Relationship Between Incremental Glucose Peak and Diabetic Retinopathy in Tertiary Hospital: Liaoning Diabetic Microvascular Complications Study (LD-MCS)

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Abstract

Background: Glucose variability may contribute to macrovascular complications of diabetes but evidence for its association with diabetic retinopathy (DR) is limited. This study is aimed to investigate the correlation between incremental glucose peak (IGP) and diabetic retinopathy (DR) in Chinese population with type 2 diabetes.

Methods: A hospital-registry, case-control study collecting data from Liaoning Diabetic Microvascular Complications Study (LD-MCS) during 2012-2018. 795 adults with DR were included and matched with two diabetic controls per case ($n = 1590$) by gender and age. IGP was defined as the peak minus baseline plasma glucose value during 5-point oral glucose tolerance test (OGTT). DR was diagnosed by two-field fundus photographs under Early Treatment Diabetic Retinopathy Study criteria and stratified into mild non-proliferative DR (NPDR), moderate NPDR, and vision-threatening DR (VTDR). Logistic regression analysis was applied to determine the relationships.

Results: Among 2385 participants (age, 58.40 ± 11.52 years; 57.11% men), higher IGP was independently associated with the presence of any DR (odds ratio [OR], 1.05; 95% confidence interval [CI], 1.02-1.07; $P < 0.001$), moderate NPDR (OR, 1.06; 95% CI, 1.01-1.12; $P = 0.020$) and VTDR (OR, 1.13; 95% CI, 1.07-1.19; $P < 0.001$), respectively. Moreover, estimated ratio for any DR and VTDR from low-quartile IGP increased by 1.33 (95% CI, 1.04-1.70; P for trend = 0.021) and 3.64 (95% CI, 1.97-6.72; P for trend < 0.001), respectively.

Conclusions: OGTT-derived IGP was independently associated with DR severity scales in Chinese patients with type 2 diabetes. More attention by health care personnel needs to be paid to glucose variability of this population.

Background

Diabetic retinopathy (DR), as one of the common complications of diabetes mellitus, is characterized by microangiopathic alterations at the fundus.[1, 2] It was estimated worldwide that the prevalence of DR and vision-threatening stage of DR was up to 34.6% and 10.2%, respectively.[3] The number of people affected by type 2 diabetes increases worldwide, with associated increases in its complications .[4] Moreover, as the most frequent cause of vision loss across many countries,[2] impaired vision triggered by DR imposed much burden on public health care resources.[5]

Great strides in the epidemiological investigations of DR, which confirmed that consistent modifiable risk factors for DR included hyperglycemia.[1, 6] Intensive glycemic control reported by the United Kingdom Prospective Diabetes Study (UKPDS) and the Diabetes Control and Complications Trial (DCCT) was proven to be beneficial of prevention of the incidence and progression of DR, and the UKPDS also reported that every 10% decrease of glycated hemoglobin A1c (HbA1c) was accompanied with one quarter decreased danger of microangiopathy for type 2 diabetes, including DR.[7, 8] The metabolic pathways initiated by hyperglycemia may explain this association.[9]

Glycemic variability (GV), the dynamic evaluation of plasma glucose changes, deepened the concept of glycemic control beyond HbA1c. However, evidence for the effect of GV on retinopathy of type 2 diabetes was limited. Sonoda et al. suggested the mean amplitude of glycemic excursions (MAGE) had no significant correlations with DR.[10] However, some evidences certified direct or indirect damage to retina caused by GV based on 72-hour continuous glucose monitoring system (CGM) or 1,5-anhydroglucitol (an indicator related to postprandial hyperglycemia and GV), especially in acute fluctuation and hyperglycemia.[11–13]

Clinically, CGM use is still considered experimental and limited, we attempted to focus on incremental glucose peak (IGP) derived from oral glucose tolerance test (OGTT), which had been considered as an appropriate alternative for approximation of GV.[14, 15] IGP refers to the glycemic difference between baseline and peak during OGTT, which has been found with significant associations with some macrovascular events.[16, 17] However, its association with microvascular complications, such as DR, has not been explored yet. Accordingly, this study aimed to investigate relationships between IGP and DR as well as its severity strata in Chinese population with type 2 diabetes.

Methods

Research design and study population

We used data from Liaoning Diabetic Microvascular Complications Study (LD-MCS). The LD-MCS is a series of hospital-based observational studies and aimed to identify risk factors and biomarkers of diabetic microvascular complications for Chinese adults with type 2 diabetes. Theory basis and methodology of the LD-MCS were reported in the Appendix (see Additional file 1).

Cases and controls defined as participants with DR or without DR, respectively. The control group sample was calculated by power of 80%, ratio of cases to controls: 1:2 and percentage of controls exposed to higher GV: 10% and Odds ratio 1.20 (both provided based on pilot investigation). Added 10% for losses, sample calculation resulted in 620 cases and 1240 controls.

After assessment in LD-MCS, 1469 cases with DR were collected from the database and subsequently some participants with incomplete data, either because an OGTT contraindication ($n = 568$; i.e. plasma glucose levels > 11.0 mmol/L before initiation of the OGTT), or ambiguous fundus grades ($n = 106$) were removed. Each remaining case was matched randomly with two controls without any history of DR by same gender and age in the database. Finally, 795 confirmed cases (54.1%) with DR and 1590 corresponding controls amounting to 2385 participants were recruited in this study.

Assessment Of Diabetic Retinopathy

Two ophthalmologists of the LD-MCS were invited to independently evaluate fundus situation of participants and confirm the diagnosis and severity scales of DR under guidelines of the Early Treatment

for Diabetic Retinopathy Study (ETDRS).[18] Each image was a two-field colorful one taken by non-mydratic digital retinal photography (CR6-45NM; Canon, Inc., Tokyo, Japan). The diagnosis of DR was ascertained by the following signature lesions: fundus microaneurysms, hemorrhage, cotton wool spots, intraretinal microvascular impairments, hard exudates, venous beading and new vascularization. Next, retinopathy was scored into five grades: 0. non-DR; 1. mild non-proliferative DR (NPDR) (ETDRS stage 20); 2. moderate NPDR (ETDRS stage 35–47); 3. severe NPDR (ETDRS stage at least 53); 4. proliferative DR (PDR) (with abnormal growth of definite new vessels).[19] Similarly, retinopathy was also divided into two categories, including none vision-threatening DR (non-VTDR) and VTDR which was certified by clinically significant maculopathy, severe NPDR or PDR.[18] The grade outcomes depended on the severity of the worse eye. In case of disagreement with the diagnosis, a third ophthalmologist would attend.

Assessment Of G Incremental Glucose Peak And Covariates

We performed comprehensive medical assessment of health profiles of each participant and collected their demographic, anthropometric information and clinical examination results on admission date. Gender, age, smoker, drinker, use of antidiabetic agents, duration of diabetes, height, weight, body mass index (BMI), heart rate (HR), blood pressures (systolic blood pressure [SBP], diastolic blood pressure [DBP] and pulse pressure [PP]) were obtained from hospital-based diabetes registry. All participants underwent a standardized 3-h 75 g OGTT after fasting overnight. The concentration of plasma glucose at baseline, 30 min, 60 min, 120 min, 180 min as fasting plasma glucose (FPG), 30-min plasma glucose (30-min PG), 60-min PG, 120-min PG, and 180-min PG, respectively. Plasma insulin levels of the five time points were processed. The peak value of post-load plasma glucose was the maximum of above glycemic concentration. IGP was the result of peak value minus FPG. Furthermore, biochemical variables included serum glycosylated haemoglobin (HbA1c), and serum creatinine. Besides, we included two calculative indices, the insulin resistance index (HOMA-IR, $\text{HOMA-IR} = \text{FPG} * \text{Insulin} [\text{baseline}] / 22.5$) and the β -cell function (HOMA- β , $\text{HOMA-}\beta = 20 * \text{Insulin} [\text{baseline}] / [\text{FPG} - 3.5]$), in which FPG was expressed in mmol/L and insulin was expressed in $\mu\text{IU/mL}$, to evaluate insulin resistance and pancreatic β -cell function.[20] Estimated glomerular filtration rate (eGFR) was calculated by the equation of Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) to evaluate renal function.[21]

Statistical analysis

All statistical analyses were performed by the SPSS version 24.0. (IBM for Windows). Continuous and categorical data were expressed as mean and SD (mean $\pm$ SD) and percentage (No. [%]), respectively. Data was verified for the normal distribution by Kolmogorov-Smirnov test. The Chi-square test and Fisher exact test was applied for discontinuous data comparison and the independent Student's t-test was applied for continuous variables comparison across different study population groups. One-way ANOVA test and the Chi-square test were used to compare variables between various subgroups. Three logistic regression models adjusted for confounding factors were carried out to estimate the odds ratio (OR) with a 95% confidence interval (95% CI). IGP and HOMA-IR were transformed into categorical data by quartiles

which were segregated by the 25th, 50th and 75th percentage as Quartile 1 (Q1), Q2, Q3, and Q4. Multiple R-squared liner regression analysis was performed to reveal the correlations between variables and IGP quartiles. In all analyses, *P* value less than 0.05 was defined as statistical significance.

Results

Study population characteristics

The characteristics of 2385 participants stratified by the presence and severity of DR were presented in Table 1. Among all participants (age, 58.40 ± 11.52 years; 57.11% men), 795 (33.3%) had retinopathy (533 mild NPDR [67.0%], 140 moderate NPDR [17.6%], and 122 VTDR [15.3%]). Compared to non-DR group (1590 [66.7%]), subjects with DR were like to be with longer duration of diabetes, and had higher level of weight, HR, SBP, PP, HbA1c, 120-min PG, 180-min PG, post-load plasma glucose peak, and IGP (all *P* < 0.05). Blood glucose variability before and after OGTT among participants with different DR severity scales were described on Figure S1 in Additional file 2. Along with the advanced retinopathy condition, HbA1c, 120-min PG, 180-min PG, post-load plasma glucose peak, and IGP showed an upward tendency (HbA1c: *P* = 0.014; 180-min PG: *P* = 0.002; all others: *P* < 0.001).

Table 1
Characteristics of included participants stratified by the presence and severity of DR

Characteristics	Total (n = 2385)	Presence of DR		P Value	Severity of DR			
		Non-DR (n = 1590)	DR (n = 795)		Mild NPDR (n = 533)	Moderate NPDR (n = 140)	VTDR (n = 122)	P Value
Male, No. (%)	1362 (57.11)	908 (57.11)	454 (57.11)	1.000	313 (58.72)	72 (51.43)	69 (56.56)	0.489
Age, years	58.40 ± 11.52	58.40 ± 11.52	58.40 ± 11.53	1.000	58.29 ± 11.54	59.41 ± 10.96	57.71 ± 12.13	0.667
Height, cm	166.31 ± 8.19	166.11 ± 8.28	166.70 ± 7.98	0.095	167.07 ± 7.86	165.59 ± 8.05	166.37 ± 8.33	0.083
Weight, kg	68.26 ± 12.20	67.88 ± 12.40	69.03 ± 11.75	0.023	69.82 ± 11.88	67.23 ± 11.56	67.59 ± 11.11	0.086
BMI, kg/m ²	24.58 ± 3.29	24.49 ± 3.30	24.76 ± 3.26	0.061	24.93 ± 3.28	24.45 ± 3.32	24.36 ± 3.10	0.046
HR, bmp	80.21 ± 10.78	79.82 ± 10.49	80.97 ± 11.31	0.026	80.65 ± 11.14	81.50 ± 12.02	81.78 ± 11.22	0.057
SBP, mmHg	135.33 ± 18.69	134.17 ± 17.88	137.65 ± 20.03	< 0.001	137.85 ± 19.63	137.06 ± 20.41	137.50 ± 21.44	< 0.001
DBP, mmHg	82.39 ± 11.08	82.36 ± 10.86	82.44 ± 11.50	0.871	82.49 ± 11.28	82.00 ± 11.56	82.75 ± 12.46	0.950
PP, mmHg	52.94 ± 15.51	51.81 ± 14.75	55.21 ± 16.72	< 0.001	55.36 ± 16.37	55.06 ± 18.34	54.75 ± 16.40	< 0.001
Smoker, No. (%)	666 (27.92)	445 (27.99)	221 (27.80)	0.923	139 (26.08)	36 (25.71)	46 (37.70)	0.071
Drinker, No. (%)	509 (21.34)	332 (20.88)	177 (22.26)	0.437	107 (20.08)	33 (23.57)	37 (30.33)	0.072
Antidiabetic agents, yes, No. [%]	2225 (93.29)	1473 (92.64)	752 (94.59)	0.073	514 (96.44)	127 (90.71)	111 (90.98)	0.007
Diabetes duration, year	8.49 ± 6.97	8.11 ± 6.87	9.24 ± 7.12	< 0.001	9.07 ± 6.97	10.52 ± 7.77	8.51 ± 6.85	< 0.001
(continued)								

In view of confounders, Fig. 1 presented bivariate and multivariate logistic regressive analysis performed to further explore the relationships between IGP and DR as well as its severity strata. Adjusted for gender and age (model 1), IGP levels had a statistically significant correlation with the presence of DR (OR, 1.05;

95% CI, 1.02–1.07; $P < 0.001$), moderate NPDR (OR, 1.06; 95% CI, 1.01–1.12; $P = 0.020$) and VTDR (OR, 1.13; 95% CI, 1.07–1.19; $P < 0.001$), respectively. Model 2 with adjustment for variables related to severity of DR and model 3 with additional potential adjustment for FPG, HOMA-IR, serum creatinine and eGFR showed similar relationships.

When study participants were divided into four groups by IGP (Q1: IGP < 7.14 ; Q2: 7.14–9.42; Q3: 9.43–11.81; Q4: IGP ≥ 11.82) in Table 2, all glycemic parameters, including HbA1c, FPG, post-load plasma glucose concentration, and HOMA-IR had strong correlations with IGP quartiles (FPG: $P = 0.002$; others: $P < 0.001$). Post-load glucose levels climbed dramatically with IGP quartiles progression, while HOMA-IR descended.

Table 2
Characteristics of included participants by quartiles of IGP

Characteristics	IGP quartiles				<i>P</i> value
	Q1 (< 7.14, n = 600)	Q2 (7.14–9.42, n = 593)	Q3 (9.43–11.81, n = 596)	Q4 (≥ 11.82, n = 596)	
Male, No. (%)	338 (56.33)	340 (57.34)	348 (58.39)	336 (56.38)	0.876
Age, years	58.12 ± 11.01	58.23 ± 11.60	58.67 ± 11.46	58.57 ± 12.01	0.813
Height, cm	166.01 ± 8.10	166.46 ± 8.50	166.83 ± 7.90	165.93 ± 8.22	0.199
Weight, kg	68.08 ± 12.12	67.95 ± 12.62	68.63 ± 11.89	68.38 ± 12.18	0.772
BMI, kg/m ²	24.64 ± 3.35	24.40 ± 3.34	24.56 ± 3.22	24.72 ± 3.24	0.363
HR, bmp	80.36 ± 10.68	80.44 ± 10.48	80.24 ± 11.56	79.78 ± 10.38	0.719
SBP, mmHg	134.77 ± 18.60	135.27 ± 19.28	135.63 ± 18.59	135.66 ± 18.32	0.830
DBP, mmHg	82.34 ± 11.16	82.20 ± 10.96	82.90 ± 10.97	82.13 ± 11.23	0.616
PP, mmHg	52.44 ± 15.24	53.07 ± 15.88	52.73 ± 15.35	53.53 ± 15.60	0.650
Smoker, No. (%)	189 (31.50)	157 (26.48)	169 (28.36)	151 (25.34)	0.091
Drinker, No. (%)	145 (24.17)	121 (20.40)	132 (22.15)	111 (18.62)	0.111
Antidiabetic agents, yes, No. [%]	564 (94.00)	560 (94.44)	556 (93.29)	545 (91.44)	0.174
Diabetes duration, year	8.28 ± 6.80	8.62 ± 7.06	8.58 ± 6.92	8.47 ± 7.11	0.840
HbA1c, %	8.62 ± 2.49	8.21 ± 2.12	8.56 ± 2.50	8.97 ± 2.04	< 0.001
(continued)					

Relationships between quartiles of IGP and DR and its severity were shown in Table 3 with same adjusted models as above. Significant correlations between the third (OR, 1.34; 95% CI, 1.05–1.72; *P* = 0.019) and fourth (OR, 1.30; 95% CI, 1.01–1.67; *P* = 0.040) quartile of IGP and DR were obtained in Model 3. Furthermore, among three models, every quartile of IGP was significantly related to VTDR referring to the lowest quartile (all *P* value for trend < 0.001).

Table 3
Multivariable-adjusted associations of incremental glucose peak (IGP) and diabetic retinopathy (DR) remodeling

		Mild NPDR		Moderate NPDR		VTDR		Any DR	
		OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value	OR (95% CI)	<i>P</i> value
Model 1	IGP Q1	1.00		1.00		1.00		1.00	
	IGP Q2	0.99 (0.75–1.31)	0.956	1.24 (0.75–2.07)	0.398	2.82 (1.50–5.29)	0.001	1.18 (0.92–1.51)	0.185
	IGP Q3	1.26 (0.96–1.66)	0.093	1.33 (0.80–2.20)	0.272	1.98 (1.01–3.86)	0.045	1.33 (1.04–1.70)	0.021
	IGP Q4	1.06 (0.80–1.41)	0.671	1.43 (0.87–2.35)	0.157	3.64 (1.97–6.72)	< 0.001	1.33 (1.04–1.70)	0.021
		<i>P</i> value for trend 0.385		<i>P</i> value for trend 0.152		<i>P</i> value for trend < 0.001		<i>P</i> value for trend 0.014	
Model 2	IGP Q1	1.00		1.00		1.00		1.00	
	IGP Q2	1.00 (0.75–1.32)	0.979	1.27 (0.76–2.12)	0.353	2.92 (1.55–5.50)	0.001	1.12 (0.93–1.53)	0.157
	IGP Q3	1.28 (0.97–1.68)	0.080	1.32 (0.79–2.18)	0.290	1.99 (1.02–3.88)	0.045	1.34 (1.05–1.72)	0.019
	IGP Q4	1.05 (0.79–1.39)	0.736	1.36 (0.83–2.24)	0.227	3.51 (1.90–6.49)	< 0.001	1.30 (1.02–1.67)	0.035
		<i>P</i> value for trend 0.425		<i>P</i> value for trend 0.233		<i>P</i> value for trend < 0.001		<i>P</i> value for trend 0.024	

Model 1: includes gender and age;

Model 2: includes gender, age, BMI, SBP, PP, antidiabetic agents, diabetes duration, and HbA1c;

Model 3: includes gender, age, BMI, SBP, PP, antidiabetic agents, diabetes duration, HbA1c, FPG, HOMA-IR, serum creatinine, and eGFR.

Abbreviation: IGP, incremental glucose peak; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy; BMI, body mass index; SBP, systolic blood pressure; PP, pulse pressure; HbA1c, glycated hemoglobin A1c; FPG, fasting plasma glucose; HOMA-IR, the homeostasis model assessment of insulin resistance index; eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval.

		Mild NPDR		Moderate NPDR		VTDR		Any DR	
Model 3	IGP Q1	1.00		1.00		1.00		1.00	
	IGP Q2	1.00 (0.75–1.32)	0.975	1.28 (0.77–2.14)	0.349	3.07 (1.62–5.82)	0.001	1.20 (0.94–1.54)	0.148
	IGP Q3	1.28 (0.97–1.68)	0.081	1.31 (0.78–2.18)	0.306	2.05 (1.04–4.03)	0.038	1.34 (1.05–1.72)	0.019
	IGP Q4	1.04 (0.78–1.39)	0.774	1.33 (0.80–2.22)	0.268	3.66 (1.96–6.87)	< 0.001	1.30 (1.01–1.67)	0.040
	<i>P</i> value for trend 0.450		<i>P</i> value for trend 0.280		<i>P</i> value for trend < 0.001		<i>P</i> value for trend 0.029		
Model 1: includes gender and age;									
Model 2: includes gender, age, BMI, SBP, PP, antidiabetic agents, diabetes duration, and HbA1c;									
Model 3: includes gender, age, BMI, SBP, PP, antidiabetic agents, diabetes duration, HbA1c, FPG, HOMA-IR, serum creatinine, and eGFR.									
Abbreviation: IGP, incremental glucose peak; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy; BMI, body mass index; SBP, systolic blood pressure; PP, pulse pressure; HbA1c, glycated hemoglobin A1c; FPG, fasting plasma glucose; HOMA-IR, the homeostasis model assessment of insulin resistance index; eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval.									

Additional Analyses

Multiple R-squared liner regression including variables about DR severity, OGTT-derived parameters, HOMA-IR, and an index of renal function were used for further assessment of the association between IGP and demographic as well as biomedical variables (Table 4). After stepwise selection of variables in model 1 and 2, only pre- and post-load index entered the models (both adjusted $R^2 = 0.908$) with remarkable significance (all $P < 0.001$).

Table 4
Associations between IGP and other variables

Variables	Model 1		Model 2	
	β	<i>P</i> value	β	<i>P</i> value
Male	-0.006	0.320	-0.017	0.030
Age, years	0.005	0.464	-0.009	0.297
BMI, kg/m ²	-0.002	0.808	-0.002	0.772
SBP, mmHg	-0.011	0.313	-0.011	0.290
PP, mmHg	0.016	0.152	0.017	0.132
Antidiabetic agents, yes	-0.007	0.276	-0.007	0.256
Diabetes duration, years	0.006	0.354	0.006	0.376
HbA1c, %	-0.008	0.330	-0.005	0.490
FPG, mmol/L	-1.026	< 0.001	-1.025	< 0.001
Post-load glucose, mmol/L	0.120	< 0.001	0.121	< 0.001
30 min	0.409	< 0.001	0.410	< 0.001
60 min	0.618	< 0.001	0.616	< 0.001
120 min	0.267	< 0.001	0.267	< 0.001
180 min				
HOMA-IR	-0.007	0.277	-0.008	0.271
Serum creatinine, μ mol/L			-0.024	0.063
eGFR, ml/min/1.73 m ²			-0.035	0.021
Included variables*				

Model 1: includes gender, age, BMI, SBP, PP, antidiabetic agents, duration of diabetes, HbA1c, FPG, post-meal glucose (30 min, 60 min, 120 min, and 180 min), and HOMA-IR;

Model 2: includes gender, age, BMI, SBP, PP, antidiabetic agents, duration of diabetes, HbA1c, FPG, post-meal glucose (30 min, 60 min, 120 min, and 180 min), HOMA-IR, serum creatinine, and eGFR.

Abbreviation: IGP, incremental glucose peak; BMI, body mass index; SBP, systolic blood pressure; PP, pulse pressure; HbA1c, glycated hemoglobin A1c; FPG, fasting plasma glucose; HOMA-IR, the homeostasis model assessment of insulin resistance index; eGFR, estimated glomerular filtration rate.

* Using stepwise method to select variables. Stepwise method ordinarily included the most significant variable ($P < 0.05$) and then excluded the most indistinctive variable ($P > 0.10$), which was aimed to obtain an optimal combine of variables based on Forward method and Backward method.

Variables	Model 1		Model 2	
	β	<i>P</i> value	β	<i>P</i> value
FPG, mmol/L	-1.033	< 0.001	-1.033	< 0.001
Post-load glucose, mmol/L	0.120	< 0.001	0.120	< 0.001
30 min	0.410	< 0.001	0.410	< 0.001
60 min	0.618	< 0.001	0.618	< 0.001
120 min	0.265	< 0.001	0.265	< 0.001
180 min				
Model 1: includes gender, age, BMI, SBP, PP, antidiabetic agents, duration of diabetes, HbA1c, FPG, post-meal glucose (30 min, 60 min, 120 min, and 180 min), and HOMA-IR;				
Model 2: includes gender, age, BMI, SBP, PP, antidiabetic agents, duration of diabetes, HbA1c, FPG, post-meal glucose (30 min, 60 min, 120 min, and 180 min), HOMA-IR, serum creatinine, and eGFR.				
Abbreviation: IGP, incremental glucose peak; BMI, body mass index; SBP, systolic blood pressure; PP, pulse pressure; HbA1c, glycated hemoglobin A1c; FPG, fasting plasma glucose; HOMA-IR, the homeostasis model assessment of insulin resistance index; eGFR, estimated glomerular filtration rate.				
* Using stepwise method to select variables. Stepwise method ordinarily included the most significant variable ($P < 0.05$) and then excluded the most indistinctive variable ($P > 0.10$), which was aimed to obtain an optimal combine of variables based on Forward method and Backward method.				

Discussion

In current study, we investigated case-control associations of IGP with DR as well as its severity in Chinese participants with type 2 diabetes. Firstly, our findings revealed the relationships between OGTT-derived indicators and the risk of retinopathy. HbA1c and 120-min PG value has previously been found to be independently associated with DR which conformed with our findings,[7, 22] while the evidence on the

association between 180-min PG value and DR were limited which might partly depend on 120-min PG. Post-load plasma glucose peak, which was described as a risk factor of cardiovascular diseases with postprandial peak,[23] also had impact of hyperglycaemia on endothelial function and retinal vascular reactivity. Previous studies had proposed that FPG could screen high risk diabetes with microvascular events and 60-min PG could prompt the onset of DR due to their close connection with insulin secretion. [22, 24] By contrast, in our study no independent association between FPG and 60-min OGTT glucose concentration and presence of DR was found. However, their use of cross-sectional design and 1- or 2-h plasma glucose concentrations by Americans or Indians entails a major different finding.

Additionally, adjusting for gender, age, and potential clinical-based confounders for DR, this present study first reported the association of IGP index with the presence of any-DR, moderate NPDR, and VTDR. This finding was also consistent with the results when IGP level was considered into categorical variable. Although there is no direct evidence on IGP levels with DR, high IGP was found to be associated with several macrovascular impairments, such as aortic stiffness, carotid intima-media thickness (CIMT), and maladaptive carotid remodeling.[16, 17] Moreover, evidences showed that when CGM was unavailable, IGP could be chosen to assess GV in replacement of coefficient of variation (CV; $SD / \text{mean} * 100\%$),[14] which was recommended as the leading measurement of variability by the international consensus of the Advanced Technologies & Treatments for Diabetes (ATTD) Congress.[25] The association between variability assessed by IGP and retinopathy was consistent with the results of the GV metrics assessed by CGM,[11, 13] while the results of some researches remained in debate and further longitudinal, population-based studies are needed to confirm our findings .[10]

Mechanistic Explanations

The biological pathway underlying the relationship between IGP and DR remains to be elucidated. In order to fill this gap, HOMA-IR which was associated with DR, was graded by quartiles, and higher level of insulin resistance increased FPG and 3-h post-load plasma glucose peak (both P value < 0.001), but decreased the level of IGP (see Figure S2 in Additional file 3). In contrast, Murata et al. got a positive correlation between glucose fluctuation and HOMA-IR due to definitional discrepancy between glucose fluctuation and IGP.[26] In our study, IGP could reflect an integrated situation of pre-load and 3-h post-load plasma glucose and seem to be related to insulin resistance, then form a relationship with DR.

In addition, DR has been traditionally recognized as a micro -vascular disease. Nevertheless, retinal neurodegeneration (neuronal apoptosis and glial activation) was also proved to be involved in the pathogenesis of DR.[27] The activation of Müller cells, the representative glial component of retina, was observed in response to GV.[28] Besides, Picconi et al. and Stem et al. demonstrated that increased glycemic excursion was connected with early structural impairments of neural retina in patients with type 1 diabetes, resulting a stable glycemic status and a decrease in GV might prevent the damage to structure and function of retina.[29, 30]

Although molecular mechanisms induced by hyperglycemia in the incidence and progression of DR have been described in previous studies, such as the hexosamine pathway, increased polyol pathway flux, protein kinase C (PKC) activation, free radicals formation and advanced glycation end (AGE) products accumulation, the metabolic mechanism of GV was unclear yet.[9] Some evidences indicated that oscillating glucose played a more destructive role on oxidative stress and endothelial function than sustained hyperglycemia.[31, 32] And a further mechanism revealed oscillating glucose left a metabolic memory, which suggested the persistence of intracellular pro-oxidant environment after plasma glucose normalization.[33] More observational and experimental evidence for the effect of GV on retinopathy was expected.

Clinical Relevance

Diabetes mellitus causes some macrovascular complications such as cardiovascular diseases (CVD) as well as microvascular complications such as retinopathy, nephropathy, and neuropathy, of which, DR is a major cause of vision loss in middle-aged and elderly people. Previous study reported that IPG was associated with aortic stiffness, which is an independent determinant of CVD. Currently, we found that after adjustment for relevant confounders risk of DR was increased with IGP increasing. Our results may imply that, even in case of well-controlled obesity, blood pressure, HbA1c, and renal function, the harmful effects of IPG on DR are still present. Future studies should investigate whether our findings translate to other microvascular complications. If they are replicated in nephropathy, and neuropathy, it would further justify therapeutic interventions that specifically target IGP.

Strengths And Limitations

In current study, a relative large sample size, standardized information collection, and a comprehensive mixed model analysis strengthened the reliability of our findings. However, some limitations should be noted. Firstly, the random measurement error might be induced by IGP, which was calculated by the indices derived from the 3-h OGTT with moderate reproducibility. Secondly, GV was an integrated definition by amplitude, frequency, and duration of the excursions,[25] but IGP merely emphasized the amplitude as a substitution of professional CGM. Thirdly, individuals this study only included Chinese hospitalized adults, which restricted the generalizability of the outcomes. Fourthly, a relatively large number of subjects were excluded due to uncompleted OGTT data. Fifth, the cross-sectional design renders us unable to rule out reverse causality.

Conclusions

In brief, the present study showed that 3-h OGTT-derived IGP was significantly correlated with the onset and progression of DR in Chinese patients with type 2 diabetes. This finding suggested that glycemic control of DR patients should be additionally targeted at IPG to approach the normally glycemic

condition. Future research is needed to elucidate the effect of IGP on DR and to what extent DR could be prevented by reducing IPG.

Abbreviations

IGP, incremental glucose peak; DR, diabetic retinopathy; LD-MCS, Liaoning Diabetic Microvascular Complications Study; ADA, American Diabetes Association; CMU1h, the First Affiliated Hospital of China Medical University; OGTT, oral glucose tolerance test; NPDR, non-proliferative diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy; UKPDS, United Kingdom Prospective Diabetes Study; DCCT, Diabetes Control and Complications Trial; HbA1c, glycated hemoglobin A1c; GV, glycemic variability; SD, standard deviation; MAGE, mean amplitude of glycemic excursions; CGM, continuous glucose monitoring system; LD-RS, Liaoning Diabetic Retinopathy Study; LD-CKDS, Liaoning Diabetic Chronic Kidney Disease Study; LD-PNS, Liaoning Diabetic Peripheral Neuropathy Study; IRB, Institutional Review Board; ETDRS, Early Treatment for Diabetic Retinopathy Study; PDR, proliferative diabetic retinopathy; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; PP, pulse pressure; FPG, fasting plasma glucose; PG, plasma glucose; HOMA-IR, model assessment of insulin resistance index; HOMA- β , homeostasis model assessment of β -cell function; eGFR, estimated glomerular filtration rate; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; OR, odds ratio; CI, confidence interval; Q, quartile; CIMT, carotid intima-media thickness; CV, coefficient of variation; ATTD, Advanced Technologies & Treatments for Diabetes; PKC, protein kinase C; AGE, advanced glycation end.

Declarations

Ethics approval and consent to participate The study adhered to the content detailed in the Declaration of Helsinki and got approval from the Institutional Review Board (IRB) of the First Affiliated Hospital of China Medical University (AF-SOP-07-1.1-01/2019-13).

Consent for publication Not applicable.

Availability of data and materials The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests No conflicting relationship exists for any author.

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Authors' contributions Contributed equally to this work

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Figures

Figure 1

Associations between incremental glucose peak (IGP) and stages of DR after controlling for confounders
 Model 1: includes gender and age; Model 2: includes gender, age, BMI, SBP, PP, antidiabetic agents, diabetes duration, and HbA1c; Model 3: includes gender, age, BMI, SBP, PP, antidiabetic agents, diabetes duration, HbA1c, FPG, HOMA-IR, serum creatinine, and eGFR. Abbreviation: IGP, incremental glucose peak; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; VTDR, vision-threatening diabetic retinopathy; BMI, body mass index; SBP, systolic blood pressure; PP, pulse pressure; HbA1c, glycated hemoglobin A1c; FPG, fasting plasma glucose; HOMA-IR, the homeostasis model assessment of insulin resistance index; eGFR, estimated glomerular filtration rate; OR, odds ratio; CI, confidence interval. Error bars represent the 95% confidence interval of odd ratios in IGP.

Figure 1

Associations between incremental glucose peak (IGP) and stages of DR after controlling for confounders
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