval no. 2012–21).

Informed consent: Written informed consent was obtained from all participants.

Approval date of registry: N/A.

Animal studies: N/A.

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